THE ROLE OF DIET

IN FELINE

INFLAMMATORY BOWEL DISEASE

by

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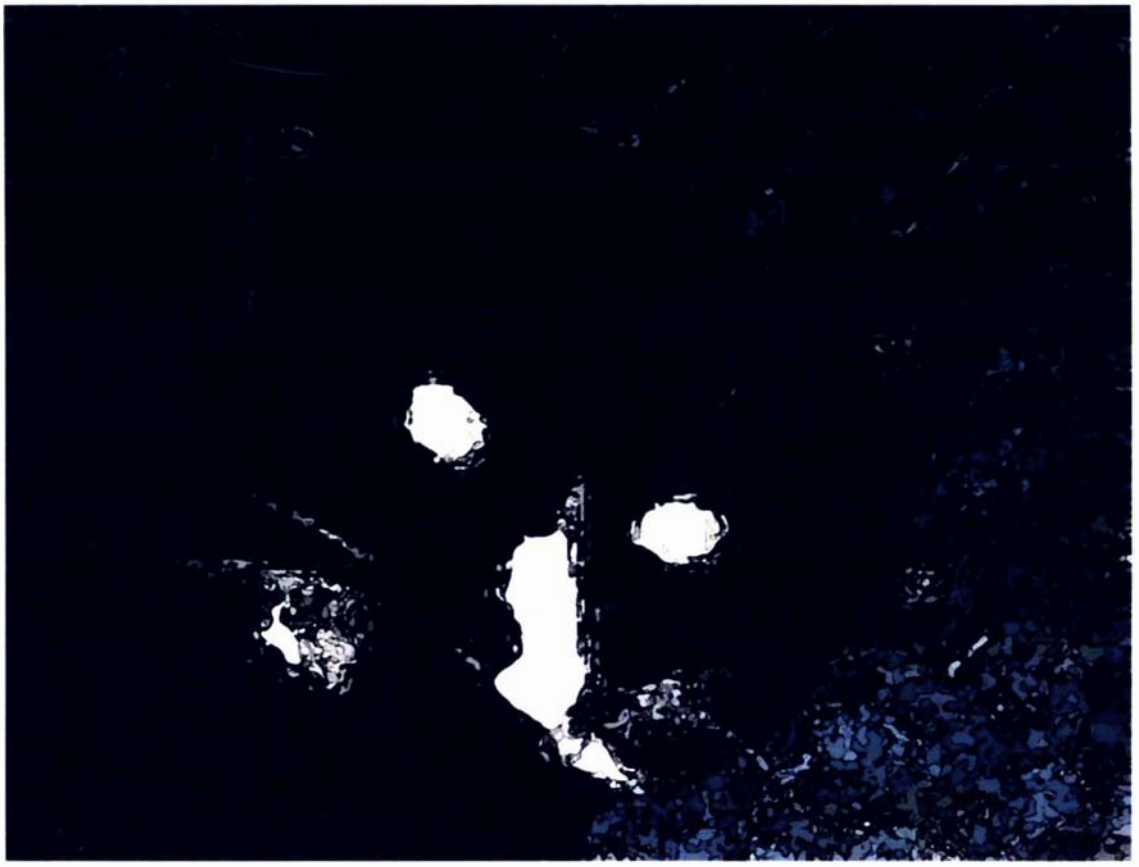
The Role of Diet in Feline Inflammatory Bowel Disease

Volume I



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ABSTRACT

THE ROLE OF DIET IN FELINE INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a chronic, idiopathic inflammatory condition of the gut mainly studied in man but also recognized in the cat. The main constraint of working with IBD is that diagnosis cannot be reached with absolute certainty. A role for diet in the initiation and/or maintenance of chronic inflammation in IBD has long been suspected among a number of other possible risk factors. On the other hand, diet is also recognized as a very important mode of therapy in IBD. There is a paucity of information on feline IBD and the effects of dietary components on gastrointestinal inflammation in the cat. This is partly due to the lack of practical and effective techniques to improve diagnosis and easily monitor therapy in a clinical patient. To test the hypothesis that diet does have a role in feline IBD, a retrospective multicentre epidemiological study and two prospective dietary clinical trials examining the influence of different sources of carbohydrates and a dietary fibre equivalent were conducted. In addition a device for easy and simple collection of colo-rectal mucosal fluid was developed and tested in a small number of cats. This device may help to improve diagnosis or to measure responses to treatment, including the response to dietary management of feline inflammatory bowel disease.

Since IBD diagnosis is a deciding factor in the inclusion criteria for both the retrospective and prospective study, it is of pivotal importance to define how this diagnosis was reached. In this thesis a diagnosis of IBD was restricted to cats with chronic clinical signs of gastrointestinal disease (anorexia, vomiting, diarrhoea, weight loss, haematochezia) and signs of leukocytic infiltration in the lamina propria of the gastrointestinal tract. In addition these cats had negative serology for

FIV/FeLV, negative faecal flotation, normal haematology and biochemistry, normal serum thyroxine concentration, a lack of response to a week-long dietary trial and a lack of abnormalities detected either by abdominal radiography or ultrasound. Other tests were included in the prospective studies as considered necessary to establish the diagnosis.

The epidemiological study investigated diet and other risk factors associated with the presence of IBD in cats. Data on the signalment, stress factors (sexual activity, length of ownership, change of address, number of cats in the house, other pets in the house, frequency of boarding and cats show attendance), environment, temperament, lifestyle, presence of disease and diet prior to the diagnosis of IBD were collected. Matched (by age, gender and breed) and random control groups were included. Cats with IBD were commonly females, 7 to 15 years old. Exotic breeds were over-represented. In addition, having only one dog in the household appeared to be associated with the disease, while more than one did not. Overall, a higher frequency of potential stress factors appeared to significantly predispose to the disease. Vomiting, diarrhoea, anorexia and dental disease were found to be common in cats with IBD before diagnosis. Skin (acne, reaction to insecticide spray, eosinophilic plaque, plasma cell pododermatitis, otitis externa) and respiratory problems (sneezing or coughing) were also more prevalent among cats with IBD. Lifestyle, veterinary care and diet were very variable between countries but none of them proved to be significantly associated with feline IBD.

The retrospective nature of epidemiological studies does not allow all possible nutritional associations to be studied. This fact along with the known nutritional idiosyncrasies of feline nutrition provided the logic to investigate diet as a mode of treatment in two prospective dietary trials. One trial involved a comparison of different sources of starch (rice, barley, tapioca and corn 18.4 – 25.8%ME) by healthy cats and cats with IBD. The rationale for this comparison was that cats do not possess all the tools to deal with dietary carbohydrates when compared to other species, and that carbohydrate malabsorption can occur during gastrointestinal inflammation and contribute to the clinical signs of gastrointestinal disease. The other prospective dietary trial tested diets supplemented with different amounts of inulin (0, 0.1 or 0.2%DM). Inulin is an oligosaccharide which effects

in the gastrointestinal tract of man and other species resemble the actions of dietary fibre. The products of fermentation of dietary fibre are considered beneficial for colonic health and have been used for the treatment of idiopathic colitis in several species.

The study on carbohydrate tolerance was a crossover study that included a control group of healthy cats from a research facility and cats diagnosed with IBD (according to the criteria mentioned above). Breath hydrogen collection, faecal grade and water content, faecal sodium, faecal potassium, faecal osmolar gap and gastrointestinal clinical signs were used to compare carbohydrates. IBD cats showed a higher area under the curve (AUC) of breath hydrogen (p=0.0001) indicating malabsorption of carbohydrate, irrespective of starch source, when compared with healthy controls. No deleterious effects on faecal characteristics, clinical signs or body weight were observed. The faecal osmolar gap did not prove to be useful to identify cats with IBD. Rice increased faecal sodium/potassium ratio when compared with the other starches. In summary, carbohydrate malabsorption seems to be a feature of gastrointestinal inflammation in the cat but in the short term it does not seem to be detrimental in terms of clinical signs or body weight. On the other hand the feline colon appears to have an amazing capacity to maintain water absorption in the midst of an increased load of fermentable material. Hence carbohydrate malabsorption cannot be judged by faecal characteristics in the cat. In addition, the use of rice as the preferred carbohydrate for dietary management of feline IBD may need to be further examined since the AUC when consuming rice was similar or higher than with the other sources of starch. The significance of the finding that the rice based diet was associated with an increase in faecal sodium is uncertain.

The inulin study was conducted in healthy cats (20 belonging to a research facility and 10 owned by the public) and a small number of cats with IBD. Changes in microdissection parameters (number of dividing cells per crypt, number of epithelial cells per crypt cell column, crypt length, crypt width and crypt area) in the duodenum, colon and rectum were studied as well as changes in histological preparations, transit of radiopaque markers and macronutrient digestibility. The addition of inulin to feline diets was associated with an increase in the number of

epithelial cells per colonic crypt (p=0.006) and colonic crypt length (p=0.025) after the cats ate the diets supplemented with inulin for four weeks with no indication of a dose response to inulin. There was also a trend towards a greater number of dividing cells in duodenal (p=0.07) and colonic ((p=0.07) crypts in publicly owned cats consuming the diet with 0.2% inulin. Much variation was found between research colony cats and publicly-owned cats before the trial started. The addition of inulin was not detrimental to faecal characteristics, macronutrient digestibility and did not cause any change in the transit of radiopaque markers in healthy cats. The increase in crypt cellularity in healthy cats is a potentially beneficial effect for the treatment of colitis but further research in cats with clinical colitis is required.

Dedicated to the

LOVING MEMORY

of my father

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At the start of this degree I was told that it would be a 'sink or swim experience'. Staying afloat was, at times, very difficult and I have swallowed a fair amount of water in the process. Little did I know when I started that besides the academic challenge, life would have a few surprises in store. However, the support of family and friends and the generosity of many people I did not know carried me through and were fundamental to my finishing of this thesis. Although this thesis will bare my name, it is the result of the time, effort and good will of many people.

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THE ROLE OF DIET IN FELINE INFLAMMATORY BOWEL DISEASE

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CHAPTER 1

INTRODUCTION

The term "Inflammatory Bowel Disease" (IBD) in the veterinary field has been 'borrowed' from human medicine. In man the term IBD is used most commonly to refer to a group of idiopathic diseases of the gastrointestinal tract: Crohn's disease and ulcerative colitis [1]. The former was first reported in 1932 as a regional ileitis by Crohn, Gizburg and Oppenheimer [2]. These investigators did not believe that the disease had been 'missed' prior to their publication because reputable pathologists were known to perform complete autopsies during the 19th century. Therefore, Crohn's disease was considered by them to be a disease of the 20th century [3]. The history of ulcerative colitis is slightly older with the first report describing the condition as a distinct clinical entity dating from 1875 by Wilks and Moxon [4]. However, both diseases were already included among the Case Reports of Giovanni Morgagni's de Sedibus in the 18th century [4].

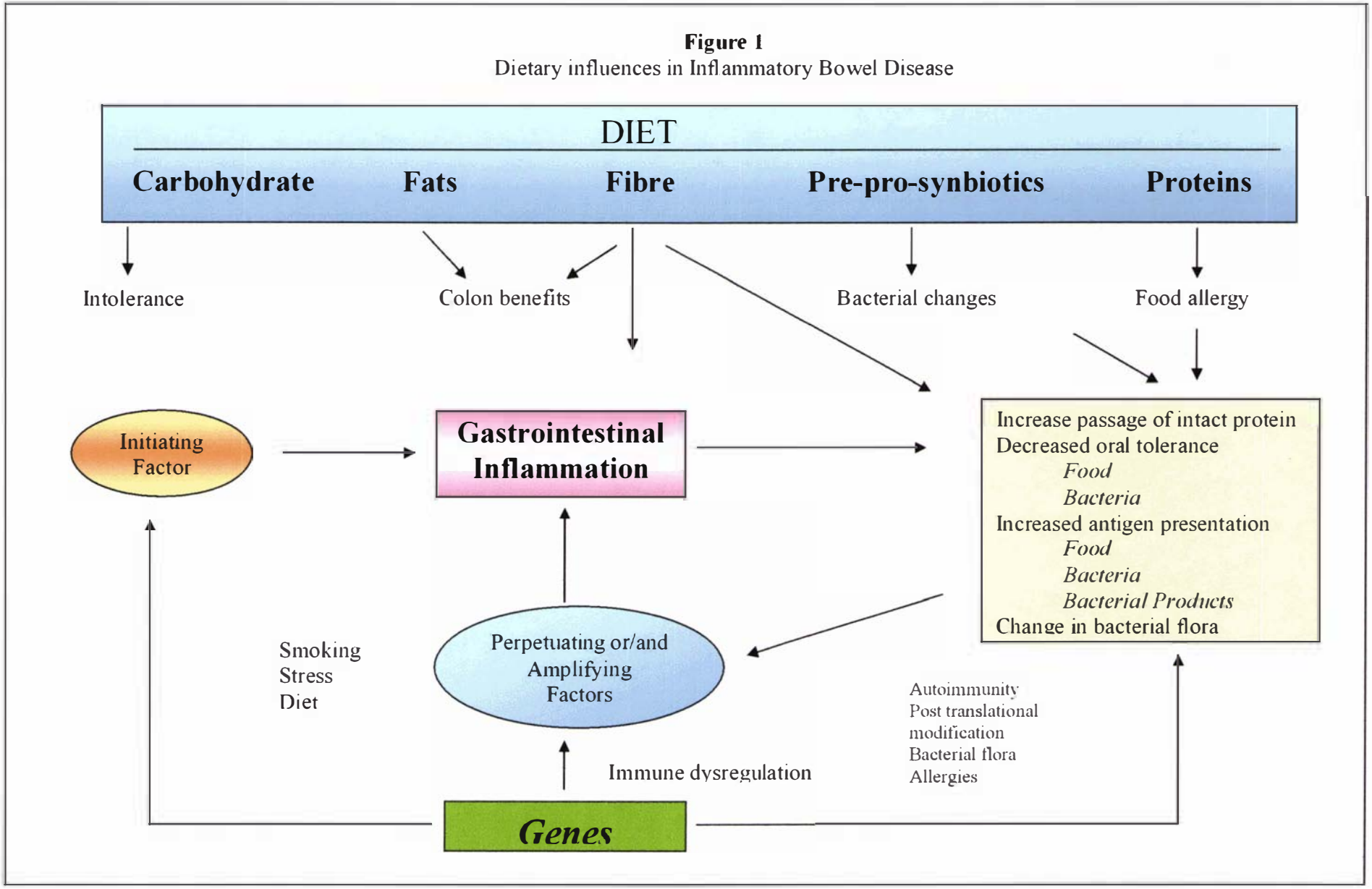
Although these two diseases are grouped together under the term IBD, evidence has mounted to suggest that they may be different diseases. They are both chronic diseases with spontaneous relapses and genetic predispositions

modified by environmental factors. However, immunologically and pathologically they are distinct diseases. Crohn's disease is a granulomatous, transmural enterocolitis whereas ulcerative colitis is characterised by superficial mucosal inflammation [1,5].

In veterinary science the term IBD is used to describe a group of gastrointestinal diseases characterised by persistent clinical signs and histological evidence of lamina propria inflammation to which no cause can be ascribed [6]. This definition does not specify the type of inflammation, type of lesion or location within the gastrointestinal tract. While this definition accurately represents the way the term is used in veterinary science, the breadth of the definition, the fact that IBD is a diagnosis of exclusion, and the reliance on subjective histological parameters for diagnosis present many problems to those wishing to reach a definitive diagnosis of IBD and differentiate the truly idiopathic inflammatory diseases of the gut from the non-inflammatory functional disorders of the gastrointestinal tract. These diagnostic problems, with particular reference to the cat, are discussed in more detail in the next chapter.

As Crohn's disease and ulcerative colitis have become more commonly diagnosed in the last few decades a greater understanding of their pathophysiology has been gained. A basic model of human IBD including important epidemiological factors has been proposed. This model includes an

Figure 1
Dietary influences in Inflammatory Bowel Disease



initiating factor, which is unknown at this stage, followed by factors that perpetuate or amplify the gastrointestinal inflammatory response. These latter factors include enteropathogens, bacterial flora and their products, dietary antigens and genetic susceptibility. Immunoregulation (influenced by genetics) is affected and tissue damage ensues [5,7,8]. In this model, diet could act as a trigger for the chronic inflammation (in a similar fashion to gliadin in celiac disease) or as a factor that maintains or enhances other phlogistic influences in the gut lumen. On the other hand, diet has also been suggested to be an effective mode of therapy in IBD of humans [9] and animals[10]. An appreciation of all dietary influences on the disease can be gained from **Figure 1**.

IBD has been reported in the cat[11]. However, no information is available on the possible risk factors for the disease in this species and it is uncertain if the human model described above applies to the cat. As a result, veterinarians must rely on information gained in other species (humans or dogs) when diagnosing and treating feline IBD. Sometimes data obtained in healthy cats is applied to the management of cats with gastrointestinal inflammation. This practice may not be reliable either. Furthermore, the cat is classified as a "strict carnivore" which implies that its natural diet does not contain large amounts of carbohydrates and dietary fibre. Although healthy cats can consume and thrive on diets with a large proportion of carbohydrates, it is known that cats do not have the full armamentarium of tools to deal with dietary carbohydrates when compared to other species[12]. Both these nutrients, digestible carbohydrates and dietary fibre,

have been considered important in the dietary management of IBD in other species. Unfortunately, their importance and gastrointestinal effects in cats diagnosed with IBD have not been studied. This is partly due to a limited range of techniques that are ethical and easily applicable to the clinical situation by which feline IBD can be studied.

Given the paucity of information on the role of diet in feline IBD a broad approach to study the subject was taken. A comprehensive review of the topic is presented first. This review is followed by an epidemiological study looking for associated dietary risk factors that could guide or direct further research into the role of diet in feline IBD. Other (non-dietary) risk factors were studied as well to make the most efficient use of the resources applied to the epidemiological study. Unfortunately, this study did not identify any dietary risk factors for IBD in cats. However, given the shortcomings of retrospective studies, the idiosyncrasies of cat nutrition and the proposed role that carbohydrates and dietary fibre play in the dietary management of IBD in other species, it was decided to study the effects of these two nutrients on feline IBD. One of these studies investigated the response to different sources of carbohydrates in healthy cats and cats with IBD. The next study looked at the structural and functional changes that follow supplementation of diets with inulin, a dietary fibre equivalent. During the literature review it became apparent that there may be significant limitations in relying on morphological parameters to assess gastrointestinal responses to nutritional trials. These limitations were confirmed in the study of the effects of inulin

supplementation. As a result, the last study of this thesis describes an attempt to develop a simple tool that may prove useful in the future to improve the diagnosis of feline IBD or to study responses to nutritional management or other modes of treatment during the course of feline IBD.

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CHAPTER 2

THE ROLE OF DIET IN FELINE INFLAMMATORY BOWEL DISEASE

Given the scarcity of information on the role of diet in feline IBD, one of the purposes of this research program was to review the literature on the topic. The diagnosis of IBD in cats and other species is controversial and can be a constraint when doing research on this disease because of the importance of ascertaining the diagnosis to make truly relevant observations. As a consequence, this review will present a discussion on the diagnosis of feline IBD and its shortcomings first. This will be followed by a description of the epidemiological features of the disease in man and animals. Subsequently the genetic predispositions and gastrointestinal environmental factors that can perpetuate and amplify the inflammatory response in IBD will be discussed. It is noteworthy that these factors are directly and/or indirectly related to the role of diet in IBD and hint at the most beneficial therapeutic interventions (particularly dietary therapeutics) that are described in the final sections of the review. Because of the breadth of the subject and the many nutrients that have been found to have an impact on the

management of IBD in general and on gastrointestinal inflammation specifically, this will be a comprehensive review. It will include all known dietary factors that can affect the appearance, expression, course and treatment of inflammatory bowel disease (IBD). The objective of the review is to provide the reader with a full understanding of how diet may affect inflammatory bowel disease before, during and after the disease has been diagnosed even though many of these factors will not be studied any further in the context of this thesis.

DIAGNOSIS OF EXCLUSION: A TRUE DIAGNOSIS?

IBD: a common case of mistaken identity

IBD in cats is not a common disease. It has been reported as occurring at a rate of 1.7/1000 admissions [1,2], but its true incidence/prevalence is unknown due to the difficulties in diagnosis. However, it is considered the most common cause of gastrointestinal disease in the cat [3].

The clinical signs presented by animals later diagnosed with inflammatory bowel disease are non-specific and variable. Clinical signs have usually been present for weeks to years at the time of presentation and have fluctuated in severity. The most common clinical signs are vomiting, diarrhoea, haematochezia, mucus in the stools, weight loss, appetite changes and changes in demeanour [1-9]. Because none of these signs are

pathognomonic for IBD, animals have to be thoroughly tested to eliminate systemic disorders (e.g. renal or hepatic conditions and thyrotoxicosis), other gastrointestinal disease than IBD (e.g. foreign body, motility disorders, neoplasia) or infectious diseases [10].

Once these tests are completed, the confirmation of a diagnosis of IBD is usually reached when intestinal biopsy specimens show gastrointestinal inflammation. There are two main aspects of mucosal anatomy to consider when assessing gastrointestinal biopsy specimens. One aspect is morphology and morphometry and the other is quantification of lamina propria inflammatory infiltrates. Both of these features ideally should be evaluated on well established reference values [11,12]. There have been reports on normal morphology and morphometry of the small intestine [13,14] and colon [15] of dogs and more recently the colon of cats [16] using a variety of methods.

Morphology is usually assessed subjectively, but there are several methods available to quantify changes in morphology. These morphometric methods have evolved from simple but time consuming measures applied to standard histological sections to more sophisticated automated computerized methods.[17,18]. The computerized methods measure surface area or mucosal volume or a mixture of the two [19]. Corazza et al. (1985) [19], compared manual and computerized methods and found that they correlated very well when assessing jejunal biopsies from patients with celiac disease. For veterinarians, there is an important difference here in that mucosal changes in feline IBD are not as profound as those in human celiac disease. Consequently, measuring mucosal differences in feline IBD may be more difficult than in human celiac disease no matter what the method used.

In veterinary science, mucosal morphological changes are considered important when deciding if pathology is present [20], but, unless the changes are severe, it is a matter of personal opinion as to whether the changes are abnormal or within expected normal variation.

A more difficult histological procedure is the quantification of inflammatory cellular infiltrates. Salzman (1989) [21], proposed an image analysis method to count nuclear density in the lamina propria. This method is compromised by the lack of identification of the type of cell present in the infiltrate. Lee (1988) [22], used a computerized point counting method that is more time consuming than the method of Salzman (1989) [21] but allows identification of the type of cell present. Unfortunately neither of these methods account for regional variations and the pathologists interpretative bias [21,22].

Infiltration of intraepithelial lymphocytes is given prominence in the diagnosis of IBD and is usually measured as the number of intraepithelial lymphocytes (IEL) per 100 epithelial cells [10,20,23]. IEL are a heterogeneous group of lymphocytes [24] that vary in number and function between the small and large intestine [25]. Cats seem to have a higher number of IEL than other species [26]. Functionally, IEL would seem to be an excellent cell type to quantify because they are the first line of immune cells that are in contact with possible antigens although the pathogenic significance of these cells is yet to be fully elucidated. Unfortunately, counting IEL in standard histopathological sections may be inaccurate because of changes in the proportions of the different intestinal cell populations when enterocytes are damaged, the problems of using flat sections to evaluate

three dimensional structures, and the variable relationship between cell volumes and cell numbers [27]. These investigators used data from patients with celiac disease in which there are prominent changes in the size and shape of villi and intestinal epithelium. This feature of celiac disease would highlight the inaccuracies reported in the counting of IEL. As mentioned previously, the epithelial changes of cats with IBD are not as severe as those of humans with celiac disease. Therefore, perhaps the degree of error in counting IEL would not be as large in cats with IBD as reported by Crowe and Marsh (1994) [27] in humans with celiac disease.

The presence of idiopathic inflammatory infiltrates in the gastrointestinal lamina propria have been identified in several species including cats, dogs [28], cheetahs [29] and primates [30] in association with a variety of gastrointestinal signs. In none of these species have specific histopathological abnormalities been identified in the gastrointestinal tract of affected animals beyond a subjective increase in the number of lymphocytes, plasma cells, eosinophils and neutrophils in the lamina propria with or without changes in villus or crypt morphology.

Unfortunately, there are many potential causes of this type of mixed inflammatory infiltrate in cats. These causes include FIV [31] and FeLV [32], *Giardia* infection [33], *Cryptosporidium* infection [34], enteropathogens [35-37], and gastrointestinal nematodes [10]. *Helicobacter* spp. has been associated with the presence of histopathological changes in cats and dogs with gastrointestinal signs [38]. However, earlier reports about *Helicobacter* spp. in healthy animals indicate that this microorganism is common and is not necessarily associated with an intestinal cellular reaction [39]. Other causes of intestinal lamina propria

inflammatory infiltration are more complex and difficult, at times, to separate from idiopathic inflammatory bowel disease e.g. adverse reactions to food [40] and lymphoma [41,42]. For instance there are case reports of animals diagnosed with IBD that went on to develop lymphosarcoma [34], as well as animals that were diagnosed twice with lymphosarcoma but later responded to a dietary change [43]. The dietary response in the latter animals suggests they suffered from an adverse reaction to food rather than neoplasia.

Feline hyperthyroidism has been considered to cause inflammatory infiltration of the intestinal lamina propria [41]. Similarly, hyperthyroidism in humans has been reported to cause oedema and infiltration with lymphocytes, plasma cells and eosinophils of the mucosa of the small intestine [44]. This infiltrate was variably responsive to treatment in cats and humans.

An association between inflammatory hepatic disease (cholangiohepatitis), pancreatitis and the presence of gastrointestinal lymphocytic-plasmacytic infiltration has been found [45,46]. In this situation it may be difficult to decide which disease is most important clinically. This poses the question: how much clinical significance should we place on the presence of lymphocytic-plasmacytic infiltration of the gastrointestinal mucosa, pancreas and liver.

Several authors have reported case series of cats showing gastrointestinal clinical signs in which the main inflammatory infiltrate cell types were lymphocytes and plasma cells [1,2,4,7,9,43,47,48]. Lymphocytic-plasmacytic enteritis, enterocolitis or colitis, is

sometimes used as a synonym for inflammatory bowel disease because it is the most common pathological diagnosis found in animals diagnosed with IBD [34,41,49]. Eosinophilic [8,50,51], histiocytic [52], suppurative [3,53], ischemic [52], and granulomatous [20,52] gastrointestinal inflammation have also been reported. However, the concept of pathologic diagnosis based on cell type prevalence is controversial [9,20] because it is subjective. The clinical signs of the patient usually hint at the site of the gastrointestinal inflammation. However, concurrent presence of inflammatory infiltrates in the small and large intestine has been reported even when clinical signs suggested involvement of only one segment of the gastrointestinal tract [1,3,9].

The above studies clearly demonstrate that idiopathic inflammatory infiltrates are frequently reported in association with gastrointestinal signs in cats. However, the significance of the reported inflammatory infiltrate is debatable for several reasons. Firstly, the assessment is subjective [12,20,41]. No studies that objectively quantify inflammatory infiltrates in cats with gastrointestinal signs have been published. Furthermore, morphometry is not used in cats with gastroenteric disease to aid diagnosis. This is unfortunate because it has been shown in people that the experience of the pathologist does little to increase reliability of the subjective assessment whereas morphometry significantly reduces inter-observer variation in the assessment of intestinal villi atrophy [54]. Secondly, the factors that affect and modify the normal range of cellular infiltration of the lamina propria are poorly understood. Thirdly, there is little correlation between the magnitude of inflammatory infiltrate in the intestinal lamina propria of cats and the severity of clinical signs or response to treatment [1]. Little correlation has also been reported between the magnitude of the inflammatory infiltrate and the severity of

macroscopic endoscopic assessment of the mucosa in people [55], cats and dogs [49,56,57]. However, Dennis, Kruger and Mullaney (1993) [2] found that most cats with petechia and hyperaemia of the colonic mucosa had severe histopathological infiltration of lymphocytes and plasma cells of the colon. It is uncertain if this is a cause and effect phenomenon or just a coincidence. Most of the cases reported in this series had severe histopathological changes. However, colonic absorptive function has been found to correlate inversely to the level of leukocyte infiltration in people [22].

Procuring biopsy specimens also presents many difficulties. Most gastrointestinal biopsy samples are obtained endoscopically. This means that the tissue samples are small and need to be of high quality to be amenable to correct interpretation [58]. Furthermore, some areas of the gut are not accessible with the standard endoscopes and localized lesions can be completely missed. In addition, endoscopic biopsy can induce some artefactual histological changes. Spinato, Barker and Houston (1990) [15] have shown endoscopic biopsy specimens of canine colon to have shorter crypts and reduced numbers of intraepithelial lymphocytic and goblet cells when compared to the colonic tissue obtained by incisional biopsy at necropsy from the same animal and from the same area of the gut.

Most veterinary case reports have used a subjective and at times ill-defined system to grade gastrointestinal inflammation. Mild, moderate and severe inflammation can have different meanings to different people and the grade assigned is often based on personal experience and not on an objective quantitative method. Although it is commendable that some authors have used and published grading systems [23] (e.g. for canine colitis), they are

hard to use in an objective manner because the method by which some parameters are assessed is not described in detail. Wilcock (1992) [20], has published also some guidelines for the interpretation of biopsy samples from the gastrointestinal tract of cats and dogs but no definitive criteria for normality have ever been published. Recently, typification by immuno-histochemistry of lymphoid cells and other leukocytes in the gastrointestinal tract of healthy dogs [59], dogs with IBD [60], dogs with other enteropathies [28], and healthy specific pathogen free cats has been reported [26,61]. These reports are important contributions to the understanding of gastrointestinal histology and pathology, but their usefulness for diagnostic purposes remains to be seen.

The last question to answer is what the meaning of cellular mucosal changes is. Even a reliable measurement of the severity of lamina propria infiltration does not necessarily represent a useful diagnostic tool. Lymphocytes and other inflammatory cells are fulfilling a defence function for a gastrointestinal tract being continuously bombarded by antigens. These antigens vary with diet [62], intestinal flora [63] and many other factors related to gastrointestinal function. Until a wider knowledge of the factors that affect the infiltration of the intestine with inflammatory/defence cells is gained, the diagnosis of IBD will be fraught with difficulty.

In summary, inflammatory bowel disease is by necessity a diagnosis of exclusion because many harmful influences on the gastrointestinal mucosa can induce gastrointestinal clinical signs, cause damage to intestinal structures and increase cellular infiltration. As there are no means of definitively diagnosing the disease, the clinician is left with testing for and eliminating other infectious or systemic causes of gastrointestinal

inflammation. The assessment of cellular infiltration is subjective and the factors that affect this infiltration are mostly unknown. Gastrointestinal inflammatory cells can fulfil a defence function and the gastrointestinal mucosa is in permanent contact with potential noxious stimuli. As a result, it is important to understand what makes these cells locate in the lamina propria, what the range of normality for the degree of cellular infiltration is and what factors produce this normal variation. Unfortunately, this information is not yet available.

EPIDEMIOLOGY

Predisposing factors. Chance or culprits?

Diagnosis of IBD in humans has been steadily increasing in the last few decades [64]. This is also true of feline IBD [41]. Epidemiological studies are done to help find the cause of diseases and to identify exacerbating and associated factors. There are more than 100 studies published on the epidemiology of IBD in humans [65]. However, to my knowledge, no epidemiological studies of large numbers of cats and dogs with IBD have been carried out. The difficulties inherent in the epidemiological study of an infrequent disease that is a diagnosis of exclusion and requires a high level of expenditure and the application of advanced technology for proper diagnosis is discussed by Calkins (1986) [64]. These problems are only the more evident in the veterinary field. Many of the earlier epidemiological reports on risk factors for IBD in people have been criticized because of the methodology used and the large number of inconsistencies in results [65]. In the last

decade, however, the use of multicentre collaborative studies has gained popularity in the epidemiological study of human IBD and has provided better epidemiological information [66]. Despite this improvement the cause of IBD has remained elusive. A summary of the most important epidemiological factors that have been studied in human IBD and the epidemiological features of the disease in animals are presented in the following section.

Sex

Crohn's disease has been found to be slightly more common in women than men while men suffer ulcerative colitis more often than women [67]. The sex predisposition does, however, seem to vary with the date of the study and the geographic location of the patients [64]. It has been suggested that these differences are due to the changing smoking habits between women and men in the last two decades [68]. Most veterinary reports do not identify a sex predisposition in cats for IBD [1,2,7,47,48]. However, in the case series with the highest number of cats, male cats seem to be affected more often [3,5,9]. The physiologic processes by which a patient's sex can affect the expression of disease are unknown.

Age

Descriptive statistics in humans reveal a bimodal age distribution in patients with IBD, with a peak in early adulthood and a smaller peak between 55 and 65 years [64,67-69]. In the veterinary field, it is commonly believed that IBD manifests more often in middle aged or older cats [41]. This belief is supported by those studies of feline IBD which disclose the age of the cats studied [1,3,5,47]. Occasionally, very young animals have been diagnosed with IBD (Tams, 1993). In this respect, there are a few points to consider. One is the waxing-waning course of feline IBD [10], which may delay the diagnosis until

later in the cat's life when the owners are sure that is not a transient gastrointestinal upset. Also of note is that it is difficult to differentiate IBD from adverse reactions to foods [40] and gastrointestinal lymphoma [34]. These diseases may confuse the age distribution of IBD because adverse reactions to food can occur at any age and neoplasia is more common in aged cats.

Race and breed

In humans the predisposition of some races to IBD has been reported [68]. It is important to note, however, that the environment has an interrelationship with the genetic predisposition. For example, Jewish people of European origin have a high prevalence of the disease but not if they have been born and live in Israel [70]. Similarly African-Americans have a higher rate of the disease than Africans living in Africa [69]. Interestingly, the geographical differences in the prevalence of IBD seem to be becoming smaller in parallel with economical and technological development [67]. It is uncertain if such 'development' truly affects the prevalence of the disease or the ease with which it can be diagnosed.

No breed predisposition has been identified in feline IBD [6,41] although the possibility that pure-bred cats are more susceptible has been mentioned [12]. It is possible that the identification of breed predisposition has been obscured by the relative infrequency of the disease and the preponderance of cross-bred domestic cats in the population.

Further detail on the genetic basis of IBD can be found in the section on causal factors.

Allergic disease and respiratory infections

Gilat et al, (1987) [71] found that atopic eczema and respiratory infections were more common during childhood in patients with IBD than in healthy controls or patients with diseases other than IBD. The former association is an interesting finding because it raises the possibility that patients with IBD may have a state of over-responsiveness to common antigens, potentially including dietary antigens. However, in the same study, the prevalence of asthma, hay fever, milk allergy and other food allergies were no different between patients and controls. In contrast, Glassman et al (1990) [72], observed an association between cow's milk sensitivity and ulcerative colitis in people with IBD. Seasonality in onset of ulcerative colitis has been reported and may indicate an association with environmental agents, possibly allergens [73]. No association between allergic disease, respiratory infections and IBD has been reported in cats although a recent study [40] demonstrated the strong similarity (both clinical and pathological) between food sensitivity and IBD in cats. Furthermore, atopic disease is not as well defined in cats making it difficult to study the relationship between the diseases. Viral respiratory infections, on the other hand, are common in the feline population. Therefore it will be hard to demonstrate if an association between IBD and viral infection exists.

Other infectious agents have also been suggested as possible culprits in the generation of chronic gastrointestinal inflammation. The measles virus has been found in lesions of Crohn's disease in people but not in controls [74]. *Mycobacterium*

paratuberculosis has also been incriminated [75,76]. However, conclusive evidence is lacking.

Breast feeding and gastrointestinal disease

It has been proposed that breast feeding is protective against subsequent development of IBD in humans [77], perhaps because breast milk is considered also to reduce the risk of gastroenteritis. . However, Gilat and coworkers (1987) [71] in a multicentre study did not find that gastroenteritis in the first year of life was more frequent in patients with IBD than controls nor that breastfeeding was protective against IBD. On the contrary, Koletzko et al (1989) [78] showed an increased risk of Crohn's disease in children fed milk formula and those who had experienced bouts of diarrhoeal disease. Only the latter group had an increased risk of ulcerative colitis [79]. In both these studies, unaffected siblings were used as controls to reduce the confounding effects of genetic and environmental background.

Stress

Stressful situations have been associated with IBD in humans and cats. Tams (1993) reports that bouts of IBD occur commonly around queening and other stressful situations. Stress is known to affect gastrointestinal motility [80,81] and neural influence on the inflammation of IBD has long been recognized [82]. However, stressful events during childhood were not found to be risk factors by Gilat et. al. (1987) [71]. A prospective study of human IBD [83] found a weak association between disease exacerbation and mood changes but no causal relationship could be established. Singular stressful life events were

also not a significant factor in disease exacerbation. However, sustained stress has been shown to be important [84].

An experimental study of rats found that colitis in of itself rendered the colon more susceptible to the effects of stress [85]. The same study documented increases in myeloperoxidase activity (a commonly used parameter of inflammation) immediately after the stressful event.

Smoking

Epidemiological studies have consistently shown that smoking is protective against ulcerative colitis but increases the risk of Crohn's disease [67,86]. Furthermore quitting smoking is associated with the onset of ulcerative colitis and smokers show a dose-response pattern of ulcerative colitis signs [87]. Childhood passive smoking decreases the risk of ulcerative colitis [88]. Exposure to cigarette smoke was found to aggravate experimental colitis in rats [86] especially in models of granulomatous inflammation such as occurs in Crohn's disease.

The mechanisms by which cigarette smoke changes the risk of IBD have not been elucidated. This lack of understanding of the pathophysiology involved and the presence of some conflicting studies has produced scepticism by some about the effect of cigarette smoke on IBD [89]. However, tobacco smoke, which contains thousands of potentially bioactive substances, has been found to inhibit the release of some cytokines [90] and inhibit mucus synthesis [91]. Nicotine has even been tried as a new complementary treatment in ulcerative colitis [92]. Nicotine has extensive effects on the gut affecting permeability,

motility and immune function [86]. The reason why cigarette smoke would affect Crohn's disease and ulcerative colitis in opposing ways is unknown.

Diet

Due to the direct contact between what a person or animal eats and the mucosa of the gastrointestinal tract, diet has long been considered an important factor in IBD. This is even more so if we consider that differences mentioned between ethnic groups can be attributed, at least partially, to dietary differences [64]. Yet, in spite of years of research, no direct relationship between IBD and diet, dietary intolerance or sensitivity has been found [93,94]. Although, cow milk allergy has been reported to occur more often during childhood in IBD patients than in controls [72].

Crohn's Disease

An early study of the diet of Crohn's disease patients found that they consumed increased levels of starch, energy and sugar when compared to healthy controls. In this study not more than a year had past from onset of disease (which reduces concern about the patients recollection of their diet) and controls were included that had been matched by age, sex and socioeconomic background with the Crohn's disease patients [95]. Other retrospective studies have shown similar results regarding sugar consumption [96-99] although they differ in the average time since diagnosis from 3 months to 10 years. In contrast, Jarnerot (1983) [100] found that the increased consumption of sugar by Crohn's disease patients occurred after onset of disease and therefore could not be considered a causal effect. Sonnenberg (1988) [101] tried to correlate the geographical and temporal incidence of the disease with the geographical and temporal variation in amount of sugar

consumed. No correlation was found. In contrast, a prospective study of patients (diagnosed within 6 months of the start of the study) showed the Crohn's disease patients to have increased carbohydrate, sugar and starch consumption in comparison to healthy controls matched for age, sex and city of residence [102]. These findings suggest that the increase in carbohydrate consumption was a result of the disease and not a risk factor.

A reduced intake of fruit and vegetables has been reported in Crohn's disease patients during childhood [71] and later in life [95,96] as well as an increase in fast food consumption [99] when compared to healthy controls. Margarine and breakfast cereal consumption does not appear to lead to any higher or decreased risk of Crohn's disease [97,99,101].

Ulcerative colitis

Dietary fibre is thought to improve the health of the large intestine but several studies indicate that sufferers of ulcerative colitis have a similar consumption of dietary fibre (as cereal, fruit and vegetable fibre in one study or lignin, cellulose, pectin and gum arabic in another study) to healthy controls [102,103]. However, one study has suggested that consumption of vegetables decreases the risk of the disease [99]. Increased consumption of energy, carbohydrates, fast foods and protein has been observed in ulcerative colitis patients when compared with healthy controls [99,102].

Constraints of dietary epidemiology

Difficulties can be encountered in retrospective dietary epidemiology studies because they must rely on memory. Pet owners may have difficulty recalling what they

had fed their pets. This is compounded by the wide availability of pet foods with frequent variation in composition and presentation within a single brand and flavour. Other factors that are likely to decrease the validity of dietary recall include the presence of several cats in the house, roaming and hunting, more than one person feeding the animal and ad-libitum feeding. Recall of food consumption in people is thought to be reliable for up to a month [104]. There is always a 'recall bias' towards the present diet and systematic errors can occur as a result of tendencies to overestimate or underestimate quantities [104]. Retrospective studies also presume a constant diet, which is certainly not the case for humans and most animals. The presence of disease could also have altered the diet Tragnone et al. (1995) [102]reported that Crohn's disease patients change their diet and avoid certain foods during the course of the disease. These biases are compounded by individual and interindividual variation in dietary intake and by the fact that in many chronic diseases (such as IBD) dietary associations are unlikely to be strong [105]. Associations between dietary factors and risk of disease are usually attenuated by measurement error [106] and calibration studies have been proposed [107]. Unfortunately, it is difficult to demonstrate the validity of a retrospective dietary intake assessment [108,109]. Good study design is critical to overcome or reduce these difficulties. Important factors include the number of patients studied, appropriate case selection, suitable control groups, awareness of confounding factors, methods to eliminate or quantify their effects and avoidance of bias originating in the recall of the dietary information or in the investigator [104,109]. The final task is to establish a plausible connection between the epidemiological findings and the pathophysiology of the disease being studied. To date this connection has not been convincingly demonstrated in humans or animals with IBD.

Miscellaneous

IBD has increased incidence and prevalence in developed countries and has followed technological development. People with higher education, living in urban areas and in professional or managerial jobs are over-represented among IBD patients [64,66,67]. Interestingly the parents of human IBD patients were most commonly of a lower social class and less educated than controls [71]. Since IBD is a disease that requires referral for diagnosis and the use of expensive diagnostic tools, true prevalence in less developed nations or regions may be difficult to ascertain. This is also true of the veterinary field. For instance, feline IBD was rarely diagnosed in New Zealand until the advent of routine use of duodenoscopy in 1990 (Guilford, 2002, personal communication)

Oral contraceptives and ingested toothpaste have been reported to increase the risk of Crohn's disease while appendectomy protects against ulcerative colitis [64,66,67,71].

A familial predisposition for both Crohn's disease and ulcerative colitis has been documented and first and second degree relatives have a higher incidence and prevalence of the same disease (concordance) [69,71,89]. However, the higher frequency of disease among family members does not separate genetic from environmental factors [110]. A complex segregation analysis of Crohn' s disease patients and their families identified the presence of a recessive gene with incomplete penetrance [111], which explains the higher frequency of IBD in offspring whose parents both suffered from IBD [110]. Twin studies have shown higher concordance for identical twins but this is not complete suggesting the environment influences the expression of the disease [64,66,112]. An interesting finding highlighting the interplay between the effects of the environment and genetic

predisposition was reported by Soderholm (1999) [113]. These researches found that spouses of Crohn's disease patients have abnormal gastrointestinal permeability more commonly than relatives that were not living in the same house as the patient. However, the response of the relatives to a mucosal barrier-disrupting agent was similar to the patients, while the spouses responded in the same way as healthy controls.

More recent research at a molecular level has identified potential genes that may be involved in IBD [114,115]. However, these genes are not widespread in the human IBD population and the presence of faults in some of these genes would most likely protect against IBD rather than predispose patients to the disease. As an example NOD2 abnormalities have been found in approximately 15% of the IBD population [114] but these gene faults would reduce inflammation not predispose to it [116]. Therefore, much research is still required in this field to be able to use this information for the benefit of IBD patients.

CAUSAL FACTORS

Genes, leaks and bugs team up against the gut.

Inherited defects

Genetic influence on the presence or expression of human IBD is an intense area of study. The familial predisposition to both of these diseases in people and the increased incidence and prevalence in certain groups, e.g. Jewish people, has already been

mentioned. Invariably the effect of genes cannot be completely separated from the environmental effects but the increased risk of disease developing in certain races [70] and in close relatives [110] indicates that genetic make-up is important in the aetiopathogenesis of IBD. Further evidence supporting a role for genetics in IBD is the knowledge that IBD accompanies other human genetic diseases like Turner's syndrome, ankylosing spondylitis and Hermansky-Pudlak syndrome [93].

Hypotheses about the actual genes involved in IBD have varied from polygenic systems [93] to simple recessive genes with incomplete penetrance [111], to the identification of chromosomes (16 and 12 of the human genome) that are linked to IBD [117].

Unfortunately in veterinary science the closest that we have come to identify a genetic predisposition to inflammatory bowel disease is the various breed predispositions that have been identified in dogs. These include the granulomatous ulcerative colitis of Boxers, which share characteristics with Crohn's colitis [118], the immunoproliferative enteropathy of Basenjis [119] and the protein-loosing enteropathies of various breeds such as Lundenhunds [120] and soft coated wheaten Terriers [121]. In cats, no breed predispositions have as yet been identified.

Animal models of IBD have also provided proof that the genetic background of an animal can initiate gastrointestinal disease alone or in combination with specific environmental factors. Some animal models of IBD are spontaneous while others are the product of genetic manipulations that affect the immune system. In many of the latter models, the *sine qua non* condition for disease to occur is the presence of bacterial flora

[63,122-124]. These models clearly demonstrate the importance in IBD of the interplay between a genetic defect and an environmental factor (in this case the normal flora).

Precisely how genetic defects lead to IBD is also the subject of intensive investigation. Some genetic studies have focused on trying to identify changes in cytokine genes that can affect the manifestation and expression of IBD. This is likely to be a fruitful approach because cytokines are regulatory chemicals that control inflammation, are genetically determined and show considerable genetic polymorphism [125]. Murine models of IBD have been created by deletion of the genes that control the production of IL-2 and IL-10 causing immune dysregulation [125]

In the search for an inherited dysfunction of the intestine, it has been reported that perhaps an inherited defect in intestinal mucosal barrier is the cause of IBD [126]. Increased permeability in Crohn's disease patients and unaffected first degree relatives has been found [127] but not conclusively proven [128]. Using the lactulose/mannitol ratio, Soderholm (1999a) [128] showed that baseline permeability in spouses of Crohn's disease patients was higher than in first degree relatives. However, the response to an agent capable of intestinal barrier disruption was higher in patients and relatives, but lower in spouses and healthy controls. Hence intestinal barrier dysfunction might be one of the ways genetic defects predispose to IBD. Further support for this hypothesis comes from one of the more recent models of IBD in mice. This model is produced by malfunction of the N-cadherin gene. As a result, there is disruption of intestinal tight junctions [63]. It is uncertain if N-cadherin dysfunction is important in human IBD. The potential

mechanisms by which intestinal barrier dysfunction could be involved in IBD are discussed in the next section.

Mucosal barrier defects

The effectiveness of the gastrointestinal mucosal barrier can be reduced by increase leakage of luminal substances, by disturbances of oral tolerance and by increased antigen presentation by the enterocytes. Each of these defects will be discussed separately in the following sections.

Increase leakage of proteins and peptides

Intact protein and peptides in quantities that could be immunologically significant cross the gastrointestinal epithelial barrier and can be detected in the general circulation in healthy animals and man [129-133]. This phenomenon was first reported in 1936 by Lippard [134]. In fact, *in vitro* experiments have shown that up to 10% of intact protein can pass intact across a human colonic epithelial monolayer [135]. Some of these proteins or peptides will cause the formation of specific immunoglobulins without causing clinically relevant ill effects in healthy people. Interestingly, no correlation was found between the levels of antigen-specific immunoglobulins (IgG and IgA) and the amount of protein antigen detected in circulation in healthy men [130]. Antigen-specific IgE has been found in the sera of healthy rats after protein feeding [136] but healthy humans did not have significant

quantities of antigen-specific IgE in their serum when compared to people suffering from adverse reactions to food after challenge [132]. Human IBD patients do not appear to show higher levels of dietary protein-specific IgE [93] although increases in IgE immunocytes have been shown in the rectum of human patients with IBD [137] and small intestine and colon of dogs with IBD [60].

The production of IgE is considered to be one of the immune responses most effectively suppressed by antigen feeding and resultant oral tolerance mechanisms [138]. In healthy individuals oral tolerance (see later) decreases absorption of antigens probably by increasing the production of IgA [134,139]. This makes sense because one of the cytokines, Transforming Growth Factor-beta (TGF-beta) that is involved in oral tolerance to dietary antigens is also involved in the switching by B lymphocytes in the Peyer Patches to IgA production [139].

Disease and or injury can increase the permeability of the intestinal barrier. The increase in macromolecular permeability has been perhaps prematurely associated with the aetiopathogenesis of several gastrointestinal diseases [140-142]. However, even if not the main cause of the disease, the increased permeability may be an important factor that perpetuates the inflammation [126]. Intestinal permeability has most commonly been measured using inert substances and sugars of different molecular weights. Unfortunately, the permeability measured by these methods has not been shown to be representative of the passage of large antigenic molecules, such as proteins [142,143]. In children with acute gastroenteritis and in dogs with diet-responsive intestinal disease, intestinal permeability has been measured by the lactulose/mannitol or lactulose/rhamnose

ratio, respectively, to assess the status of the gastrointestinal barrier [144,145]. Similar techniques have been also tested in healthy cats [146]. However, in rats the gastrointestinal uptake of intact protein does not correlate well with the lactulose/rhamnose excretion ratio [147]. Nor is there a good correlation in Crohn's disease patients between dextran and ovalbumin intestinal uptake, even when they have a similar diameter and molecular weight [128]. These methodological concerns contribute to the difficulty in determining the importance of intestinal permeability in chronic gastrointestinal diseases such as IBD.

Turner et al. (1988) [147] and Berin et al. (1997) [148], showed that non-specific and antigen-specific systemic sensitization to ovalbumin and horseradish peroxidase respectively, increased the uptake of intact protein by the enterocyte. Gut hypersensitivity reactions [147] (with mast cell activation) that occur after feeding antigenic protein (to which an animal is sensitized) had the same effect but there is also an increase in the uptake of antigen via the paracellular route. This enhanced antigen transport has recently been associated with the presence of IL-4, IgE and CD23 expression on the enterocyte [149]. Crohn's disease patients have been reported to show an increase passage of intact protein (horseradish peroxidase and ovoalbumin) through the upper small intestine mucosa and the ileum [113,150]. Interestingly the study with horseradish peroxidase found that the increase passage of protein occurred mainly in patients with a moderate to severe index of inflammation and the defect was not present in non affected areas [150]. The inference is that the presence of inflammation may have been the determinant factor of the increase of intact protein crossing the intestinal barrier, but if a hypersensitivity reaction was involved it was not investigated. However, the study using ovoalbumin found that the passage of

intact protein was present also in healthy ileal mucosa from Crohn's disease patients suggesting that an increase in passage of intact protein could be a primary defect [113].

Chronic inflammatory conditions of the intestine are characterized by the production of many different cytokines that affect the immune cells of the gut and the intestinal epithelium. Interferon-gamma (IFN-gamma) has been found to directly affect the paracellular route of epithelial transport by inhibiting the Na-K-ATPase which downregulates the tight junction proteins occludin and ZO-1 [151,152] and by altering the actin cytoskeleton in the apical region of the epithelial cells [152]. This explains earlier observations that IFN-gamma increases the flux of mannitol and inulin through intestinal monolayers [151,153]. Although there are some initial reports that indicate that IFN-gamma does not increase the passage of macromolecules through the intestinal mucosa [154]. However, more recently it has been found that IFN-gamma does increase the formation of peptides from exogenous proteins in a human intestinal cell line, an indication of increased transepithelial transport [135].

It should be noted that IFN-gamma is one of the main inflammatory products in human IBD [155] and its concentration is strongly related to severity of disease [156]. In addition, it also has important effects on the efficacy with which epithelial intestinal cells can act as antigen-presenting cells (see later).

In summary, if an increase in intestinal permeability is part of the pathogenesis of chronic gastrointestinal diseases, it appears that the increased permeability needs to be

part and parcel of a dysregulated gut immune response, (including the abrogation of protective mechanisms like oral tolerance) for clinically significant disease to develop.

Disturbances of oral tolerance

The phenomenon of oral tolerance was recognized at the beginning of the century when Wells (1911) showed that feeding egg protein to guinea pigs reduced anaphylactic reactions to ovalbumin [157]. Since that time, more understanding of the mechanisms of oral tolerance has been gained but it is surprising that the control and intricacies of this vital process are only now starting to emerge. Oral tolerance refers to the process by which systemic administration of an antigen that has been previously given orally causes no recognizable immune response [158]. Considering that the gastrointestinal system is in intimate contact with microorganisms and their metabolic products and a large number of dietary proteins and peptides, it is fortunate that a system is in place to differentiate the dangerous from the innocuous. The importance of derangements of oral tolerance in chronic inflammatory gastrointestinal diseases is unknown but it seems likely that dysfunctional oral tolerance could contribute to the chronic inflammation seen.

For example, it has been shown in mice that pre-treatment with cyclophosphamide or 2'-deoxyguanosine to inhibit suppressor cells (as described later these are an important cell mediator of oral tolerance) eliminates the normal lack of response to fed protein and produces a cell-mediated inflammatory response in the gut wall [158,159]. Migration inhibition of mesenteric lymph node lymphocytes was also observed in mice with cyclophosphamide abrogated oral tolerance [160] indicating immune activation.

Oral tolerance involves recognition of dietary antigens. In this respect, it is important to note that although most antigens are proteins and the main sources of proteins in foods are meats, eggs and dairy products, other components of feeds can also provide antigenic material. Cereals contain small amounts of proteins that can be the source of immune responses in the gut as it occurs in celiac disease in humans [161] and gluten hypersensitivity of Irish Setters [162]. Oral tolerance to wheat and maize protein in mice showed different fractions produce different antibody responses and different levels of oral tolerance [163]. Some of these fractions have been involved in food anaphylactic reactions and atopic dermatitis in children [164]. Cereal protein food adverse reactions have been reported in dogs and cats [40,165,166]. In addition, dietary fibre can also be a vector of antigens; small amounts of peptides are present in gum arabic, which are also subject to oral tolerance [167].

Abrogation of oral tolerance has been shown also as a result of non-specific stimulation of the immune system by oestrogen injection, induction of graft vs. host reaction, or intraperitoneal injection of an adjuvant. In these models a delayed type hypersensitivity reaction in the gut wall was confirmed [168,169]. Parasitic infections have also been shown to abrogate oral tolerance [170]. Zhang and Michael (1990) [171], showed that IFN-gamma abrogates oral tolerance possibly by heightening the capacity of enterocytes to present antigens (see later). In addition, *in vitro* exposure of T cells from tolerant animals to a pro-inflammatory cytokine such as interleukin-2 [90] restores antigen responsiveness [157,172]. Recently it has also been reported that IL12, the most important determinant of tissue damage in Crohn's disease, can abrogate tolerance by acting as a

third signal in the activation of naive cytotoxic lymphocytes (CD8+) by peptide antigens abrogating tolerance [173]. Collectively, these observations show that various inflammatory processes can abrogate oral tolerance.

Gut wall cell-mediated-immune (CMI) reaction produced by the abrogation of oral tolerance has been measured indirectly using the microdissection technique [Mowat, 1981; Mowat, 1986] and compared with the changes observed in two other models of cell-mediated immunity in the intestine -allograft rejection and graft-versus-host reaction [174]. The microdissection technique was able to detect significant histological differences that were not seen in standard histological preparations. The CMI reaction is characterized by an increased number of IEL, increased mitotic activity of crypt cells and an increased crypt cell production ratio. These were the most early and sensitive changes. In addition, increased crypt depth was also observed. The microdissection technique is not commonly used in the assessment of gastrointestinal pathology but may be able to identify animals with non-specific changes of lymphocytic/plasmacytic enteritis from those with a delayed hypersensitivity reaction to luminal antigens.

If a failure of oral tolerance is important in the pathophysiology of IBD, the missing link is the method by which sensitization to luminal antigens occurs in clinical cases. Most experimental work on oral tolerance has involved systemic sensitization except for some recent models of oral sensitization with dietary antigens using cholera toxin as adjuvant [175]. Some differences between systemic and mucosal sensitization were shown. Dose of antigen, mode of tolerization (gavage or drinking water) and type of immunization (systemic or oral) were important variables. Specific and bystander antigen

directed production of IgE was reduced more effectively with lower doses of antigen administered orally and repeatedly for 3 weeks. As this work shows, dietary and bacterial antigens seem to be treated differently by the gastrointestinal immune system. Fed bacterial products have been found to be very effective in producing systemic antibody responses due to their lectin or lectin-like binding activities [176,177]. Importantly bacterial antigens may be able to interfere with tolerance to dietary antigens, as the previous work with cholera toxin seems to suggest.

There are three mechanisms of oral tolerance that are well documented: active suppression, deletion, and anergy [157,178]. The separation between these three does not mean they do not overlap *in vivo* [179,180]. The dose of the antigen fed is one of the principal determinants of the mechanism of oral tolerance that predominates. Active suppression usually results from single or repeated low doses of proteins or haptens. In this situation the exposure to the antigen gives origin to a specific population of suppressor T cells in Peyer Patches (PP) [181] that produce non-specific suppressive cytokines like (TGF-beta), and interleukin 4 and 10 (IL-4 and IL-10) [157,181,182]. Dendritic cells in PP can drive the production of IL-10 by T cells [180]. IL-10 in turn induces tolerance of dendritic cells to specific peptides [183]. The lack of antigenic specificity of the cytokines allows bystander suppression [157]. It is generally accepted that it is more difficult for oral tolerance to prevent humoral responses [184,185] than cellular immune responses, with the exception of those involving IgE. Anergy or clonal deletion of lymphocytes involved in both arms of the immune response: (humoral and cellular) is produced only by feeding large doses of antigen [138,157]. The active mechanism of oral tolerance (that requires repeated low doses of antigen) is the mechanism that predominates with dietary antigens [178]. The active

mechanism of oral tolerance is also, because of its bystander suppression effect, the one that offers more promise as a therapeutic tool in gastrointestinal disease.

Exactly how oral tolerance is initiated is not known, but CD4 T lymphocytes are indispensable to the process [172] as are healthy enterocytes [171]. Lack of intraepithelial lymphocytes (IEL) in animals that have had cholera toxin given in conjunction with a tolerogenic antigen is associated with failure of oral tolerance [186]. IEL are the closest immune cells to intestinal contents and are suspected to undertake immune regulatory functions. Interestingly, IEL are polyclonal in the newborn but become oligoclonal in adults perhaps because selection by enteric antigens has taken place [122,178,187]. Around half of the human population of IEL are gamma-delta T lymphocytes, which, besides being protective to the intestinal epithelium [188], seem to also be indispensable for the maintenance and induction of oral tolerance [189]. It is interesting that this set of lymphocytes seems to be particularly reactive also to microbial molecules [190]. Reduced numbers of gamma-delta T lymphocytes are found in some inflammatory diseases of the intestine [122,190] but this cell type increases in the proximity of IBD lesions in people [122]. In contrast, no difference was found in the number of gamma-delta T lymphocytes in the mucosa of dogs with IBD and controls [28].

Karlsson et al (2001) [191] have shown that structures they referred to as "tolerosomes" mediate the production of oral tolerance. These are exosome-like structures released from the enterocyte into general circulation. They carry MHC II complexes attached to peptides sampled from the gastrointestinal lumen. "Tolerosomes" can transfer oral tolerance to naive animals. Interestingly, if the MHC II complexes are

eliminated, oral tolerance does not occur. In this context, the increase in the expression of MHC-II complexes by gamma-delta lymphocytes following bacterial stimulation is important [192].

Oral tolerance has been used as a therapeutic strategy in the hapten [193,194] and cell transfer [195] models of murine colitis. The offending antigen was fed prior to sensitization in both models. Clearly, this strategy is impossible in a clinical setting. Neurath et al. (1996) [193] used haptenized colonic proteins as the tolerogenic antigen to treat colitis in mice. A pattern of cytokine production that is typical of the oral tolerance resulting from low doses of antigen was observed. Production of TGF-beta, IL-10 and IL-4 was increased.

Neurath et al. (1996) [193], demonstrated that it is possible to elicit oral tolerance in a mouse model of colitis even after the inflammatory reaction had already started. The use of oral tolerance to suppress already established inflammation was also tested in mice that have been sensitized to ovalbumin. Feeding ovoalbumin after systemic immunization was successful at reducing cellular and humoral immune responses to it, but the oral tolerance produced in these circumstances was dose and time dependent. Only large doses given no more than a few days after immunization were effective. Feeding of ovoalbumin more than 7 days after sensitization was ineffective at re-establishing oral tolerance no matter what dose of ovalbumin was used. Because large doses of ovoalbumin were needed in this model to induce oral tolerance, it was speculated that clonal deletion and not active suppression was the mechanism by which the oral tolerance was re-established [185]. These findings lessen the therapeutic potential of this technique

because suppression of the immune response to 'bystander proteins', in addition to the protein to which the animal has become sensitized only occurs when active immune suppression underpins the oral tolerance. Bystander protein immune suppression is particularly important in clinical disease because the antigen to which the immune response initially occurred is usually unknown. However, when sensitization occurs through the mucosal route active mechanisms (and bystander suppression) are suspected to usually occur as discussed before [175], but this model of oral sensitization to produce oral tolerance needs to be tested under inflammatory conditions.

Aside from oral tolerance to dietary antigens, it has become evident that tolerance to one's intestinal flora is part and parcel of the regulatory mechanisms that prevent 'gastrointestinal chaos'. The gut immune 'recognizance team' not only has to respond to or ignore food antigens but it also has to deal with a high concentration of microorganisms and their products, especially in the distal parts of the gastrointestinal system. The proposed association between bacteria and IBD is long standing [93,196]. Duchmann et al (1996) [197], showed in hapten induced experimental murine colitis that tolerance to autologous bacterial sonicates is abrogated. Administration of IL-10 or antibodies to IL-12 restored tolerance and resolved the colitis. As will be discussed later (in the section on bacterial flora) it has been shown that human patients with Crohn's disease lack tolerance to their own flora [198].

Enterocytes as effective antigen presenting cells

As previously discussed, inflammation can not only increase the quantity of intact proteins crossing the intestinal barrier but can also abrogate oral tolerance to foreign

proteins. In addition, inflammation can change the way that protein antigens are presented by the enterocyte [199,200]. Enterocytes can act as antigen presenting cells [183] but are naturally inefficient at the job. It has been shown that enterocytes are unable to present proteins to sensitized lymphocytes but can present 'immunogenic peptides'. It is thought that unlike the usual APC in the intestine, enterocytes are incapable of the intracellular processing of proteins necessary for presentation of 'immunogenic peptides' to lymphocytes [201]. Deficiencies of certain antigen-processing proteases have been described [199]. Enterocytes do not form compact antigen presenting complexes (class II major histocompatibility complexes or MHC-II) due to lack of expression of the invariant chain [200]. This deficiency of expression does not occur in other lympho-epithelial tissues like the thymus or the skin [202]. As a result of this constraint large doses of antigen are required before effective antigen presentation by normal epithelial gut cells can take place. However, in the presence of IFN-gamma enterocytes become very effective APC as a result of increased expression of MHC-II molecules [199,200]. Interestingly, greater expression of MHC II by the gut epithelium has been reported in cats with IBD when compared to cats with other gastrointestinal diseases [203]. On the other hand SPF cats (used commonly for research) do not express epithelial MHC II [26].

Gut inflammation and dietary perspective

Mucosal barrier defects have the potential to produce and/or enhance gastrointestinal mucosal inflammation as discussed in the previous sections. Of all potential causal factors of IBD mucosal barrier defects are the ones directly related to some of the purported deleterious or beneficial effects of diet in the pathophysiology of IBD in people and animals. Original publications and reviews on the immunological

basis of IBD abound [122,125,196,204-206]. However, most of the original research focuses on narrow aspects of the immune response. This has contributed to the presence of many contradictory research findings in the literature, which complicate our understanding of gastrointestinal mucosal inflammation. A brief summary on the immunological basis of inflammation in IBD followed by how gut inflammation can affect the immunological response to dietary antigens and viceversa will be presented.

Cytokines regulate the cell function [207], of immune and non-immune cells like the intestinal epithelium [208]. They are small molecules that act as cell messengers. In an inflammatory reaction opposing messages are the norm and the use of the common paradigm of cytokine production that separates immune reactions into TH1 and TH2 categories has been criticized [209]. The end result of an immune reaction will always be the algebraic sum of the messages present at one single time. Keeping this in mind, Crohn's disease is considered a TH1 type of immune reaction characterised by increases in IFN-gamma secretion and low levels of IL-4 and IL-5. Conversely, ulcerative colitis is considered to be dominated by a TH2 immune reaction with high levels of IL-5, low levels of IL-4 and normal levels of IFN-gamma. IL-2 has been found to be low in both diseases [155] although contradictory results have been reported [125].

As discussed, some cytokines strongly influence the outcome of the contact of cells with potentially deleterious antigens. For example, IFN-gamma affects the passage of intact and processed protein through the intestinal barrier, abrogates oral tolerance and increases the ability of enterocytes to present antigens to lymphocytes in the lamina propria. Yet, intestinal inflammation can be induced in IFN-gamma deficient mice [156],

which indicates that IFN-gamma may be important in enhancing an already established inflammatory response but not the aetiological culprit in IBD. IL-12 secretion leads to up-regulation of IFN-gamma and TNF-alpha secretion and severe tissue injury ensues [210,211]. Both IL-12 and IL-18 are secreted in increased levels in Crohn's disease [212] while IL-4, a regulatory cytokine, is reduced [213]. IL-18 increases the concentrations of IFN-gamma only but does not appear to cause tissue injury [210]. Most importantly, the secretion of specific cytokines by lymphocytes is induced by the presence of other cytokines originating in the initial response to an antigen. Thus, IL-4 will induce further production of IL-4 while IFN-gamma will induce IL-12 [207,210]. IL-10 and TGF-beta act as differentiating factors for regulatory T cells that drive immunosuppression [195], although this so called 'inductive differentiation' in lamina propria T-cells has been contested [214].

The complexity of the inflammatory response in IBD is increased when the role of adhesion molecules, homing receptors and their interaction with cytokines is considered. Lymphocytes homing to the gut express a specific integrin, alpha-4-beta-7, which is induced on activation of lymphocytes. TNF-alpha up-regulates the binding protein (MAdCAM-1) for this integrin on the vascular endothelium [215] allowing further lymphocytic infiltration. Another important integrin is alpha-E-beta-7, which binds to E-cadherin in the intestinal epithelium and plays an important role in the adherence of IEL to epithelial cells and their function in maintaining a healthy epithelium [216]. Alpha-E-beta-7 has been found to be defective in the gut of Crohn's disease patients in areas of inflammation as well as in non-inflamed tissue [217]. An interesting aspect of these molecules is that they might be controlled by factors present in the intestinal microenvironment [218]. They may play a role in driving the inflammatory reaction in

intestinal mucosa but they are also important in maintaining a healthy barrier through their functions in epithelial proliferation and differentiation [219]

Although our understanding of the complicated circuits, cascades and networks that underpin immune regulation and inflammation in the intestinal mucosa is improving, the conundrum of the original insult in inflammatory bowel disease has not been resolved. Increased permeability, abrogation of oral tolerance and exaggerated antigen presentation by the enterocyte can all be phlogistic but none of these factors have yet been identified as the main cause of the disease. In spite of this, the knowledge of the inflammatory mediators responsible for tissue damage has allowed the development of novel means of therapeutic intervention. Adhesion molecules and inflammatory cytokines have been targeted with the aim of controlling inflammation and tissue injury. IL-10, IL-12, IL-4 and TGF-beta have also been used in recombinant and gene therapy techniques [124]. The use of specific antibodies to TNF-alpha is already being used in the treatment of human IBD [220,221] and antibodies to adhesion molecules have proven successful in a model of murine colitis [222].

The immunological response to dietary antigens

Of more relevance to veterinary patients than advanced manipulations of cytokines is the use of dietary therapy. Even if the dietary treatments used in IBD are not necessarily specific for the disease, it seems likely that they may avoid inadvertent enhancement of the inflammatory cascade. There is a clinical impression that recovery

from gastrointestinal disease can be followed by food intolerance or sensitivity in children with diarrhoea or other gastrointestinal diseases [223,224] and in veterinary patients [225]. However, the role of food sensitivities in human IBD has been questioned [226,227]. The presence of adverse reactions to food in human patients with Crohn's disease was observed on introduction to normal foods after being on an elemental diet for 4-8 weeks [94]. However, healthy controls showed the same problem and none of the signs were long lasting or affected the length of remission. Milk was the main offending food [94]. Furthermore, the B lymphocytes from lymph nodes draining IBD lesions do not react with food antigens [228]. In addition, histamine, a mediator of acute allergic reactions, has been found to inhibit the production of IL-12, an important mediator of tissue injury in Crohn's disease [229]. In contrast, IBD patients show a high number of mast cells especially in inflamed tissue [230]. In this study the mast cells in IBD patients were stimulated to release histamine by binding immunoglobulin G4, which is suggested to be allergen specific [227,230] and suspected of initiating allergic reactions to food [231].

There is some evidence, at least in people with intestinal signs due to cow's milk allergy, that it is the intact protein rather than the degraded protein that produce the immune reaction [232]. In IBD, given the pathophysiological abnormalities already mentioned, the passage of increased levels of intact protein into the mucosa is a very real possibility. It remains uncertain if dietary sensitivities can be acquired this way.

Some people that show adverse reactions to foods have been found to have serum IgE specific to the food proteins suspected of causing their disease. These people were also found to have circulating immune complexes, which were detected at the same time

than the clinical signs appeared. [132]. On the other hand, in another study patients with IBD showed similar serum antibody responses to fed proteins as normal volunteers, with a high level of inter-individual variation [167]. Unfortunately, measurement of serum concentration of antigen specific IgE in dogs and cats with positive challenge dietary trials has not been found to be of diagnostic value to assess abnormal immune responses to dietary proteins [40,233,234]. Therefore, the serum concentration of food specific IgE is unlikely to provide any insight into the link between IBD and food allergy in domestic animals. However, other immunoglobulins such as IgG4 have been suggested of mediating food allergies in people [231]. Their involvement and importance in allergic reactions to food in companion animals remains to be determined.

Some of the murine models of IBD include the use of a barrier-breaking agent in conjunction with an hapten [63,124]. These models support the view that leakage of dietary proteins (or other luminal factors) into the lamina propria may be capable of causing chronic inflammation. We have discussed that a cell mediated reaction occurs when oral tolerance is abrogated. However, the existence of a chronic form of allergic enteropathy remains to be proven [235]. Certainly, animals with adverse reactions to food can present with gastrointestinal signs [165,166,236,237]. However, to dissect what came first, the food sensitivity or the gastrointestinal inflammation, is difficult in most clinical situations as the diagnosis of food sensitivity can be complex and cumbersome. The 'gold standard' diagnostic method of oral elimination challenge studies can take several weeks [237] and may not detect subclinical intestinal hypersensitivity reactions. Furthermore, identifying the protein responsible for the allergy may not be straightforward. Therefore, although there is evidence of a fast response to diet change in cats considered to be suffering a true

primary food allergy [40,237] the differentiation between this condition and adverse reactions to food secondary to IBD is difficult.

Food allergy can also result in dermatoses in animals [166] and people [164,238]. Although, skin disease has not commonly been reported in animals with a diagnosis of IBD, the difficulty in distinguishing between IBD and food allergy in domestic animals suggests that an association between skin disease and IBD with secondary dietary sensitivity may be under-diagnosed. Interestingly, a cross-reaction between contact hypersensitivity cells in the skin and IBD effector cells has been found in an hapten model of murine IBD [239]. This observation indicates that sensitization to antigens in patients with IBD could occur through other surfaces than the gastrointestinal tract with the latter just behaving as the target organ. Cross-reactivity between pollen allergens and food allergens has already been proposed [240]. It is known that lipocalins are among the most important allergens of mammalian origin to which people are allergic. Lipocalins are usually found in cow milk [175] but also in the environment as aeroallergens from urine, hair, dander [241,242]. Any of the latter could easily be found in some pet foods. In addition, intestinal challenge with allergens in atopic and asthmatic human patients results in a small intestine secretory response and an increase in intestinal cellular infiltrate in the lamina propria [243,244].

It is obvious that dietary antigens could have an important role in IBD. The strategies available to avoid the potential aggravating effects of diet will be discussed in the section on dietary management.

The bacterial flora and IBD

The intestinal bacterial flora is established at birth and influences the development of the gastrointestinal immune system [245]. It also affects the function of the healthy intestine, protects against invasion or injury and regulates immune activity [246,247]. Defining what species constitute the bacterial flora has proven difficult and there are still elements that have not been cultured. Changes in the bacterial flora and intestinal microenvironment provoked by diet and disease have been studied [248].

The bacteria that colonise the intestine originate in the birth canal, the dam's large intestine and the environment [249.250]. Kittens and puppies already have a bacterial faecal density similar to adults within the first 24 hrs of life [251]. In nursing children, the predominant bacterial flora is *Lactobacilli* and B*ifidobacteria*. This population changes to enterobacteria and gram-negative organisms if children are bottle-fed [249,250]. These studies demonstrate that the microflora that will prime the immune activity of the intestine is modified by the diet before weaning. These changes in intestinal flora have been considered an important predisposing factor for gastrointestinal clinical disease in infancy [77,250,252] and even for the development of Crohn's disease [78]. It is not known if the suspected beneficial effects of the bacterial flora of nursing children extend into adulthood. However, the bacterial flora is believed to be important in the development of the immune system, and the immune activity of the gut [245]. The flora also influences the development of specific epithelial receptors to bacterial lectins [252]. Newborn cats and dogs are slightly different from newborn human infants in that kittens have mainly

Bifidobacteria and puppies mainly enterobacteria [253] in their gut. It is not known what changes occur to the bacterial flora if kittens and puppies are hand-reared.

The gastrointestinal tract of germ-free rodents is lighter in weight, has a reduced surface area, thinner villi, a decreased villus epithelial cell renewal and a thinner lamina propria than conventionally reared rodents. In addition they show a decreased inflammatory gut response [248]. The development of organised lymphocytic structures like Peyer Patches (PP) is genetically determined but the number of diffuse lymphocytes in mucosal surfaces increases with antigenic stimulation [254]. Germ-free animals develop germinal centres in the PP, IgA producing B lymphocytes in PP and lamina propria (LP), and are capable of producing IgA when colonised by bacteria or virus [245,255]. The primordial IgA response down-regulates the long-term response to the same antigen by binding the specific antigen to avoid adherence (the first step in colonisation and invasion) or by eliminating antigen from the epithelium in the form of immune complexes [139]. The development of the IgA system (dependent on the bacterial flora) is most important since it has been shown that oral tolerance cannot be produced in mice in their first week of life [169,256]. However, multiple feedings can later overcome this priming effect [169]. These observations then suggest that the bacterial flora helps to protect against gut reactions to antigens including food antigens.

The development of PP that occurs when germ-free animals are colonised involves the development of M cells. Specific Pathogen Free (SPF) animals show an increase in M cells [257] once they are transferred to a conventional environment. M cells contain receptors that bind bacterial lectins, which facilitate antigen sampling. M cells are also

the route by which many pathogens cross the intestinal barrier. The lectin receptors vary according to the intestinal segment. This variation has been attributed to induction by the microflora in each gut segment [258]. For example, *E. coli* and lipo-polysaccharide (LPS) can stimulate the production of their own receptors on the M cells of the intestine [180,259].

The original response by the intestinal immune system to bacterial determinants or lectins (inulin, phosphocolin and beta-galactosyl among others) is delayed in the newborn in accordance with the 'immune status' of the dam. Immunoglobulins in milk from normal dams coat the bacteria so that they cannot interact with immune cells to produce the IgA response. Mice whose dam is immune deficient react to their intestinal flora earlier. This immune reaction is amplified to include non-specific immune responses and it is much more effectively generated with a bacterial mixture rather than a single bacterial species [245]. It is likely that this bacterial driven immune response also occurs in kittens. Kittens and adult cats have a high number of bacteria present in their gut. Although queens have a very low content of IgA in their colostrum and milk compared to other species [260] they have higher concentrations of IgG that are maintained throughout the lactation period [261]. Perhaps this is the reason why the development of the small intestinal villi, crypts and lamina proprial cells occurs at a slower pace in cats than in dogs [251].

Therefore, the main result of microbiota colonising the intestine of a healthy individual is controlled activation of the intestinal immune system. It is tempting to think of this as a process by which oral tolerance develops to the normal microflora, especially as it involves similar suppressive cytokines as are involved in the immunoglobulin switch

to IgA production (i.e. TGF-beta and IL-10) [139]. The importance of this interaction between a healthy immune system and the normal microflora in the maintenance of gastrointestinal health has been shown in several models of IBD. Several animal models of IBD are based on the interaction of a genetically engineered dysregulated gastrointestinal immune system and the intestinal flora [63,122-124]. In one model of IBD, colitis is produced by transfer of activated lymphocytes from mice with colitis to immune deficient animals. Although the cells mediating disease populate the length of the intestine disease appears only in the colon. Proliferation of the donor cells in the intestine of the recipients was directly related to the size of the bacterial population in the recipient's gut suggesting the T cell reactivity is stimulated by bacteria or bacterial products [262]. In addition, a model of IBD in Guinea pigs involves the administration of oral carrageenans shows more reliable lesions if the animals are sensitized to *Bacteroides vulgatus* concurrently with the feeding of carrageenans [263]. Furthermore, when epithelial, food and bacterial antigens were compared in a model of spontaneous murine colitis, antibodies specific to the bacterial flora were present in serum, (especially to aerobic bacteria) but epithelial and food specific antibodies were not detected [264].

A connection has also been made between increased reactivity to the intestinal bacterial flora and clinical IBD in people. Lymphocytes harvested from the lamina propria of affected areas of the gut [265] and mesenteric lymph nodes [266] in IBD patients show increased proliferation when incubated with bacterial antigens compared to lymphocytes from controls. Furthermore, the proliferative response is stimulated by the autologous bacterial flora and not by a similar bacterial population from other individuals [267]. Further definition of the antigens involved in this reaction was sought but revealed

cross reactivity between different species and bacterial groups [268]. *E. coli* has been implicated as the antigen to which the reactions in some of these studies are directed. Increased adherence of *E. coli* strains recovered from patients with Crohn's disease has also been noted [269] but the significance of this finding in clinical disease is unknown. More recently it has been reported that IBD patients have significantly increased numbers of bacteria on the mucosa of the intestine when compared to controls supporting the view that an increase in adherence of bacteria may be part of the pathogenesis of IBD [270]. Because MHC-II was involved in the lymphocytic reaction to autologous flora it is suspected that bacterial antigens (especially superantigens) rather than non-antigenic secreted bacterial products underpin this reaction [149,267]. Further evidence for the importance of bacteria in the pathogenesis of IBD is provided by the observation that bacterial proteins are the target of increase IgG produced in Crohn's disease and ulcerative colitis [271].

Other bacterial products like formylated peptides (FNLP, FMLP and F-met) are known to be strong chemotactic stimuli for neutrophils [272]. In addition, bacterial cell wall polymers (peptidoglycan-polysaccharide complexes) have been shown to cross the colonic barrier in higher quantities after injury and to attract polymorph-neutrophils [273]. However, if the barrier was healthy, a lesser amount of the polymers crossed the barrier and the resultant cellular infiltration was dominated by mononuclear cells. It is unknown if these products are important in the pathogenesis of IBD in cats or dogs. This seems

unlikely given that neutrophilic infiltration of the lamina propria is not the most important pathological feature in most cases.

Other bacterial products can also affect the course of IBD. For example, endotoxaemia has been reported as a common finding in active Crohn's disease and elimination of luminal contents by whole gut irrigation speeded up the recovery of patients [274]. Mercaptides, sulphur-containing compounds produced by bacteria, have been shown to decrease butyrate oxidation in colonocytes [275]. This metabolic abnormality has been suggested as to be important in ulcerative colitis. It is interesting that some animal models of murine colitis use sulphur containing irritants like dextran sulphate and carrageenan to produce intestinal injury [275].

In addition to the evidence already presented above to support a central role for intestinal bacteria in the pathogenesis of IBD, it is noteworthy that IL-12 (an important cause of tissue injury in IBD) is thought to be produced mainly in response to bacteria and their products [276]. Likewise, the reaction to the autologous flora seen in IBD patients was driven by IL-12 and IFN-gamma [267].

The cat has a higher number of bacteria in the small intestine than other species and these bacteria more closely resemble the typical colonic flora. Do these peculiarities of the cat increase the risk of protracted intestinal inflammation in this species? Perhaps, but it should be noted that the normal intestinal microflora also can provide benefits to the host. It is the presence of gastrointestinal disease that produces changes in the microenvironment (pH, substrate availability, motility alterations, and abnormal bacterial

species growth) that can be detrimental to the symbiotic relationship between bacterial flora and host e.g. bacterial overgrowth in people [246] and dogs [277]. Studies in germ free animals indicate that the normal flora can protect against colonisation with opportunist intestinal pathogens [245]. It has been shown that protection against Salmonella in conventional animals usually requires the presence of inflammation elicited by LPS binding to its receptor in the epithelial cells [278]. Some specific genera of bacteria seem to influence gastrointestinal function in a more positive way. For example, the presence of Lactobacilli spp. and Bifidobacteria spp. decrease the risk of diarrhoea in breast fed infants [77,250,252] possibly due to their adherence to enterocytes [279]. Bifidobacteria have also been able to restore the susceptibility of the IgE 'production system' to oral tolerance in germ-free mice [280]. This influence on the pathogenesis of allergy has subsequently been determined to be attributable to a single species of Bifidobacteria in children with atopic eczema [281]. Bifidobacteria has also been shown to promote healing and protect the intestinal mucosa from the damage induced by zinc deficiency in rats [282]. In zinc deficiency the intestine develops lesions similar to IBD including ulcerations, edema, inflammatory cell infiltration and dilatation of blood vessels. Lactobacilli have been shown to improve colonic barrier function and decrease the passage of mannitol through the intestinal mucosa, while other bacterial species had the opposite effect [283]. Lactobacilli have also been shown to decrease the absorption of intact macromolecules [284]. Other beneficial effects of these bacteria on the gastrointestinal tract have been described including immunoregulation, restoration of normal microflora and repression of viruses [249,279]. In addition to these effects on gastrointestinal function administration of Lactobacilli have prevented colitis in murine models of IBD [282,285,286]. Administration of

a mixture of several bacteria including Bifidobacteria and Lactobacilli have improved barrier function (mannitol flux), decreased inflammatory mediators [90] and improved histological grade of intestinal specimens in IL-10 deficient mice [287]. Even non-pathogenic *E. coli* has been used to treat ulcerative colitis with some success [288].

Recent studies have also been able to document the effects of bacterial colonization on genetic transcription in the host. These studies suggest that nutrient absorption, mucosal barrier function and intestinal maturation are modulated by the commensal flora and differences in these intestinal functions can be explained in part, by the colonizing bacterial species [289,290].

In summary, a full understanding of the relationship between the microflora and its host is only starting to emerge. Nevertheless, gastrointestinal bacteria have an important role in gastrointestinal health and development through their actions on the gastrointestinal immune system, their protective effects against potential pathogens, and their support of oral tolerance and an effective gut barrier. How this relationship is deranged in IBD is uncertain but results from experimental animals and people with IBD indicate that an abnormal reaction to the presence of autologous bacteria in the gastrointestinal tract may be very important in the pathogenesis of IBD. Dietary manipulation to favour the growth of Bifidobacteria and Lactobacilli is possible and will be discussed further in the section on probiotics, prebiotics and symbiotics, as well as in the section on dietary fibre.

DIETARY MANAGEMENT

Food for thought

The importance of feeding during disease has been realized since the time of the ancient Egyptians who used the rectum as a route to administer food! This practice was maintained until the beginning of last century [291]. Good nutrition is very important during gastrointestinal disease. The specific and special requirements of the species being treated (the cat in this instance) the special requirements conferred by the disease and the nutritional status of the animal at presentation, all need to be taken into account when planning nutritional intervention in IBD. The nutritional management of patients with gastrointestinal disease has three principal aims: 1.- the nutrition of the individual, 2.- the nutrition of the gut and 3.- the avoidance of further injury to the bowel.

Malnutrition can affect 30 to 80% of human IBD patients [292]. Malnutrition alters immune function and in IBD it can compound the existing immune dysregulation that accompanies the disease [293]. Protein-energy malnutrition decreases the number of intra-epithelial lymphocytes and the number of IgA producing cells in the mucosa [294]. These changes are likely to compromise the health of the epithelium and to reduce antigen exclusion by the mucosa. Oral tolerance is also impaired during protein deprivation [295,296] and permeability to large macromolecules and particles is increased [297]. As discussed previously, barrier function, oral tolerance and the ability of enterocytes to exclude antigens are already disturbed in IBD. Undernourished Crohn's disease patients show lower number of circulating lymphocytes, lower production of immunoglobulins and

impaired macrophage function [298], but it is uncertain if this is related to the nutritional status of the patient or the clinical stage of the disease.

Several reports indicate that patients with Crohn's disease do not have increased energy requirements [292]. One study on patients with active Crohn's disease that had not had prednisone treatment for 3 months showed that their resting energy requirements were neither increased or decreased. However, these patients adopted a starvation pattern of substrate metabolism increasing the utilization of fats and decreasing the utilization of protein and glucose [299]. Another study of people with IBD has shown that disease activity increases resting energy requirements of these patients but reduces physical activity energy requirements, maintaining the total energy requirements equal to patients with a lower disease activity [300]. Importantly, the starvation pattern is quickly normalised after enteral feeding [299] and a good correlation exists between the attainment of nitrogen balance and remission in Crohn's disease [301]. Malnutrition in human IBD is considered to be the results of anorexia, malabsorption and increased intestinal losses [300,302] with anorexia being considered the most important of the three [292]. These observations in people with IBD agree with the clinical impressions in veterinary patients [12]. It is noteworthy, that the cat cannot adapt to a decrease in energy intake with a decrease in nitrogen loss [303,304] as a result the negative impact of malnutrition on vital bodily functions is likely to have a rapid onset compared to other species. Some adaptation of protein catabolic enzymes has been suggested in a recent report that compared protein oxidation in cats consuming different amounts of protein [305]. However, both diets had protein content above requirement and therefore this mechanism of adaptation may not be available during anorexia when protein intake is markedly reduced.

The presence of nutrients in the intestinal lumen is known to have a trophic effect on the gastrointestinal tract. Food acts directly by nutrition of the epithelial cells and indirectly by stimulation of systemic and gastrointestinal hormones like gastrin and cholecystokinin [306,307], gastrointestinal secretions, gastrointestinal nerves and motility [308]. Certain nutrients such as glutamine or SCFA act as trophic factors for the epithelium and help to maintain the high level of renewal of this tissue [293]. Furthermore, lack of feeding during total parenteral nutrition (TPN) has been associated with atrophic changes of the bowel in rats [306,309] and cats [310]. Cats on TPN showed atrophy and fusion of villi, crypt dilation and hypercellularity of the lamina propria after 2 weeks of intravenous feeding. These changes reverted to normal after 3 weeks on standard diets. Similarly in human patients with IBD TPN results in reduced height of microvilli and lower activity of brush border enzymes, but did not produce severe atrophy and recovery occurred rapidly. [311,312]. Further significance to nutritional therapy has been afforded by the finding that certain nutrients may reduce inflammation, 'normalize' intestinal function and bacterial flora, or act as immunomodulators. These compounds include glutamine, SCFA, arginine, n-3 fatty acid, probiotics and prebiotics, and zinc [291] will be discussed in the pertinent sections below.

It is also important to consider the degree of malassimilation occurring during gastrointestinal disease. Undigested and/or unabsorbed food promotes intestinal disturbances due to fermentation, bacterial overgrowth and the formation of inflammatory or secretory compounds [313]. Nevertheless, bacterial overgrowth was not a feature in a series of cats with chronic gastrointestinal disease [146]. Furthermore, recent *in vitro*

experiments indicated that pro-inflammatory interleukins can increase nutrient transport [314]. This effect of interleukins may help counter the adverse effects of malabsorptive changes in IBD but this is unlikely to be a clinical significant beneficial effect. Notwithstanding the above observations, malabsorption is potentially a common complication of IBD and patients with the disease are likely to benefit from highly digestible diets that maximize nutrition for the patient and decrease the chances of further immune reactivity [10]. The correct diet is also likely to benefit the IBD patient by promoting an appropriate intestinal flora and overcoming the 'bacterial imbalance' that can accompany deranged gastrointestinal function [246].

Method of feeding and diet characteristics

Feeding animals and people with IBD can take place through the gut or parenterally. Enteral nutrition includes the use of liquid elemental or polymeric diets or controlled solid food elimination diets (polymeric in nature). Parenteral nutrition can take the form of total parenteral nutrition (TPN) in which the patient is fed all their nutrient needs through a central vein, or Peripheral Parenteral Nutrition (PPN) in which a peripheral vein is used to partially supply the patient's daily nutrient requirements. The choice of any of these feeding methods is influenced by such considerations as the degree of bowel rest required, the value of low or high residue diets and the dietary antigenicity. These properties will be used to compare different feeding methods and different types of diets below.

Total parenteral nutrition (TPN) is commonly used in the treatment of Crohn's disease but not in ulcerative colitis [300]. Total parenteral nutrition and enteral nutrition have been repeatedly tested and compared in human patients with IBD [315-320] but no comparisons of these feeding methods have been reported in the veterinary field. The use of TPN outside of veterinary referral institutions is difficult because of the need for intensive nursing care and the risk of possible metabolic and infectious complications [321]. These complications are also considered disadvantages of TPN in people and, as a result, enteral feeding is favoured when the patient has a functioning gastrointestinal tract. However, today many experts in human nutrition consider that there are no nutritional advantages of enteral over parenteral nutrition in man when nitrogen and energy intake are equal between the two methods [322,323].

In the veterinary patient, the choice of the different routes of feeding is influenced by the costs and infrastructure available [324]. Although the use of TPN has been reported in healthy cats [310] and in some cats with gastrointestinal disease [325], TPN is usually reserved only for severely compromised animals with persistent vomiting or anorexia [10]. It has been reported that the use of TPN in domestic animals is feasible for up to a week [10] but in cases with moderate malnutrition peripheral parenteral nutrition (PPN) may be all that is needed [324]. PPN is probably better suited to a private clinical practice and its use is indicated in animals that are not seriously malnourished and for which complete enteral nutrition is not desirable [324]. PPN in dogs has shown protein sparing capacity [326] but the same may not be true in cats because of this species metabolic idiosyncrasy in relation to

protein metabolism. In the veterinary IBD patient parenteral nutrition is the exception rather than the norm and therefore no further discussion will follow.

The type of enteral diet that is best suited to the IBD patient is controversial and many reports have been published comparing the use of elemental and polymeric diets in Crohn's disease [315,317-320]. The best enteral diet for veterinary IBD patients is also controversial [225,327]. Bowel rest has been standard advice after gastrointestinal disease or injury [302]. Bowel rest can be provided with total parenteral nutrition or highly digestible diets [10]. The rationale for bowel rest has been to reduce the digestive and absorptive load placed on the bowel to minimize vomiting and osmotic diarrhoea. Additional goals of bowel rest are to reduce bowel secretion resulting from malabsorbed fatty acids and bile acids, to reduce bowel bacterial numbers and to decrease passage of food proteins through the faulty mucosal barrier to reduce further inflammation [315,316,328]. Accordingly, bowel rest is best suited for osmotic diarrhoea and cannot be used in chronic processes unless another method is utilized to feed the patient. In repeated bouts of acute diarrhoea in children it has been recognized that bowel rest can be detrimental and 'feeding through diarrhoea' has been observed to better maintain body weight [144]. Guilford (1994) [225] argues that this may not be applicable to acute vomiting and diarrhoea in the veterinary patient because of the different type of diarrhoea occurring in children (mostly infectious and secretory) to the ones commonly affecting pets (non-infectious) and the need to control the vomiting which frequently accompanies diarrhoea in acute gastroenteritis in cats and dogs. Greenberg (1988) [316] showed in a controlled trial that bowel rest did not play a major role in achieving remission after an acute bout of Crohn's disease and did not affect the long term outcome. In this study, one group ate palatable meals without

restrictions and had the same outcome to the TPN and elemental diet groups. Dietary intolerance after an acute bout of Crohn's disease has been reported in other studies. The offending foods have usually been milk, cereals and vegetables, but the adverse reactions to these foods do not seem to be a serious long term concern [94,315]. Bowel rest in ulcerative colitis has proven not to be beneficial [315,329].

Cats with IBD usually present with chronic vomiting and diarrhoea, rather than bouts of acute clinical signs. As a result bowel rest, other than by use of highly digestible diets, would not offer therapeutic advantage. Most highly digestible diets still contain intact proteins and so can only to a limited extent reduce the exposure of the mucosa to antigens that may perpetuate inflammation or allergic responses by the gastrointestinal mucosa. However, as previously discussed there is as yet little convincing evidence in humans for a clinically important role for food sensitivity in IBD.

Cats with gastrointestinal clinical signs diagnosed with adverse reactions to food and idiopathic gastrointestinal disease have been reported [8,40,237,330]. Most of these cats had gastrointestinal histological lesions consistent with IBD, concurrent skin lesions, and responded to dietary change. Guilford et al. (2001) showed that cats with gastrointestinal signs and adverse reactions to food and cats with gastrointestinal signs but no adverse reactions to food could not be differentiated by any other means than food trials. Specifically designed gastrointestinal food allergy diagnostic techniques like gastroscopic provocation tests in humans [235] and dogs [331,332], and colonoscopic allergen provocation [333] in people show promise to detect food allergies. However, gastroscopic provocation tests were not helpful in cats [40]. The resolution or improvement of the clinical signs of

idiopathic colitis with the use of hypoallergenic diets in cats [47] and dogs [334] suggests that dietary antigens may participate in the process of gastrointestinal inflammation as does the success of hydrolyzed proteins in dogs with IBD [335]. However, it remains to be definitively established if food sensitivities are part of the pathogenesis of feline IBD.

The use of elemental diets has been postulated to be beneficial in people with a relapse of IBD because it confers partial bowel rest reducing antigenic stimulations and decreases mucosal permeability [336]. However, the clinical advantage of these diets has not been demonstrated convincingly. More recent work using the lactulose/mannitol permeability test failed to find a difference in intestinal permeability between TPN and enteral nutrition [337]. A comparison between a complex diet and enteral liquid diets with different nitrogen sources (amino-acids, oligopeptides or protein) showed that all the liquid diets reduced the expression of epithelial MCH-II and the number of intraepithelial lymphocytes but these changes were not related to the source of nitrogen administered [338]. Several studies have compared the use of elemental liquid diets with polymeric diets [317,318,320]. Although polymeric diets were found to be inferior to elemental diets in one study [317], the consensus is that polymeric enteral nutrition is well tolerated, cheap and effective [317-319,339]. Some authors have even claimed that polymeric diets are as effective as corticosteroids at inducing remission of Crohn's disease [319]. Unfortunately, a European cooperative study and a meta-analysis of randomized controlled trials made the opposite conclusion that enteral diets are less effective than drug treatment in active Crohn's disease [320,340]. The former trial was based on objective assessments of disease activity whereas the latter trials utilised the subjective parameters of the Crohn's disease activity index but nevertheless, it is difficult to reconcile such starkly different conclusions.

The use of elimination diets in IBD in people has been advocated with the aim of avoiding potentially offending antigens [315]. The presence of adverse reactions to food in Crohn's disease patients [94,341,342] lends credit to their use. Jones et al. (1985) reported a reduced relapsed rate of Crohn's disease in patients on elimination diets. Moreover, in a controlled trial comparing the effectiveness of an elimination diet and prednisolone after remission of Crohn's disease was achieved with elemental diets, diet alone gave the best outcome [343]. However, several trials have disputed this view. Pearson et al. (1993) [94] concluded that there was no advantage of elimination diets in a controlled trial in which Crohn's patients and healthy controls showed no difference in the presentation of adverse reactions to food after elemental diet feeding. Most of the adverse reactions to food were short lived and there were no differences in relapse rate between the patients that showed these adverse reactions to food and those that did not. Moreover, Crohn's disease patients on standard diets have shown similar long term remission rates to patients on TPN or elemental diets [316,341]. Experimental data in rats indicate that there is no difference in gut associated lymphoid tissue reaction to different forms of nitrogen (amino-acids, peptides or intact protein) [338]. In addition, a favourable outcome of nutritional therapy in Crohn's disease has been associated with replenished total body protein stores rather than with the type of diet used [301].

Low residue diets have been prescribed for the treatment of gastrointestinal disease, especially of the small intestine [302,328]. However, no difference in outcome was found between Crohn's disease patients that consumed a low residue diet and those that consumed a normal diet including fibre-rich foods [328,341]. In view of the numerous

beneficial effects that fibre has on the gastrointestinal tract (see the section on dietary fibre), in particular the large intestine, it is difficult to support the view that dietary fibre should not be included in the dietary management of IBD. Furthermore, short chain fatty acids (i.e. fermentation products of dietary fibre) have been used in the treatment of ulcerative colitis with good results [292]. However, diet as a primary mode of treatment in ulcerative colitis has not been exhaustively studied in controlled randomized trials [300]. A small trial of patients with ulcerative colitis in which sulphasalazine and a high fibre diet were compared suggested that the relapse in the group that consumed the dietary fibre was not better than if they would have been treated with a placebo [344]. In fact, a recent review of nutritional therapy in human IBD failed to raise the issue of the role of dietary fibre in the dietary treatment of IBD [292]. The same reviewer suggested that given that pharmacological therapy is as or more effective than dietary treatment and less costly, the use of diet as a primary therapy should be restricted to those patients that are refractory to the effects of steroids or have only mild signs of disease.

Controlled, randomized, prospective dietary trials in veterinary patients with IBD have not been carried out. Therefore a large part of veterinary nutritional advice has been 'borrowed' from the human literature, derived from the theoretical considerations about the aetiopathogenesis of IBD or based on anecdotal evidence. Feeding controlled, elimination diets is the most common form of nutritional treatment in veterinary patients with IBD [10]. Liquid diets may be used in animals with persistent anorexia [345] or vomiting [10]. Forcefeeding commercially available polymeric diets formulated for cats, dogs and humans is a common way of dealing with anorexic cats with IBD in clinical practice. The typical recommendation for cats with IBD that are still willing to eat voluntarily is the use

of a highly digestible, low residue, low lactose, nutritionally balanced and palatable novel protein diet [10,12,34,41,308,327,346]. If involvement of the colon is suspected dietary fibre supplementation has been recommended [12,34,41,225,308,327,346,347]. However, very few clinical trials on the use of fibre in veterinary patients with gastrointestinal disease have been performed. The results of dietary fibre supplementation in dogs with colitis have varied according to the type of fibre used. Insoluble fibre did not improve colitis when compared with a hypoallergenic diet [347] but soluble (i.e. fermentable) fibre supplementation to a highly digestible diet was considered advantageous [348]. The use of sterculia gum for a range of gastrointestinal ailments in a group of cats has been described as beneficial [349] and high fibre diets have been used successfully in some cats with colitis [2]. In contrast lymphocytic-plasmacytic colitis in cats has been reported to respond to hypoallergenic diets without fibre supplementation [47]. This is similar to the reported findings in a group of dogs with lymphocytic-plasmacytic colitis in which a hypoallergenic diet gave better results than a high cellulose diet [347]. The evidence in support of the use of hypoallergenic diets in IBD is not restricted to clinical cases of colitis in cats and dogs. A small number of dogs with small bowel IBD responded clinically and histologically to the exclusive feeding of a diet containing an enzymatically hydrolyzed soy protein [335]. Although preliminary these results are interesting and suggest further investigation of the role of hydrolysate diets in IBD is justified.

In summary, it is clear that our understanding on the benefits of controlled, elimination, low residue diets in cats with IBD is in its infancy and so is our knowledge of the benefits of dietary fibre supplementation. However these diets have proven beneficial

in preliminary trials and certainly comply with the first principle of medicine 'do no harm'.

Protein, amino-acids and nucleotides

Proteins are large molecules with a primary, secondary, tertiary and quaternary structure. The importance of these different levels of structure becomes clear when considering protein functions. The protein fed to animals and people with IBD has to be considered not only from the nutritional standpoint but also from the perspective that dietary protein has the potential to cause further injury to the bowel through its antigenic properties and fermentation products.

The building blocks of proteins are amino acids and their sequence (primary structure) determines the shape of the molecule [242]. Approximately ten amino acids are essential nutrients and therefore determine the biological value of a protein i.e. its quality or how well a certain protein can provide for the needs of an individual. Consequently high biological value proteins are recommended for the treatment of gastrointestinal disease [313,327]. Excessive dietary protein should be avoided [327] not only because of the allergenic potential but also because protein fermentation gives origin to mercaptides [275], ammonia [10] and other products which may be detrimental to the health of the colonocytes.

Proteins are digested to amino acids and small peptides in the gut lumen. These digestion products are then absorbed by sodium coupled transporters [350]. Peptides of a certain length (8 to 16 amino acids) [351] are effective antigens and/or allergens and depending on their shape will interact with antigen presenting cells [242]. The recommendation to use highly digestible protein (e.g. above 87%) in the treatment of gastrointestinal disease is made in part with the aim of reducing the absorption of intact protein and the amount/size of potential antigenic peptides [327]. Small peptides have lesser affinity for specific antibody than proteins [242], although IgE mediated reactions can be precipitated by very small doses of allergens [352].

Dietary peptides can be modified intracellularly. This occurs to gliadin in patients with coeliac disease and T-cell recognition is heightened [161]. The effects of post-translation modification of peptides can increase the binding affinity of the peptide to a MHC complex, or cause cross-reactivity between allergens, or abrogance of tolerance.[351]. Most interestingly, peptide binding to MHC II complexes is not as specific [353,354] as once thought giving ample room for cross-reactivity between two dietary proteins and between dietary proteins and self.

Some antigens are more likely to induce allergic reactions than others. Size, solubility and stability of the molecule in addition to the protein folding characteristics are important in determining whether a protein is allergenic. Common food allergens are stable in the presence of digestive enzymes in an *in vitro* model of gastrointestinal environment [242,355]. Glycolisation, deamination and other posttranslational modifications can affect allergenic potential but are not critical factors [351]. Although structural features

are important determinants of allergenicity other factors (like mode of entry) are important as well. Structural features are the main determinant of cross-reactivity, although structural similarity does not guarantee cross-reactivity [242]. In general, for cross-reactivity 70% homology is required [242]. The ability to predict if a protein will be allergenic is poor because of our lack of understanding on how allergy develops. Mapping protein folds goes someway towards allergen recognition but so far its main merit is its negative predictive value and not the identification of allergenic proteins [242].

In cases in which the allergens are known, chemical and structural modification strategies to escape allergy have been proposed. The aim is to avoid presentation of the antigen by IgE, at the same time as preserving linear epitopes needed for presentation to T cells [241.352] and by so doing change the nature of the inflammatory cascade. Genetic modification of food allergens at source by agricultural biotechnology has been tried but better methods of assessing allergenicity, as opposed to immunogenicity, are still needed before this strategy will be successful [355].

This allergenic potential of proteins is the reason why it is frequently recommended to feed one or more novel protein diets to companion animals with IBD. A novel protein is a protein source the animal has not been exposed to previously and therefore the animal has less chances of being allergic to it. The first novel protein an animal with IBD is given during treatment is sometimes considered 'a sacrificial protein' because the animal is exposed to it while the gut barrier is dysfunctional and the possibility of adverse reactions to it is heightened. Diets that contain a small number of novel protein and carbohydrates are called by a variety of names including 'novel

protein', 'controlled', 'restricted', 'limited antigen', 'selected protein' or 'elimination' diets [313,327].

The use of novel proteins and sacrificial proteins in IBD is based on the belief that food sensitivities can be important in the pathogenesis of IBD [10,327]. As explained before, there are enough alterations in barrier function and immune regulation in IBD to theorize accordingly and there is preliminary clinical data (see above) to support this practice. However, it is noteworthy that the connection between IBD and food sensitivity in humans has not been proven and therefore the use of novel protein diets remains speculative in human IBD [356]. The situation is similar in the veterinary field, and made more so because, as discussed before, the distinction between IBD and food sensitivity can be incredibly difficult [34,40]. This fact alone perhaps justifies the precautionary use of hypoallergenic diets in patients suspected to have IBD. A report on the protein digestibility of current 'hypoallergenic' commercial diets in America indicated that most feline diets had a high apparent protein digestibility [357]. On the contrary 'home-made' hypoallergenic diets were found to be nutritiously inadequate for adult maintenance [358]. However, a study in ileal-cannulated dogs indicated that apparent digestibility can be quite different from ileal digestibility in raw and rendered animal by-products used in the petfood industry [359]. If the differences are present and of significant magnitude in the cat is uncertain but cats have shown reduced apparent digestibility of most macronutrients when compared to dogs consuming the same commercial foods [360]. Nevertheless, the practicality of commercial 'hypoallergenic' diets in clinical practice [361] and the fact that they are complete and balanced for adult maintenance support their use in IBD. However, their efficacy as 'hypoallergenic' diets has never been proven.

In recent years, hypoallergenic diets based on protein hydrolysates have become available for use by veterinarians in IBD patients. The hydrolysed dietary proteins provide all the required amino acids necessary for the animal but prevent effective presentation of allergens. In IBD patients this may curtail any on-going food allergy and prevent the acquisition of further allergies. It also reduces the difficulty identifying novel protein diets in pets with a history of being fed a variety of protein sources and supersedes the concept of feeding a 'sacrificial' protein. Hydrolysate diets have been used extensively in the management of cow's milk allergy in children and clinical benefits have been reported [362]. This approach is logical because it is the intact cow's milk protein and not the peptide fragments product of intestinal processing that releases the inflammatory mediators [232]. However, hydrolysates can cause allergic reactions [363]. For this reason hydrolysates are usually carefully tested *in vitro* for their residual allergenicity compared to the parent protein [364-366]. This testing is a sensible precaution prior to use of hydrolysates in clinical patients but is marred by the fact that the test protocol involves raising antibodies in an artificial way (intraperitoneal or subcutaneous injection); also the ELISA inhibition assays that are the cornerstone of the assessment measure antigenicity rather than allergenicity [242]. Hence, efficacy and safety of these diets can only be definitely tested in clinical trials. The use of dietary hydrolysates is new in veterinary medicine and their value in treating food allergies and associated diseases is unknown [367] although results in canine intractable IBD, although limited, are promising [368]. Feline commercial diets based on hydrolysates are available [346] and await critical appraisal of their efficacy as hypoallergenic diets.

Protein requirements in IBD

Humans with IBD show an increased whole body protein turnover that correlates with the severity of disease [369]. Enteral feeding results in a higher rate of protein retention in the body of human neonates and pigs than intravenous nutrition [370,371]. It is estimated that the portal-drained viscera account for up to 50% of the turnover of essential amino acids and 10-20% of the whole body energy expenditure [372]. Thus, glutamine, glutamate, aspartate, serine, glycine, arginine, proline, leucine, isoleucine and valine are substantially extracted by the intestinal mucosa and the dietary content of these amino acids is not fully available to the extraintestinal tissues [373]. It is not yet known if gastrointestinal disease increases the total requirements for these amino-acids.

However, protein metabolism is quite different in strict carnivores like the cat, when compared to other species. Cats have high requirements for protein and are susceptible to amino acid deficiencies, some of which do not occur in other species. Animal flesh is rich in all amino acids but particularly in arginine, taurine and sulphur amino acids [304,374], amino acids for which the cat has obligate requirements.

Most omnivores can increase intestinal transport of amino acids when consuming a low protein diet. This is not so in the cat [374]. In addition, a low protein diet does not reduce feline protein metabolism because the cat cannot downregulate aminotransferases and the enzymes participating in the urea cycle [375]. The increase in whole body protein turnover that can occur during IBD [369] and the higher total protein requirements in the cat raise the possibility that this species may be more sensitive to protein malnutrition during

IBD than other species. Weight loss is a common clinical complaint in cats with IBD [12] and hypoproteinaemia and hypoglobulinaemia have been reported [1,3,9]. However, these clinical signs and clinical pathological abnormalities are not particular characters of cats with IBD and they have many potential explanations including anorexia and malabsorption.

Arginine, ornithine and polyamines

An important role for arginine in the nutritional support of human IBD patients, especially children, has been proposed [328]. Arginine is believed to be important in tissue healing and immune function [376]. Moreover, arginine has a role in the maintenance of the intestinal epithelium through the provision of polyamines. Ornithine, derived from dietary arginine, can be converted into polyamines like putrescine, spermidine and spermine, albeit usually in small quantities [377]. The intestinal microflora is a major source of polyamines in the large intestine, especially when fermentable carbohydrates have been added to the diet [378]. Ingested polyamines have shown a maturational influence in rat intestine increasing sucrase and maltase activity and reducing lactase [379,380]. In addition, polyamines have been reported as been essential for cell growth and protein synthesis.

Intestinal polyamines decrease during fasting and increase on refeeding as well as during intestinal recovery from injury. Ornithine, added to an enteral diet after fasting, increased the level of sucrase and lactase, crypt length and villous height in rats [381]. Thus the provision of arginine and ornithine may be important during gastrointestinal disease. This may be more so in cats, which -as opposed to rat and dogs- are exquisitely sensitive

to arginine deficiency [375,382] because of low activity of the enzymes of the arginine-ornithine-glutamate cycle [383,384]

Arginine is also a substrate for the synthesis of nitric oxide (NO) by action of the nitric oxide synthase [385]. The list of gastrointestinal functions modified by NO is long and not completely understood. It includes gastrointestinal motility [386], the dysmotility present after ischemia-reperfusion injury in the rat [387], maintenance of microvascular integrity and mucosal barrier [388], mucosal blood flow [389], Th1 T cell differentiation in mice [390] and the production of IgE-IgG in a model of food sensitivity in guinea pigs [391]. In cats, NO has been shown to play a role in modulating gastrointestinal permeability [392], colonic motility [393] and neutrophil adhesion [394]. Kubes et al. (1995) [388] showed that excessive NO does not cause any dysfunction in the cat small intestine.

Nitric oxide has also been found to hasten the recovery of jejunal transport mechanisms after exposure to superantigens [395]. Alternatively in the presence of oxidants it is believed that NO mediates cellular injury. Inflammation can induce the expression of inducible nitric oxidase synthase in macrophages and neutrophils [122,385]. Nitrates, the stable end products of nitric oxide, are increased in the serum and intestinal biopsies of patients with ulcerative colitis and Crohn's disease [396-398]. Macrophages and neutrophils are common in human IBD but not so in feline IBD [20]. Therefore the importance of this path of tissue injury is debatable in cats.

The multiplicity of actions of NO was suspected to be the reason arginine supplementation in guinea pigs with peritonitis was beneficial at low levels but

catastrophic at high levels [399]. More research is needed into the effects of arginine supplementation of the diet of animals with gastrointestinal disease, especially in species like the cat that has a high requirement for this amino acid.

Nucleotides and nucleosides

Nucleotides and nucleosides are required for the cell renewal that occurs constantly in tissues such as the intestinal epithelium. Dietary nucleotides have been shown to have an effect on growth and maturation of the gut in young rats [400] and can be derived from intestinal desquamating cells and the diet. There is a *de novo* synthetic pathway in the enterocyte that utilizes glutamine but is not usually active [401]. Nucleotides and nucleosides are needed by all rapidly dividing cells including lymphatic tissue [401]. For this reason diets free of nucleotides/nucleosides are considered immunosuppressive [10,328,370,401]. While this may be theoretically advantageous in IBD because of the suspected immune basis of the disease, mucosal healing would also potentially be compromised [328].

A recent report indicates that nucleotides accelerate intestinal recovery from fasting in older rats [402]. Furthermore, oral nucleotides have hastened histological recovery from osmotic diarrhoea in rats [401] and intravenously administration of nucleotides has been reported to heal indomethacin intestinal ulcers [403]. In contrast, administration of nucleotides aggravated dextran sulphate sodium induced colitis in rats [404]. In IBD, nucleotide free diets and 6-mercaptopurine treatment have been beneficial in the treatment of Crohn's disease [328]. Because of these contradictory findings high concentrations of

nucleotides should not be added to the diet of IBD patients until further research is carried out.

Glutamine

Glutamine is considered a non-essential amino acid in all species, yet it has been found to be an important metabolic fuel to rapidly dividing cells like enterocytes and immune cells [405] and may become essential during the body's response to burns, trauma and sepsis [406]. Glutamine has a rapidly exchangeable amino group and a carbon chain. Therefore, it acts as a crossroad between nitrogen and carbon metabolism. In most species it is part of a group of inter-converting amino acids including glutamate, proline, arginine and ornithine [407]. In the cat this potential conversion into another amino acid is curtailed because of the lack of enzymes necessary to convert glutamate into ornithine and proline [384].

The gut removes up to 25% of the systemic flux of glutamine [372]. In accordance with this observation the addition of a stable deceptive of glutamine to TPN solutions has been found to improve morphometric intestinal parameters and reduce permeability in rats [408]. However, the beneficial effects of dietary supplementation with glutamine have been less spectacular and conflicting reports are frequent in the literature [372]. Reeds et al. (2000) [409], reports that enteral glutamate, enteral glucose and arterial glutamine contribute 36, 6 and 15 % of the CO_2 production in the portal drained viscera. These observations establish that glutamate is the most important substrate for the metabolic activity of the enterocyte.

Several actions have been ascribed to glutamine in the gastrointestinal tract. These include oxidative protection, trophic effects on the mucosa, maintenance of blood flow, reduction in mucosal permeability and bacterial translocation, improved cytotoxic T cell function and increased IgA secretion [370,405,410-412]. Glutamine supplementation during gastrointestinal disease has not been exhaustively researched in veterinary science. In a model of ulcerative colitis in rats, glutamine feeding before the application of trinitrobenzene sulphonic acid was effective in reducing the concentration of inflammatory cytokines in the colonic mucosa, colonic injury and bacterial translocation [411]. Conversely, the administration of enemas with glutamine after the induction of colitis failed to improve the inflammatory response in the same colitis model [406]. Glutamine administration to dogs undergoing radiation therapy compared with a placebo group has been reported to be beneficial [405]. However, the addition of glutamine to a purified diet fed to cats with methotrexate induced enteritis did not reduce permeability or bacterial translocation [413].

Limited research has been done on the clinical advantages of glutamine administration in animals with gastrointestinal disease and the results are contradictory. It is possible that the methotrexate model of enteritis in cats is too severe to show an advantage of glutamine supplementation, especially because little recovery time was allowed in that report. Therefore further research into the benefits of glutamine administration to patients with gastrointestinal disease is warranted.

Taurine, cysteine and methionine

The cat requires the sulphur containing amino acids cysteine and methionine at a higher level than other mammals. These amino acids are interconvertible and can be utilized to synthesize two other amino acids, taurine and felinine, which are important only in the cat. Taurine is a beta-sulphonic amino acid required by cats in their diet. Cats can only partially fulfilled their taurine needs by synthesis from sulphur containing amino acids cysteine and methionine [375] because of the low activity of two enzymes in the metabolic pathway that converts cysteine to taurine [304]. Felinine is an amino acid that is produced in very high amounts in entire male cats but in minor amounts in female cats [414]. Hence, the synthesis of these two amino acids cannot explain the increased requirements of sulphur containing amino acids by the cat.

Taurine deficiency in cats causes retinal degeneration, developmental abnormalities, reproductive failure, dilated cardiomyopathy and compromised immune function [415]. Losses of taurine are assured in the cat because cats conjugate bile acids only with taurine and not with glycine [416] unlike other species such as rats and dogs [417,418]. However, the bile acids conjugates are usually efficiently recovered by the entero-hepatic circulation [304], but the nature of the diet affects this recovery. Canned food increases bile acid excretion, especially secondary bile acids and need to be supplemented with a higher level of taurine than dry diets [417,419,420,421]. The source of dietary protein can also affect taurine status in cats [422] as can the quality and quantity of protein [423]. Slowly or poorly digested proteins appear to increase the loss of taurine possibly due to increased bacterial metabolism on the luminal protein remnants.

The immunological consequences of taurine deficiency are many. Taurine deficiency in the cat decreases the number of white cells, reducing neutrophil oxidative burst, increases gamma-globulins and alters splenic structure [424]. Taurine has also been shown to be a strong antioxidant protecting membranes from oxidative damage [419]. Taurine is found in high levels within cat leukocytes [424]. It has been suggested that this may be a protective mechanism because taurine can sequester hypochlorous acid in neutrophils and hence protects cell membranes against cellular peroxidation. Taurine deficiency increases the expression of MCH II antigens in cat lung macrophages [425]. These effects of taurine deficiency may one day be shown to affect the course of gastrointestinal diseases such as IBD but to date no such association has been demonstrated.

Fats

Traditionally, animals with diarrhoea are provided diets with a restricted fat content because malabsorbed bile acids and fatty acids are known to promote colonic secretion and increased permeability [313,426,427]. However, steatorrhoea is not a common complaint in cats with IBD [3,5,9,41,428]. Furthermore, the provision of fats during gastrointestinal disease allows administration of much needed calories in a small volume and enhances palatability, both of which are important in cats with IBD because two of the most common clinical signs are anorexia and weight loss [345]. Cats appear to be more tolerant than dogs to a high fat diet [313]. Nevertheless, because fat malabsorption can

contribute to osmotic diarrhoea, and high fat diets induce delayed gastric emptying (and thus may result in increased nausea and vomiting) [313], it has been recommended that cats with gastrointestinal disease do not consume a diet with more than 22%DM fat content [327].

In addition to the calories provided by dietary fats, these nutrients have important structural and immune functions. A proportion of dietary fatty acids are incorporated into cell membranes and utilized later for the production of eicosanoids. Eicosanoids vary in their pro-inflammatory nature in accordance with their parent fatty acid. The effect of dietary fatty acids on inflammation was first considered when epidemiological studies of human populations that consume diets rich in fish oils revealed a marked reduction in the prevalence of chronic inflammatory and circulatory diseases [429]. Since that time, considerable research effort has been devoted to better understanding the mechanism of action and clinical importance of the phenomenon. This is briefly summarized below.

Fatty acids from animal, vegetable and fish differ in their chemical structure, mainly in their length, and the number and position of their double bonds. Changes can be conferred on the molecules through the activity of several desaturases (add double bonds) and elongases (lengthen the carbon chain). However, the first double bond on the non-carboxylic end of the molecule cannot be changed. The position of this double bond determines the 'series' of fatty acid to which a particular fatty acid belongs. Fatty acids from different series are not interconvertible [430]. The most important fatty acids series are called n-3, n-6 (includes the essential fatty acids such as linoleic acid) and n-9 (non-essential fatty acids) [431]. There is still debate as to whether n-3 fatty acids are essential

[432-434]. Most of the structural fatty acids belong to the n-6 series. These can be exchanged for fatty acids from the n-3 series, even though the latter cannot fulfil all the functions of the n-6 series e.g. dermal integrity, renal function and parturition [432].

Most mammals require linoleic acid in the diet (18:2 n-6) from which the other essential fatty acids can be manufactured. Linoleic acid is found in vegetable and seed oils. However, the cat has low activities of delta-6 desaturase [304,435], the enzyme that converts linoleic acid to gamma-linolenic (18:3 n-6). Cats also have low levels of delta-5 and delta-8 desaturase and as a result cannot synthesize arachidonic acid (20:4 n-6) [431,433]. Gamma-linolenic acid can be provided by such supplements as evening primrose oil [430]. Arachidonic acid is obtained by the ingestion of the structural lipids in meat i.e. lean meat [431].

The composition of the structural lipids in cellular membranes is mainly determined by the dietary ratio of n-6:n-3 fatty acids [432]. Structural lipids are important because they can influence cellular membrane function, affecting display and activity of transcellular receptors (including MCH II) and also barrier function [430,433]. However, the principal therapeutic effect of n-3 fatty acid supplementation is thought to operate by modification of the type of eicosanoids produced from these structural lipids. Thus, Ling et al (1998) [436], concluded that the main antiinflammatory effect of fish oils in rats was principally through the reduction of arachidonic acid in plasma phospholipids. When n-3 fatty acids are incorporated into structural lipids, the eicosanoids produced (prostaglandins of the 3-series and leukotrienes of the 5-series) are less inflammatory than those derived from membrane lipids formed from the n-6 fatty acids (prostaglandins of

the 2 series and leukotrienes of the 4 series). There is also a pool of free, non-esterified fatty acids available for eicosanoid synthesis, which has a higher composition of n-3 unsaturated acids and responds faster than membrane phospholipids. This pool of non-esterified fatty acids is dependent on the dietary intake of fatty acids [432,434].

The dose and duration of supplementation of fatty acids have been very variable in the small number of published reports of the therapeutic use of fatty acids in the veterinary field [433,434,437]. In humans, von Schacky (1985) [429] showed that several variables affected the results of fatty acid supplementation including dose, length of supplementation period and tissue involved. In addition, a 'ceiling effect' of dietary supplementation with n-3 fatty acids has been reported [432,436]. There is a non-linear relationship between dietary n-3/n-6 actions in the maintenance of eicosanoid precursors in tissues. Thus, large changes in the administration of a particular oil (as percent dietary energy) above a certain threshold produces little further change in the proportion n-6:n-3 of tissue fatty acids. However, the study of this response is biased by the fact that most diets in the western world contain mainly n-6 fatty acids and most tissues are at their maximal capacity in n-6 fatty acid content [432].

Human intestinal cells supplemented *in vitro* with different fatty acids, especially eicosapentaenoic acid (EPA) from fish oil, have shown modulation of fatty acid desaturases and decrease of the production of arachidonic acid [438]. However, since the cat has very low activity of these enzymes this effect of EPA in cats is likely to be negligible. Other reports on the *in vitro* effects of n-3 fatty acids suggest that they promote

proliferation, migration and restitution of the intestinal epithelium after injury in rats [439] and lower the expression of MHC II complexes and integrin molecules in monocytes [440]. Interestingly, EPA seems to be more effective than docosahexaenoic acid (DHA) although in a pro-inflammatory situation (addition of IFN-gamma) DHA does show similar effects to EPA [439,440].

From the *in vitro* data above, it would be logical to believe that direct incorporation of EPA and DHA in intestinal cellular membranes may be beneficial. A change in the composition of cellular membranes has been shown to occur in human patients with IBD [441] and colorectal cancer [442]that were given dietary supplementation with fish oils. However, cell proliferation in the colon of the patients with colon cancer was not normalized in contrast to the *in vitro* data. Interestingly, it was not only the n-3 acids but also the n-6 acids that showed beneficial effects in promoting intestinal healing *in vitro* [439] and in malnourished piglets [443].

Some eicosanoids derived from n-6 fatty acids have been incriminated in the pathophysiology of IBD in humans including thromboxanes, prostaglandins and leukotrienes. Prostaglandin E_2, thromboxane B_2 and leukotriene B_4 predominate in ulcerative colitis [208,444-446]. These mediators have been directly correlated with an increased secretory response in the colon [444]. Furthermore, the tissue damage and clinical signs of colitis are effectively treated with corticosteroids and sulphasalazine, both of which inhibit eicosanoid production [122,447]. Moreover, colonic eicosanoid concentrations have been postulated as reliable predictors of relapse in ulcerative colitis [448]. Mice deficient in most types of prostanoid receptors do not develop colitis when treated with

dextran-sodium-sulphate [449] and reduction in eicosanoid production in the colon following fish oil supplementation has reduced progression of disease in a rat model of colitis [450]. Similarly a reduction in eicosanoid production and an improvement in clinical signs have been reported to occur in ulcerative colitis and Crohn's disease in people as a result of fish oil supplementation [441]. It is noteworthy, that similar clinical improvement (without a change in eicosanoid production) was also seen in the latter study following supplementation with olive oil (77% oleic acid 18:1n-9 and 7% linoleic acid 18:2n-6). This study points to an unknown beneficial effect of olive oil on inflammatory processes and raises the possibility that the benefits of fish oil may not be mediated entirely by the n-3 fatty acids-mediated reduction of inflammatory eicosanoid production. Recently it has been reported that the phenols present in olive oil are absorbed by the intestine and can behave as effective antioxidants [Vissers, 2002].

Despite some clinical success, supplementation with fish oils remains controversial in the management of IBD. The results of some trials in patients with ulcerative colitis and Crohn's disease have shown promise [441,451] whereas other trials have not been successful [452]. Further controversy arises because high levels of polyunsaturated fatty acids, including arachidonic acid (20:4 n-6) and DHA, have been found in the plasma of IBD patients with both active and inactive disease [451,453]. This observation questions the need for essential fatty acids and fish oil supplementation in IBD.

The dietary provision of fatty acids may be useful in reducing the side effects of corticosteroids and non-steroidal antiinflammatory drugs. These medications inhibit formation of eicosanoids and impair the physiological benefits eicosanoids have in several

organs and tissues including the gastrointestinal tract [439,449]. Much research has been done recently on the enzymatic selectivity of antiinflammatory drugs to avoid affecting the homeostatic functions mediated by eicosanoids but gastrointestinal damage is still possible [328,454] and may be amenable to fatty acid supplementation. It has been suggested that the effectiveness of fish oils in clinical trials has been reduced by the excessive intake of n-6 fatty acids [432]. Even when this is taken into consideration, however, fish oils have not proven to be as clinically effective as expected [292,300]. In the future it may be established that the primary clinical benefit of n-3 fatty acids in animals will be to permit clinicians to use lower doses of antiinflammatory drugs [327,356,455]. This 'steroid-sparing' effect has been shown in ulcerative colitis in people [300]. Given the sometimes serious side-effects of the drugs used in the treatment of IBD in people and pets, this potential 'steroid-sparing' effect may be of significant clinical value.

Fish oil supplementation of cats is complicated by the need to provide arachidonic acid to meet nutritional requirements. Competitive interactions between n-6 and n-3 fatty acids such as arachidonic acid and EPA are thought to determine the 'ceiling effect' mentioned before in membrane phospholipids composition [432] and eicosanoid production [456]. Arachidonic acid and EPA behaved as antagonists when given together [456]and arachidonic acid negated the effects of EPA. These results might be explained by the fact that, as explained before, both series of fatty acids, n-3 and n-6, compete for the same enzymes but the n-3 cascade is weaker and slower than the n-6 cascade [432]. In addition dietary arachidonic acid is specifically and preferentially incorporated into phospholipids [456]. Perhaps an optimal ratio between arachidonic acid and n-3 fatty acids can be found in cats that will demonstrate to be beneficial in cats with gastrointestinal disease. Results of

the use of different fatty acids in cats with dermatitis indicate poor effectiveness of dietary supplementation with n-3 fatty acids and positive effects of linoleic or gamma-linolenic fatty acids (18:2 and 18:3 n-6) supplementation [433].

Other type of fatty acids present in foods, such as conjugated linoleic acid (CLA), trans-fatty acids and medium chain fatty acids, can affect gastrointestinal structure and function. Conjugated linoleic acid is a mixture of isomers of linoleic acid and is produced by rumen fermentation. It is found in meat, milk and other dairy products. CLA has shown immunomodulatory effects in pigs affecting mainly the circulating gamma-delta T cells and possibly having antiinflammatory effects [457]. *In vitro,* trans-10 CLA have been shown to affect the distribution of tight junction proteins and to increase permeability to mannitol [458]. Other trans-fatty acids have shown no beneficial effect on intestinal healing *in vitro* [439]. These observations are interesting because epidemiological studies have linked IBD to the consumption of fast foods [67]. Trans-fatty acids are formed during food processing, they are not only biologically inactive but they are also antagonists of essential fatty acids [459]. Despite this, healthy cats fed oxidized lipids (trans fatty acids increase during fat oxidation) did not show any deleterious effects but they did need to consume more food to maintain body weight [460].

Lastly, medium chain fatty acids (MCT), usually as coconut oil, have been used to provide extra calories to animals and people with gastrointestinal disease because their absorption theoretically is simpler than that of long chain fatty acids. These fatty acids have 8 to 10 carbons and are thought to be mainly absorbed directly into the blood stream with no need for esterification in the enterocyte [327]. However, there are reports in dogs

showing the absorption of some MCT via the lymph [461]. Furthermore, when MCT are given in excess to people they do become incorporated in chylomicrons [462]. More worrisome is that they have been associated with hepatic lipidosis in the cat [463].

The use of MCT in different models of trauma in rats has shown that MCT are most effective at increasing body weight, jejunal mass and gastrointestinal absorptive function when MCT is not more than 40 % of the total lipids administered orally. [464]. Higher levels were deleterious [464]. It is usually recommended to feed MCT to veterinary patients at no more than 30% of daily calories although palatability and diarrhoea due to malabsorption are common complications [327].

In summary, fats are a concentrated source of energy and supply essential fatty acids that if deficient, decrease the adaptive response of the intestine to injury [328,465]. Essential fatty acids, such as linoleic acid, have shown beneficial effects in people with IBD and cats with inflammatory conditions of the skin that are equal or superior to those shown by 'antiinflammatory' n-3 fatty acids. The therapeutic benefit (if any) of providing n-3 fatty acids to patients with IBD requires further research, including the determination of the appropriate ratio of n-6/n-3 fatty acids and perhaps the ratio of n-3 fatty acids with arachidonic acid. It has been recommended that not more than 4% of energy intake be included in the diet as n-6 and n-3 fatty acids in small veterinary patients. Changes in the cellular membranes of the intestine are expected to occur after 4 weeks of supplementation and will take several weeks to disappear [434]. The relevance of trans-fatty acids in petfoods and their link with disease remains to be elucidated.

Minerals and vitamins

Several vitamin and mineral deficiencies have been reported in patients with IBD, principally in those with Crohn's disease affecting the small intestine [300,302,328]. However, the relationship between these deficiencies and the severity of disease, as well as the importance of dietary vitamin and mineral supplementation has not been established [292]. Malnutrition because of lower food intake is common in humans with IBD and may be the main cause of these deficiencies [292]. Other potential contributing causes may be poor absorption, increased losses, increased requirements or side effects of medication. For example, it is well known that sulphasalazine decreases the absorption of folate, corticosteroids affect calcium metabolism and antibiotics can affect the provision of vitamin K [300,302].

Fat soluble vitamins

Lipid malabsorption may adversely affect the absorption of lipid soluble vitamins like vitamin A, D, E and K [300]. However, malabsorption of fats is not common in human IBD patients [300] and it has been reported only sporadically in cats with IBD [41]. The perceived association between feline IBD and pancreatitis [45], and between feline pancreatitis and exocrine pancreatic insufficiency [466] increases the possibility of fat malabsorption in cats with IBD. Another factor that may affect the fat soluble vitamin status of IBD patients is the adverse effects of IBD on protein metabolism, which can decrease the concentration of serum binding proteins of vitamin A [300,328]. The use of fat restricted diets may be another factor compromising fat soluble vitamin status in IBD

patients. The intake of vitamin A and E, as a risk factor for the development of Crohn's disease, has been investigated [467]. Consumption of vitamin E was found to have a strong negative association with the onset of the disease.

Both vitamins A and E have an antioxidant function and for this reason they have been attributed importance in chronic inflammatory diseases [468]. These vitamins are efficient scavengers of free radicals [469-472]. Vitamin E is found in cellular membranes closely associated with polyunsaturated fatty acids, which are very sensitive to free radicals damage [471].

Clinically overt vitamin E deficiency has been described in cats as a result of long term consumption of oily fish [473] and has been also experimentally produced [474]. However, high quality fish oil does not seem to increase the requirement for the vitamin [475]. There seems to be a large inter-individual variation in sensitivity to vitamin E deficiency in the cat [419,473]. The clinical presentation of vitamin E deficiency is distinctive in the cat [350] but it has not yet been reported in cats as a consequence of gastrointestinal disease.

Some human patients with IBD have developed vitamin A deficiency [300,328,476]. Clinically overt vitamin A deficiency has been experimentally produced in cats using purified diets [477]. To my knowledge, however, vitamin A deficiency has never been reported in cats as a consequence of gastrointestinal disease.

Carotenoids (vitamin A precursors in some species) have antioxidant properties of their own (not by conversion to vitamin A) and by comparison are much better antioxidants than vitamin A [468]. Carotenoids have been found to act as immunomodulators in people [478 468] and dogs [479]. Animal tissues contain very small amounts of carotenoids (unlike plants) and cats show a very low activity of the enzyme that converts carotenoids to vitamin A (carotene dioxygenase) [304,477]. Despite this, cats absorb carotenoids (lutein) from food to much higher serum concentrations than dogs, and do incorporate them into lymphocytes and neutrophils. However, if carotenoids act as immunomodulators in cats it is still unknown [479]

Vitamin K has been reported to be frequently deficient in patients with IBD as a result of malabsorption or antibiotic treatment [328]. Similarly, two cats with lymphocytic-plasmacytic enteritis, malabsorption, probable vitamin K deficiency and a haemorrhagic diathesis have been reported [480]. Feline IBD is sometimes associated with hepatic and pancreatic disease [45] and is not possible to discount the possibility that vitamin K deficiency was the result of advanced liver [166] or pancreatic [481] pathology.

Vitamin D deficiency and bone loss are common in human patients with IBD [292,300]. Corticosteroid therapy is considered the main cause of bone loss in ulcerative colitis, but in Crohn's disease the bone loss is correlated with disease activity and may be mediated by TNF-alpha [292]. The cat has an obligatory dietary requirement for vitamin D because of the high activity of 7-dehydrocholesterol-delta 7-reductase that converts the skin precursor of pro-vitamin D to cholesterol, so by-passing the vitamin D synthetic

pathway [304]. Unfortunately, vitamin D status of cats with anorexia or severe gastrointestinal disease has not been studied.

Water soluble vitamins

Deficiency of water soluble vitamins has been reported in small numbers of human patients with IBD but as a rule their supplementation is not warranted [476]. Low serum concentrations of vitamin C, thiamine, folate, vitamin B_2, vitamin B_6, vitamin B_{12}, biotin, niacin and pantothenic acid have been described in human IBD patients [292,482]. Folate absorption is affected by sulphasalazine and is usually supplemented in human patients with IBD [300,328,482]. Vitamin B_{12} has been reported to be decreased in a group of Crohn's disease patients with severe involvement of the ileum or short bowel syndrome without ill-effects [292,482,483]. In one report, bacterial overgrowth, ileal resection and disease activity did not increase the risk of vitamin B_{12} deficiency [483]. Caution is necessary in interpreting serum values of vitamin B_{12} because they may not be representative of the intracellular values [484]. High levels of homocysteine have been observed together with normal serum concentrations of vitamin B_{12} in humans with IBD, perhaps indicating intracellular deficiency of vitamin B_{12} [483].

Two studies have been published reporting the serum concentrations of folate and vitamin B_{12} in cats with IBD. In one study the cats were found to have normal serum folate concentrations but half of the cats had low serum concentrations of vitamin B_{12} [485]. In the second study, some cats had high serum concentrations of folate along with reduced serum levels of vitamin B_{12} [486]. Cats with moderate to severe gut IBD lesions in intestinal biopsies consistently show a decreased vitamin B_{12} concentration [486,487]. The cause for

the lower levels of vitamin B_{12} in the cats with IBD is unknown. However, cats with IBD given vitamin B_{12} parenterally show rapid depletion of this vitamin when compared to healthy cats [486]. Also an association between IBD and pancreatitis was observed in the cats with decreased vitamin B_{12} concentration [486] suggesting that the accompanying pancreatitis may be the reason for the low concentration of vitamin B_{12} in these cats. In this context, it has been reported that feline intrinsic factor, which is required for vitamin B_{12} absorption, originates in the pancreas [488]. It is uncertain if alterations of the bacterial flora in cats with IBD contribute to the decreased level of vitamin B_{12} seen in the serum of cats with this disease. However, it has been shown that the administration of antibiotics reduces bacterial counts in the duodenal fluid and this change is accompanied by an increase in serum concentrations of vitamin B_{12} and albumin [489]. The interrelationship between proteins and vitamin B_{12} is interesting because abnormalities of amino acid metabolism have been reported in cats with low serum concentrations of vitamin B_{12} [490]. Nevertheless, no clinical impact of the lower levels of vitamin B_{12} has been reported in cats with IBD. Retardation of growth rate was the main consequence of deficiency of vitamin B_{12} in kittens fed vitamin B_{12} deficient purified diets[491].

In most species niacin is provided by the diet and by endogenous synthesis from the amino-acid tryptophan [350]. The cat has all the enzymes necessary for synthesis but the high activity of alpha-picolinic carboxylase removes an intermediate of the synthetic pathway too rapidly for any synthesis of niacin to occur. Thus, tryptophan is converted to glutamic acid and not to nicotinic acid [492]. Consequently, the dietary requirements of nicotinic acid are increased for the cat. Under normal circumstances this should not cause problems in a strict carnivore because tryptophan and niacin are abundant in animal flesh.

Cats do not survive if the diet is devoid of niacin even when high levels of tryptophan are present [492]. Pellagra, the clinical state of niacin deficiency, has been reported in two human IBD patients [476] but not in cats with IBD or other gastrointestinal diseases.

Vitamin C has been reported to be low in patients with Crohn's disease [328]. In epidemiological studies a high intake of vitamin C has been found to have a strong negative association with the disease [467]. Cats synthesize vitamin C and have no daily requirement for this compound [350]. Vitamin C deficiency is, therefore, unlikely to play a significant role in the pathogenesis of feline IBD.

Although thiamine deficiency has not been reported in cats with gastrointestinal disease a combination of factors suggest that such cats are likely to be at significant risk of this deficiency. Cats have a high requirement for thiamine compared to other species and the vitamin is poorly stored compared to fat soluble vitamins. In addition, many IBD cats are anorectic to some degree. Lastly, the process by which commercial pet foods are produced increases the losses of vitamin B_1, although this loss is usually offset by addition of extra thiamine [350,419].

Minerals

Decreased serum concentration of iron, magnesium, selenium, calcium, potassium and zinc are commonly seen in humans with IBD. However, serum concentrations are not always a direct indication of deficiency [292,300,328,482,493] as the concentration of minerals can be influenced by alteration of serum protein concentrations. [328,494]. The

value of mineral supplementation of humans with IBD is unknown [292] with the possible exception of zinc supplementation [494].

The importance of mineral deficiencies in cats with IBD is unknown but depletion of potassium is possible given that anorexia is common in feline IBD and can be accompanied by increased losses of potassium through diarrhoea and vomiting [10,166].

Antioxidants

Reactive oxygen metabolites (ROM) are thought to be an important cause of tissue damage in IBD [122,208,495,496]. A significant part of the knowledge gained about oxygen radical damage has been obtained through models of ischemia, some of which have involved ischaemia and reperfusion in the intestine and stomach of the cat [497-500].

Xanthine oxidase (small intestine contains the greatest activity of this enzyme) and/or the activity of neutrophils [499,501-503] are considered to be the main sources of ROM in the gastrointestinal tract. The gastrointestinal tract also contains considerable amounts of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) [504] that bind and degrade the ROM to less reactive forms.

Oxygen radicals produce DNA damage, degradation of cell proteins and oxidation of cell membranes, leading to metabolic derangements and eventually cell death [503,505]. Damage to the duodenal villi with epithelial loss and increase mucosal permeability to

albumin was documented in a model of ischemia in the intestine of the cat. However, the increase in permeability is now believed to be caused by disruption of the epithelium during neutrophil transmigration and not by the release of oxygen radicals [503]

The importance of reactive oxygen metabolites has been studied in experimental colitis in rats [502,504]. The study by Keshavarzian et al (1990) [502] suggests that superoxide anion is the most important oxygen radical in this model. The effectiveness of sulphasalazine in experimental models of colitis and in human colitis also supports a role for ROM in colitis. Numerous publications have shown the value of this drug as a radical scavenger [502,506,507]. Dallegri et al (1990) [507] showed that the cytoprotective effect of sulphasalazine is due to its effect on hypochlorous acid. These findings indicate that neutrophils are an important cause of oxidative damage in this model of colitis.

The role oxygen radical damage plays in feline IBD is unknown. While neutrophils are present in the mucosal infiltrates of feline IBD they are not the main inflammatory cell [12]. This reduces the likelihood that ROM play a major part in feline IBD but does not eliminate this possibility because of the other potential mucosal sources of ROM.

The risk of tissue damage from the increased production of ROM in IBD is compounded by decreased concentrations of antioxidants and reduced activities of antioxidant enzymes. In experimental colitis in rats and in human patients with IBD glutathione, SOD, metallothionein concentration and activity were reported to be reduced [496,504]. Besides, the production of eicosanoids (main inflammatory mediators in IBD) by

oxidation of arachidonic acid reduces the amounts of vitamin E, vitamin C and reduced glutathione [508].

Glutathione synthesis inhibition is known to cause intestinal tract damage in mice [509]. It is noteworthy that glutathione status is responsive to nutritional management. Oral glutathione is effective at increasing glutathione levels in the intestine of rats [510]. Conversely, a diet poor in protein or sulphur containing amino acids reduces the levels of glutathione present in rats and therefore predisposes to oxidative damage [510]. This observation may have importance in IBD because many people and cats with the disease are malnourished. Selenium is an important mineral in this respect because it forms part of glutathione peroxidase and other tissue peroxidases [511]. However, cats have been reported to have much higher serum concentrations of selenium than other species [512]. Anyhow, selenium status in cats requires further studies before any conclusions can be drawn.

Zinc deficiency is commonly reported in human patients with IBD [292,493,494]. Zinc forms part of SOD together with copper but the activity of this enzyme is not decreased during zinc deficiency and the signs of zinc deficiency can only be partially reverted by supplementation with vitamin E in rats and chickens [511]. Zinc deficiency does cause severe gastrointestinal lesions in rats, which respond to the use of antiinflammatory [282]. However, it is not known how exactly zinc deficiency causes tissue damage but it decreases metallothionein, which is already deficient in IBD human patients [496]

In summary, research on ischaemic-reperfusion injury has demonstrated that the cat gastrointestinal tract has all the machinery necessary to produce oxidative damage. Whether ROM play an important role in feline IBD is uncertain but, if so, the diet may be an important factor in averting a mismatch of oxidants and antioxidants. Nutrients capable of influencing the oxidative/antioxidative balance in cats include niacin (a component of reducing substrates like NADH/NADHP) [471], riboflavin (a component of GSH reductive which regenerates oxidated glutathione) [508], Vitamin E (the principal antioxidant of cell membranes), sulphur-containing amino-acids (for which the cat has a higher requirement and which help maintain the levels of glutathione) and glutamine (which enhances gut glutathione production) [513]. The importance of selenium, zinc and copper as antioxidants have not been studied closely in the cat but evidence from other species suggests these nutrients might also be important in cats.

Probiotics, prebiotics and synbiotics

The importance of the gastrointestinal bacterial flora in health and disease has already been discussed. As early as 1908, Metchnicoff postulated that some gastrointestinal bacterial species, specifically *Lactobacillus bulgaris* might have beneficial effects on their host [514]. Further research on the normal microbiota of the gut revealed that some species are more beneficial than other and, more importantly, that diet could encourage these beneficial effects by modifying the species composition of the microflora [515].

Changes in the gastrointestinal bacterial population are not easy to produce and maintain long term [246]. Individual people have a relatively stable flora even with different diets [516,517]. However, a high level of variability in the composition of the flora occurs between individuals[516]. The variability in composition of the gut flora has been also noticed in cats [518] and dogs [519,520]. The adaptive changes of the gastrointestinal flora to diet have been better demonstrated by measuring the activity of bacterial enzymes rather than by quantifying bacterial species. Bacterial enzyme systems are inducible according to the substrate present [246,521,522]. Unfortunately, studying the response of gastrointestinal microflora to dietary changes has been particularly difficult because of methodological constraints (e.g. suitability of faeces versus colonic contents, appropriate control diets, failure to use comparable bacteriological methods, limitations of bacteriological techniques). These constraints need to be kept in mind when considering the results of studies examining the effects of dietary manipulations on the gut flora [246,253,517,521,523]. It is important also to consider the complex mechanisms responsible for the control of the bacterial flora when attempting to manipulate its composition by diet. Gastrointestinal motility, gastric acid secretion, antibacterial products (substances secreted to inhibit the growth of other species) and production of short chain fatty acids [246] can affect the gastrointestinal flora. These and other factors can determine the success or failure of probiotics or bacterial substrates in the diet.

Changes in protein and fat consumption do not appear to greatly influence the microbial composition of the faecal flora [246]. On the contrary, different dietary fibres have been reported to change the metabolic activity of the bacterial flora but not to exert a profound effect on the bacterial composition of the bacterial flora of the healthy gut [517].

Despite this general statement changes in the quality of the bacterial flora in humans has been reported as a result of the consumption of dietary fibre equivalents. Gibson et al (1995) [515] confirmed that inulin and oligofructose increase the numbers of Bifidobacteria in stools without changing total bacterial counts. Bifidobacteria and Lactobacilli are considered beneficial to health and their benefits have been mentioned before. This has encouraged research into ways in which supplementation with the bacteria or its substrates could be used to promote health or modify disease.

Probiotics

Probiotics are defined as a live microbial food supplement that beneficially affects the host by improving its microbial balance [524]. Microorganisms most frequently used for this purpose are *Lactobacilli* and *Bifidobacteria*, which are commonly found in fermented milk products [249]. *Streptococcus* spp. and *Saccharomyces boulardii* have also been used as probiotics [525]. These bacteria and yeasts resist gastric acid conditions, adhere to and colonize the gut and have been associated with clinical improvement of some diseases [525]. Unfortunately their effects are transient [249,524-526] and they require constant administration for long term benefits. Importantly, bacteria to be used as probiotics should have been isolated from the mammal species in which they are intended for use, otherwise colonization of the gastrointestinal tract is unlikely [249]. Adherence to enterocytes is fundamental for colonization to occur and is this adhesive property that is associated with inhibition of invasion of intestinal pathogens [527]

Probiotics are believed by some to improve the outcome of certain gastrointestinal disorders including antibiotic-induced diarrhoea, *Clostridium difficile* infection, rotavirus

diarrhoea, hepatic encephalopathy, disaccharidase deficiency, lactose intolerance, IBD, irritable bowel syndrome, bacterial overgrowth, enteral feeding, neonatal necrotizing enterocolitis and cancer [524,525,528]. They are also purported to beneficially modulate the immune system, improving humoral immunity, the pattern of cytokine production, phagocytosis and non-specific immunity [529].

The use of yogurt has been recommended for treatment of many ailments in people despite a lack of understanding about its mechanism of action or proof of beneficial effects [530]. Yogurt consumption has been used in the treatment of cancer, gastrointestinal disease and allergies [514,530]. The lactic acid producing bacteria in yogurt have been considered by some to mediate its presumed beneficial effects [525]

However, conventional yogurt cultures may not survive gastric acid as well as the Lactobacillus and Bifidobacteria that have been shown to colonize the human intestine [514,524]. Furthermore, Bernet et al (1994) [527], showed that different strains of *Lactobacillus acidophilus* show very different adherence properties to intestinal epithelial cells. These observations question the value of yogurt in gastrointestinal disease in humans and other species. However, they do not eliminate the possibility that this time honoured strategy is of some value perhaps through non-probiotic mechanism.

More recently, another dimension has been added to the use of probiotics. Probiotic bacteria have been genetically modified to deliver desirable antiinflammatory cytokines. Steidler (2000) has successfully used this strategy in two models of murine colitis by administering *Lactobacillus lactis* capable of producing IL-10 [531].

Prebiotics

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth, activity or both of one of a limited number of bacterial species already resident in the colon [532]. Most of the search for prebiotics is aimed at substances that selectively enhance the proliferation of bacteria considered to be beneficial to the host (e.g. *Lactobacilli* spp. and *Bifidobacteria* spp.) [249]. Oligofructose and inulin have shown this capacity *in vitro* [533], in the human colon [515] and in cat faeces [534]. Resistant starch (RS), including some novel chemically-modified varieties, can behaved similarly but RS is not yet considered a prebiotic because their effects on the microflora may lack specificity [535]. Both, soluble and insoluble dietary fibres have been shown to increase faecal density of Bifidobacteria for a short time [522] but they are considered to be non-selective in their action and hence do not strictly comply with the definition of prebiotics [535]. Oligosaccharides containing galactose, xylose, mannose or glucose, lactulose and lactitol also function as prebiotics [249,535].

Oligofructose is a product of the hydrolysis of inulin and both together are commonly referred as fructooligosaccharides (FOS) [532]. They have been called 'functional foods' because they do not strictly meet the definition of dietary fibre as stated by the Food and Drug Administration and the United States Department of Agriculture. Acceptance of FOS as dietary fibre varies between countries [526].

The effects of inulin on gastrointestinal functions are similar to those of dietary fibre (see later), although inulin does not form viscous solutions as do other fermentable

fibres [526]. In humans, inulin is completely fermented in the colon and passes through the small intestine unchanged [536]. Adaptation to inulin by the bacterial flora is required and inulin fermentation is slower than for other fructans [533]. Inulin has some effects usually associated with dietary fibre (e.g. lowering serum lipids) that only occur with higher doses but not with moderate doses [537]. It has been reported that inulin produces a dose-related increase in *Bifidobacteria* growth [533].

Inulin is preferentially fermented by Bifidobacteria to acetate, propionate, butyrate and lactate [537]. The acetate fermentation product is partly responsible for the inhibition of some species of bacteria by Bifidobacteria [515]. However, other bacterial species are also capable of fermenting inulin and produce higher amounts of gas than Bifidobacteria [526,538]. High levels of inulin included in human diets have been reported to cause abdominal discomfort and there is individual variability in tolerance to inulin [526].

Inulin decreases the absorption rate and total absorption of glucose [539] and increases absorption of calcium, phosphate, magnesium [537], zinc and iron [540] in rats. These effects have yet to be confirmed in humans [541]. The mechanism by which inulin decreases glucose absorption is unknown. Inulin does not affect gastrointestinal transit time significantly [526] perhaps because it is considered not to increase viscosity significantly [542]. Moderate to high doses of inulin or oligofructose cause a small increase in faecal bulk [540]. An increase in bacterial biomass is considered to be the main cause of the increased faecal bulk [526].

Bifidobacteria produce several vitamins of the B group such as biotin, thiamine, riboflavin, niacin, pyridoxine, cyanocobalamin and folic acid, which can be an additional benefit to the host [526,532].

The effects of inulin and oligofructose reported in the literature are discussed further in the dietary fibre section of this thesis to facilitate comparison with dietary fibres as many publications include and compare FOS with dietary fibres.

Synbiotics

Synbiotics are a mixture of probiotics and prebiotics fed together in order to improve the chances of survival of beneficial bacteria and their colonization of the gastrointestinal tract [532]. A variety of combinations have been suggested including Bifidobacteria with FOS, Lactobacilli with lactitol and Bifidobacteria with galacto-oligosaccharides [249]. Synbiotics are not always more beneficial than their component parts. For example inulin administered with *Bifidobacterium* did not enhance the effects on bacterial flora of the probiotic alone [526].

In summary, the use of probiotics, prebiotics and symbiotics in IBD needs to be further explored. In the veterinary field these therapeutic approaches have been studied only in a few trials involving healthy animals (discussed later with the dietary fibre section). Unfortunately, the effects of probiotics, prebiotics and synbiotics on the bacterial flora are difficult to assess microbiologically. Molecular approaches for

studying bacterial diversity and bacterial population changes offer advantages over standard microbiological techniques. The use of 16S ribosomal RNA is a promising technique and probes for detection of Bifidobacteria in faecal samples have been described. This may be a more reliable tool to firmly confirm the effects of probiotics, prebiotics and synbiotics on colonic flora [249]. However, a full understanding of the implications of administering these dietary supplements will require future studies assessing the effects on disease course and severity in clinically affected patients.

Carbohydrates

Carbohydrates are not considered essential nutrients even though they are an important source of energy in cats or dogs [543]. However, healthy cats adjust well to a diet rich in carbohydrates. Cats show very high apparent digestibility for a variety of carbohydrates (glucose, sucrose, lactose, dextrin and starch) [544]. In addition, Kienzle (1993) [545] reported 72% prececal digestibility for maize starch in the cat indicating that in healthy cats some starch is not digested in the small intestine and will reach the colon to be fermented.

The ability of the cat to digest and absorb carbohydrates is based on the provision of digestive enzymes by the pancreas and the epithelial brush border, in addition to the necessary transporter systems. Adult cats have similar transport systems for glucose in the intestinal brush border than other mammals, which show a higher transport velocity and affinity than in other species [546]. However, an increase in total glucose uptake could not be demonstrated [547] and a decrease on total glucose uptake occurred in kittens by 60

days of age at the time that a carnivorous diet was being established [547]. In addition, digestive enzymatic adaptation to a high dietary level of carbohydrate does not seem to occur in cats. Rats [548] and other omnivores [374] have shown the inductive nature of brush border disaccharidases when consuming carbohydrate rich food. On the contrary, cats have not only shown considerable variation in disaccharidase activity, but also lack of increase in enzymatic activity when on a carbohydrate rich diet [374,549]. Kienzle [550] also reported low to moderate levels of pancreatic amylase and poor adaptive change to long term carbohydrate change. It is uncertain if this lack of flexibility in carbohydrate handling renders cats, especially cats with gastrointestinal disease, more susceptible to carbohydrate malabsorption and its nutritional and clinical consequences.

Further adaptations of the cat to a low carbohydrate diet are seen on the lack of salivary amylase, a lower amount and activity of pancreatic digestive enzymes and in the higher response of insulin to amino acids [543,551].

Unfortunately, carbohydrate malabsorption is not easy to evaluate and quantify directly. Carbohydrate malabsorption has been measured by different tests, among them total faecal carbohydrate, faecal reducing substances and faecal pH [552,553], faecal osmolar gap (refer to Chapter 3 and 5), breath hydrogen (See chapter 3) and starch tolerance test [554,555]. However, these tests do not correlate well with each other [556,557]. Understanding the factors that affect carbohydrate digestion and absorption, and which are more prevalent or important as a whole in cats with IBD will help understanding of the pathophysiology of the disease and assist with the correct choice of nutritional therapy. Further discussion on dietary carbohydrates in the cat is presented in Chapter 3.

Dietary Fibre

Dietary fibre is defined as the chemically heterogeneous group of non-starchy structural plant polysaccharides and lignins that are resistant to the host intestinal enzymes during passage along the gastrointestinal tract [558,559]. However, dietary fibre not only includes the cell wall of vegetable matter but also some food additives like gums and modified starches [560]. Dietary fibres have multiple effects on gastrointestinal structure and function which are mostly related to their chemical and physical properties [561]. Therefore, a short discussion on the difficulties in studying dietary fibre and interpreting research on the topic followed by a brief summary of the chemical substances classified as dietary fibre will be presented. Lastly, the diverse nutritional properties of dietary fibre and the effects of dietary fibre on the gastrointestinal tract will be discussed. This section will conclude with a summary of the published reports describing the effects of dietary fibre in the cat.

Dietary fibre research: a complex arena

The gastrointestinal effects of dietary fibre have been recognized for centuries. Hippocrates about 600 BC recommended coarse flour (with the bran) for the treatment of constipation [562]. Unfortunately, in spite of the early interest in fibre, this nutrient was largely ignored until the studies by Walker, Trowell and Burkitt in African rural people in the 1960s raised the likelihood that fibre consumption reduced the risks of some diseases [563].

It was apparent from this work that the prevalence of certain disease was different in populations that consumed a considerable amount of vegetable matter and those that did not [562]. Constipation, appendicitis, colon cancer, gall stones, diverticular disease and coronary heart disease were rare in the former population. Furthermore, it was observed that these differences in prevalence became less distinct when the populations consuming high amounts of fibre began to consume diets with less dietary fibre [564]. Other diseases are also known to be 'fibre responsive'. These include obesity, diabetes mellitus and disorders of lipid metabolism [565,566].

Dietary fibre is not an homogeneous substance but a group of chemically and physically diverse polymers [558] as it will be described in the next section. Cellulose, lignin, hemicellulose, pectin, gums and other oligosaccharides are considered to be dietary fibre. Resistant starch (indigestible) acts also as a dietary fibre [558,559,567]. Some water-soluble or water-dispersible polysaccharides that are commonly used as food additives in humans and pet foods behave like dietary fibres. They act as gelling agents by forming a three-dimensional network when dissolved in water [568]. The most common of these additives are pectins, carrageenans (from algae) and gums [569], which have been a component of pet foods for many years [570].

Dietary fibre has been added to human and pet foods following general nutritional advice to increase dietary fibre intake for health purposes [571]. However, in certain circumstances, some dietary fibres have shown deleterious effects on the intestinal mucosa. Dietary fibre added to basic diets has been associated with damaged mucosal cells in the

cecum of rabbits [572], the jejunum and colon of rats [573], the jejunum and ileum of pigs [574], and the colon of dogs [575]. Carrageenans particularly have shown an adverse effect on the intestine [576]. Ulceration, an abnormal villous pattern, and lymphoid hyperplasia were observed in rats after consumption of carrageenans in the drinking water for a month. The lesions were worse if prior subcutaneous sensitization to carrageenans had taken place [577]. Crypt abscesses, lymphocytic infiltration and gross ulceration of the colon have also been produced by carrageenans administered in the drinking water to guinea pigs [118]. Interestingly more consistent responses were obtained when the protocol in guinea pigs included sensitization with *Bacteroides vulgatus* [263]. However, carrageenans have never been shown to have toxic effects in the human population [578].

The chemical complexity of dietary fibre leads to difficulty in measuring the quantity of dietary fibre in foods. Preferably, both quantitative and qualitative methods should measure characteristics of fibre that are of practical relevance in assessing the nutritional impact of the fibre. Unfortunately, the more complete methods of evaluating dietary fibre are laborious and expensive. Simple methods, such as crude fibre or detergent analysis, give a good indication of the content of insoluble fibre in a food but limited information on the amount of soluble fibres present. Comparisons between different methods of measuring dietary fibre in petfoods have indicated that crude fibre analysis correlates well with other more expensive and laborious methods of measuring insoluble dietary fibre [579]. Crude fibre analysis is the accepted method to quantify fibre for declaration on the label of foods, including petfoods [350]. However, if the formulation of the food involves the addition of soluble fibres then crude fibre analysis will not reflect this fact accurately. Unfortunately, the common methods of measuring soluble fibres (i.e. Englyst

and Prosky) do not correlate well, which further compromises the ability to understand the functionality of a food from its label [579]. Thus, these chemical analysis methods neither give exact information of the chemical composition of dietary fibres [571,580] nor a full indication of their physical properties [561,568]. As we will see later, these physical properties determine the functional value of dietary fibre and its effects in the gut [561].

At this stage unfortunately is not possible to accurately predict the behaviour of a fibre from its chemical composition or physical arrangement, nor from *in vitro* studies [561,581]. Thus, *in vivo* testing is required to gain full understanding of the effects of any dietary fibre on the gastrointestinal tract and any other body systems.

Composition of dietary fibre

The chemical composition of the different fractions of the cell wall (i.e. dietary fibre) varies with the type of tissue and cell being studied [350,560,571]. A brief description of the different components of dietary fibre follows

Parenchymatous tissues

The cell wall in parenchymatous tissues of fruit and vegetables contains pectic polysaccharides, cellulose, hemicelluloses, glycoproteins and phenolics [560]. ***Pectic polysaccharides*** contain monosaccharide residues that interfere with hydrogen bonding resulting in a much more water-soluble and readily hydrolyzed fibre. In contrast, *cellulose* is a linear polymer of glucose that forms crystalline fibrils. The structure of the beta-glycosidic bond allows strong intermolecular binding rendering celluloses insoluble and poorly fermentable. ***Hemicelluloses*** mainly differ from cellulose in the fact that they

are made soluble by alkali. ***Glycoproteins and phenolics*** are present in low amounts and they are usually of low apparent digestibility [576]. Phenolics render the fibre very difficult to be degraded by heating while cooking [560].

The cuticular layers of the cell wall of fruit and vegetables usually contain waxes, suberine in roots [560], and cutin. All of these are complex lipid material poorly degraded, usually recovered together with lignin [576]. Secondary thickening of the cell wall occurs in fruit and vegetables due to the deposition of cellulose, xylans and lignin decreasing fermentability. Lignin is resistant to chemical degradation and is recovered virtually unaltered from faecal material [582].

Seeds

Dietary fibre composition also varies according to the seeds it originates from, two types are mainly described i.e. monocotyledons and dicotyledons[560]. *Dicotyledons seeds* mainly include legumes. Legumes can store two types of product, starch or a cell wall polysaccharide. If starch is the main storage product, the cell wall is very similar to the one already described above for the parenchymatous tissues. In the other type of legumes, extraction of the cell wall polysaccharide produces ***guars*** and ***gums***, mainly used as food additives. Chemically these fibres are mainly galactomannans, readily soluble in water and fermentable. Lignin is not an important component of the secondary thickening of the cell wall of legumes and therefore legumes do not show an increase in indigestible material with age.

Monocotyledons seeds include cereal grains which can be separated mainly in two groups, one with wheat, and the other with barley and oats; rye being intermediate between the two groups. Wheat endosperm cell wall contains mainly hemicelluloses and small amounts of beta-glucans. A significant part of wheat bran contains also the aleurone layer, which is rich in beta-glucans. In barley and oats 70 % of the cell wall is composed of beta-glucans. *Beta-glucans* form viscous solutions like the galactomannans of guar. *Beta-glucans* are water soluble and ferment readily in the large intestine. Oat bran is mainly composed of *beta-glucans.* Rice is an exception in several ways. Its content of fibre is low and the endosperm cell wall contains mainly cellulose with smaller amounts of xyloglucans, pectic polysaccharides and *beta-glucans.* [560]

Resistant starch

Starch that is not digested and absorbed in the small intestine is called *resistant starch.* Approximately around 20 % of total starch intake in a normal human diet- is resistant digestion [583,584]. This starch is fully fermented in the colon [567] and hence can act as dietary fibre [559]. Resistant starch includes inaccessible starch (whole grains and pastas), crystalline granules (raw potato and banana starches) that are less susceptible to gelatinisation and digestion, retrograded starch (re-crystallized) which is formed when cooked starch cools, and chemically modified starch used as a stabilizer in the food industry [584].

Oligosaccharides

Oligosaccharides are polysaccharides with usually only 2 to 9 monosaccharide units [585] that are not considered strictly dietary fibre [526] and they are commonly referred as 'prebiotics' because they selectively promote a specific bacterial population as they are fermented in the colon.

Legumes are rich in some oligosaccharides, namely raffinose, stachyose and verbascose [586], while some vegetables and fruits contain fructans like inulin and oligofructose. However, inulin is most commonly obtained from the chicory root [532]. Fructo-oligosaccharides (FOS) are widely distributed in nature and are also present in many petfood ingredients. For example wheat by-products have high concentrations of FOS. In contrast, rice and corn have low content of oligofructose [587].

Chemically, inulin is a beta-(2-1) fructan, with 2 to 60 units, which typically has a glucose moiety at the end of each chain of fructose. This type of linkage prevents digestion in the small intestine [538]. Inulin is added to many human foods because of a fat-like mouth-feel. It does not add flavours and does not increase viscosity [585]. Food texture and mouth-feel are important characteristics of food preferences in cats [345] and perhaps inulin has potential to be used in petfoods to improve their acceptability. Inulin is totally fermented in the human colon and stimulates the growth of *Bifidobacteria* in the large intestine of humans, has a laxative effect and increases faecal nitrogen loss by increasing bacterial biomass [532]. More on the effects of inulin can be found in the section on prebiotics.

As it can be appreciated from the previous paragraphs the chemical composition of dietary fibre varies according to botanical source [588]. Geographical origin, seasonal variation, maturity at harvest, environment and mode of commercial extraction are other factors that affect the composition of dietary fibre [558,588]. In addition, processing modifies the organization of these polysaccharides and it can produce artifacts during analytical quantification [560]. Crude fibre determination is still used to quantify dietary fibre for food labelling purposes but it recovers only variable amounts of the hemicelluloses, cellulose and lignin [559].

Dietary fibre properties

The physical properties of dietary fibre -viscosity, water holding capacity, cation exchange, organic acid adsorption, gel filtration, and particle-size distribution- are the primary determinants of the physiological effects of dietary fibre [558,561]. The disruption of the fibre structure by processing or by traversing along the gastrointestinal tract is necessary before some of these physical properties become evident. For example, mechanical shear, heat and pressure can result in cross-linking, change particle size and disrupt the fibre structure with release of starch or water-soluble components [589]. The need for disruption of the fibre before some of these properties are manifest explains, in part, why analysis of the chemical composition of dietary fibre, is an inexact predictor of the physical properties of fibre.

The solubility and viscosity of many polysaccharides are salt and pH dependent [558]. Fibres that are water-insoluble (e.g. some cereals) are relatively inert and they are fermented less by bacterial enzymes and tend to contribute less to dietary viscosity than water-soluble fibres. The effects of insoluble fibre such as cellulose on viscosity depend on fibre length [586]. Water-soluble fibres and gels (e.g. some fruits and vegetables) are more viscous, have some chemical activity in the gut lumen and are susceptible to fermentation [589]. Viscosity (resistance to flow) depends on concentration of the polysaccharide and molecular weight. The concentration of the polysaccharide can be modified inside the gastrointestinal tract and therefore viscosity is difficult to predict *in vivo* by *in vitro* assessments. Furthermore, other macromolecules present in the digesta can contribute to the overall viscosity i.e. proteins, starch and mucus glycoproteins [561].

Dietary fibre is fermented to different extents in the colon and this susceptibility to fermentation is an important property from the nutritional and therapeutic perspective. The fermentability of the different dietary fibres by the colonic flora determines their caloric nutritional value (if any). In addition, the products resulting from fibre fermentation are responsible for many of the effects of fibre in the gut. However, adaptation of the colonic flora to use dietary fibre as a fermentation substrate is an important consideration when studying dietary fibre fermentability and its consequences on bowel structure and function. The type of fibre and the amount of fibre fed are also important factors to consider [561,590]. Unfortunately, measurement of fermentability have shown large variation between animals and laboratories [591]

The susceptibility to fermentation determines, among other things, the amount of residue left in the faeces and the water holding capacity of the residue. In addition, fermentability determines the size of the faecal bacterial mass. Both, water holding capacity and faecal bacterial mass are directly related to stool weight [561,581].

The water content of a fibre can be separated in water holding capacity and water binding capacity. The first is water trapped by the fibre and depends on the physical structure of the fibre whereas the water binding capacity depends on the chemical composition and it is not readily removed [592]. Water holding and water binding capacity of a fibre varies with the method of analysis [592,593]. The relationship between the in vitro measurement of water holding capacity and the actual ability of these foods to hold water in the intestine is unknown for most foods [589] and difficult to predict with *in vitro* experimentation [581].

Another important property of fibre is cation exchange and binding. This property is the result of the number of carboxyl groups on the sugar residues and the uronic acid content of the fibre. However, there is doubt that cation binding by dietary fibre would be an important cause of nutritional mineral imbalance [589]. Adsorption of organic molecules is also known to occur in the presence of dietary fibre, specially the binding of bile acids by lignin or carcinogens by wheat bran.[589].

Nutritional value of dietary fibre and its effects on gastrointestinal structure and function

Dietary fibre has many effects on gastrointestinal structure and function. Some of the effects are well documented and others remain poorly understood or controversial [538,594]. Much research effort has been expended to harness the effects of fibre to improve health. The following is a summary of the professed effects of dietary fibre on the gastrointestinal tract.

Food intake

Modification of the fibre content of the diet can affect food intake at least in the short term [595]. Energy density and palatability are important factors influencing food intake in humans and these can be modified by the addition of fibre [596]. Fibre has been hypothesized to induce satiation and satiety by bulking and increasing the viscosity of gastrointestinal content [595,597]. Increased bulk and viscosity of the digesta reduces energy density and affects the regulatory mechanisms that determine satiation and satiety. As with many other effects of dietary fibre, the type of fibre involved and its quantity are important. Rats did not consume all the food offered when it was mixed 1:1 with ispaghula mucilage [598]. However, soluble fibre added to the water in the form of oligosaccharides and gum at lower concentrations (approximately 5-7 %DF w/w) did not alter water or food consumption in mice and rats [599]. Similarly, two diets with 5.4 %DM and 11.25 % DM of soluble types of dietary fibre (gums blend) did not modify food intake in calorie-restricted dogs [600].

Insoluble fibres added to the diet have been reported to have the opposite effect on food intake to soluble fibres. For example, increased intake has been reported in rats consuming a diet with high amounts of fibre (40% of the diet as dry alfalfa) [601]. Similar results were seen in rats fed moderately high quantities of fibre (13 to 14.4 %DM) as wheat bran, oat bran or pea fibre [590]. Adaptation to fibre supplementation occurs with time and food intake returns to baseline levels in rats [602]. Calorie-restricted dogs fed up to 11.7 %DM insoluble fibre did not show any change on food intake either [603].

Conversely, the absence of dietary fibre seems to inhibit food consumption in rabbits [572]. Similarly dogs showed lower acceptance of 0% dietary fibre diets when compared with the same diet with added fibre [588].

Body weight

Changes in body weight have been observed in humans and animals fed high fibre diets. In some studies an increase in weight has been reported whereas in others weight loss has been shown. The reasons by which high fibre diets can produce weight gain are complex. In some species, fermentable fibre may provide significant calories leading to an increase in body mass. In other cases, the increased body weight may be due to an increase in the weight of the gut contents or weight of the gastrointestinal tract itself.

The fermentation of fibre contributes to energy balance in ruminants and to a lesser extent in monogastric species (5-10% of energy requirements in men on a Western diet) [567]. This is most remarkable in patients with short bowel syndrome or severe malabsorption in

whom the importance of colonic preservation for nutritional well being has been noted [604]. However, the efficiency of dietary energy utilization is reduced in the colon. Pigs fed 9.3 and 16.3 %DM soluble and insoluble fibre respectively, showed a utilization of energy equal to 73 % of the energy obtained by enzymatic digestion in the small intestine [605].

Another reason why animals fed a diet rich in dietary fibre can show weight gain is the effects of fibre in increasing volume of digesta and weight and length of gastrointestinal organs. Weight and length changes in different parts of the gastrointestinal tract were some of the first observations made about the effects of dietary fibre on the gastrointestinal tract. Addis (1932) [601] reported increases in length and weight of the stomach and colon of rats fed a diet composed of 40% alfalfa and 10 % agar when compared with a control group. Similarly, dogs fed soluble, insoluble and moderately soluble fibres (9 to 10.5 % DM) showed and increase in colon weight as a proportion of body weight when consuming the soluble fibres [575].

Increase in the wet weight of digesta at all gastrointestinal sites with a decrease in dry weight have been shown in pigs fed a diet containing pea fibre (35 %w/w) and pectin (2.5 %w/w) [606]. In another study pigs on a high fibre (pectin and pea fibre, both rich in soluble fibre) diet (26 %DM), had also an increase in body weight when compared with animals fed a lower fibre diet (5%DM). However, after correcting for gutfill, there were no differences in the daily gain or the final weight of the pigs. This result shows the bulking capacity of dietary fibres. In addition, when the gastrointestinal tract of the pigs was emptied and weighed, the weights of the stomach and the cecum and colon were doubled in

the high fibre group. There was also a considerable increase in colonic length in the latter group as well [605].

Although fermentable soluble fibres seem to have a more pronounced effect on the weight and size of the gastrointestinal tract than non-fermentable fibres, an increase in gastrointestinal tissue weight is still seen in germ free rats fed fibre [607]. These results demonstrate that fibre has a direct trophic effect on the gut. The products from the fermentation of dietary fibre stimulate intestinal epithelial proliferation [608], thus the composition of the increase in organ weight in germ free animals may be different. Kaolin has been found to increase the weight of the colon by increasing colonic muscle weight, the same effect observed with bulky wheat bran [598]. Stark (1995) [609], found an increase in the muscular cross section of the small intestine and colon after consumption of a diet high in cellulose (15% w/w) for 8 weeks. In the same experiment, pectin caused no significant difference in organ weight yet hypertrophy of muscular cells was obvious. The latter finding is not universal since fibre sources with a high content of soluble fibres, oat bran and pectin, have been shown in another study to decrease the size of muscle cells [598].

Nonetheless, not all studies show clear differences between soluble, insoluble and moderately soluble fibres in their influence on weight gain [590]. Studies in miniature swine showed that these animals gained equivalent weight when on diets with equal quantities of cellulose, wheat bran, oat bran or corn bran [610]. Similarly, dogs fed different amounts of fibre (moderately soluble at 11%DM, or insoluble fibre at 9, 10 and 13.6%DM), in otherwise similar diets, for 3 weeks showed no significant trend on changes in body weight [588].

In contrast, some studies have reported weight loss in animals consuming high amounts of dietary fibre. The effects of life long (18 months) consumption of dietary fibre were evaluated in rats. The rats on the high dietary fibre level (13.3% w/w,) were lighter than the controls (1.7% w/w). The large intestine contents were heavier and their cercal and colonic tissue weights were higher when compared with the ones fed the control diet [602]. This effect of fibre is of particular interest in the veterinary field because of its application to the control of obesity in cats and dogs. The use of high fibre diets for weight control is controversial [611] especially if used with the aim of reducing intake and the sensation of hunger. In humans, soluble viscous fibres are considered to suppress hunger and cause greater satiety while insoluble fibres increase satiation and stomach fullness. These effects result from a reduction in energy density and palatability reducing the rate of nutrient absorption and forward movement of the digesta [595]. The role of food energy density in energy intake has been clearly demonstrated in humans [612]. However, the long-term control of body weight by increasing the consumption of dietary fibres in humans is still unclear [595]. Several studies have reported on the dietary intake in dogs fed different amounts of dietary fibre. Dogs did not show a reduction in food intake when eating a diet high in dietary fibre if they were energy restricted [597]. However, dogs consuming diets with 12 to 21 %DM of insoluble dietary fibre did show an increase in satiety, and decreased daily energy intake [611]. No similar studies have been carried out in cats. However, cats did not modify their food intake when soluble and insoluble fibres at approximately 11%DM were added to a basic diet [613].

Morphology of intestinal mucosa

Changes in intestinal morphology and morphometry of the small and large intestine have been observed in animals consuming diets with different types of fibres [614]. For example, rats fed 10 % cellulose or pectin for 6 weeks showed an increase in the height and width of intestinal villi (associated with an increase in the number of intestinal cells) when compared with rats fed a fibre free diet [615]. However, an earlier study by Schwartz and Levine, (1980) [616] found no morphological differences (mucosal thickness, villus height, crypt depth and villus/crypt ratio) in the proximal jejunum of rats fed 10% cellulose or 5% pectin (w/w), in comparison to rats fed a control diet. Rabbits also show marked variation in villi morphology when given different levels and sources of crude fibre [572]. In addition, pectin feeding has been associated with villi shortening [617] and crypt elongation in rats [617,618].

The morphology of the large intestine has also been affected by dietary fibre. For example, the crypts of miniature swine were deeper in the cecum when bean fibre was fed compared to the feeding of a wheat bran and potato diet. [610]. In contrast, the distal colon of these pigs showed deeper crypts with the bran diet. Fermentable oligosaccharides (xylo-oligosaccharides and fructooligosaccharides) and gum arabic produced modest to no effects on the depth of the colonic crypts in rats and mice [599]. In rabbits, a diet with 14.5 % crude fibre (CF) (mainly alfalfa meal) resulted in damaged cecal mucosa cells. Conversely, a level of 5.5 % CF (mainly wheat bran) in the diet produced a flattened colonic mucosa [572].

Morphological changes in the intestinal mucosa are considered intricately related to epithelial proliferation. [85]. In the small intestine it is accepted that villus morphology

depends on the cell turn over in the crypts, any factor that affects the multiplication of crypt cells would alter villus morphology [619]). However, dietary fibre has not been found to affect epithelial proliferation rate in most of the length of the small intestine [608] and hence morphological changes cannot be explained by an increase in proliferative activity of the epithelium.

Changes in morphology and/or morphometry have been accompanied by signs of mucosal disturbance in the jejunum and colon of rats [573], jejunum of pigs [574], colon of dogs [575] and jejunum, cecum and colon of rabbits [572] after fibre consumption. The normal elimination of cells at the villus tips does not produce breaks in the epithelial barrier [573], but in all the latter reports loss of epithelial cells and denudated villi or intestinal folds were seen. In dogs cellulose (as opposed to pectin/gum arabic and beet pulp) caused the most damage. In rats, pigs and rabbits alfalfa most seriously affected the mucosa. The magnitude of mucosal surface cell damage has been advanced as being directly related to the ability of some fibres to bind bile acids, which are known to be injurious to the epithelial cells [573.614].

In none of these reports, the morphological changes observed after dietary fibre consumption have been studied in terms of how they affected gastrointestinal function, if they did at all. Therefore the significance of these observed changes remains uncertain.

Proliferation of intestinal epithelium

The everyday proliferative response of the intestinal epithelium is compromised without exposure to intestinal luminal contents [293]. This response is seen in the small as

well as the large intestine, although it occurs more slowly in the latter [619]. Furthermore, intravenous nutrition does support a proliferative response in the gut [309], and a fibre-free enteral diet produces very little proliferative activity in the colon [598,620].

The mechanisms by which the presence of digesta stimulates the proliferative response are direct as well as indirect (through the activity of enteroglucagon and other hormones) [598,619,621]. Goodlad et al (1987 and 1989) [598,607] demonstrated that different fibres promote epithelial proliferation in different sites of the gastrointestinal tract.

The proliferative response of the gastrointestinal epithelium to dietary fibre is thought to be a response to the products of fermentation of dietary fibre, specifically, short chain fatty acids (SCFA)[622.623]. This hypothesis is supported by the finding that germ free rats fed a mixture of dietary fibres did not show any proliferative response [607] until their colon was irrigated with SCFA and then a normal proliferative response was obtained [622]. However, cell proliferation studies of the cecum of miniature pigs showed no significant correlation between the amount of soluble fermentable fibre and cellular proliferation [610].

The individual effects of SCFA (acetic, propionic and butyric acid) have been explored as has the effect of the mixture of SCFA usually present in the gut contents. Butyric acid has shown the highest proliferative effect, followed by propionic and acetic acid [622,623]. On the other hand, some reports have not shown a high correlation between the concentration of SCFA and the mucosal growth at the same site [624,625]. However, the value of measuring SCFA concentration in faeces or intestinal digesta is equivocal because most

of the SCFA produced in the intestinal lumen are absorbed through the colonic mucosa [626,627].

Jacobs and Lupton (1984) [628] highlighted the difficulties of trying to understand the effects of dietary fibre on epithelial proliferation parameters. Interpretation is difficult if mucosal weight, DNA mucosal content, crypt length and width, total number of epithelial cells per crypt, number of mitotic figures per crypt, mitotic index and size and position of the crypt proliferative zone are measured in the same sample to determine proliferative response [619]. Techniques that assess a crypt as a unit [629-631], and not a crypt section [632], have definitive advantages. Assessment of the size of the proliferative zone [633] can be misleading when crypt sections are used as can counting the number of dividing cells [619,631]. However, newer techniques with superior image analysis tools such as confocal microscopy [633] along with crypt microdissection [634] may improve our understanding of the kinetics of the intestinal epithelium and better define therapeutic strategies.

Epithelial proliferation can also be affected by other luminal factors like calcium, bile acids, long chain fatty acids [442,625,635,636] and ammonia [637] that can confound the study of dietary fibre and its effects on epithelial proliferation.

A summary of the effects of fibre on the mucosal proliferative response in different regions of the gastrointestinal site follows:

Stomach: Goodlad (1995) [608] reported increases in crypt cell production rate (CCPR) in the stomach of conventional and germ free rats that had been fed with an

elemental diet supplemented with 30% dietary fibre (a mixture of soluble and insoluble fibre). The effect was more marked in the fundus than in the antrum. The stimulus for this change is unknown since the diets had been sterilized prior to feeding to the germ-free rats and both conventional and germ-free groups of rats showed similar changes. These circumstances make fermentation of food an unlikely cause of the proliferative response observed. This effect of dietary fibre, regardless of its solubility and fermentation characteristics, on the proliferative activity of gastric epithelium had been reported before [638]

Small Intestine: The proliferative response of the small intestinal mucosa seems to be different according to the type of fibre fed. Starved rats have shown a higher CCPR response to feeding with a fibre-supplemented diet (30 to 50% w/w of a mixture of soluble and insoluble fibres) than the rats fed a control diet. The response was seen only in the more distal parts of the small intestine. Interestingly, the increase in CCPR response was not observed with an insoluble fibre like cellulose or with ispaghula husk mucilage [598,607,608]. Similarly, wheat bran (contains mainly insoluble fibre) has also been reported to not produce any proliferative response in the jejunum of rats [617] whereas pectin and guar (soluble fermentable fibres) have been reported to increase proliferative activity [617] in this region of the bowel. It is uncertain how the proliferative response originates in the cranial parts of the gut.

Large Intestine: It is in the large bowel where the effects of dietary fibre on dietary proliferation have been most extensively studied. Feeding rats a diet with 50% w/w dietary fibre caused an increase in crypt cell proliferation rate in the proximal, middle and distal

colon [598]. In this experiment the fibre fed was a mixture of soluble and insoluble fibre derived from wheat grain (hemicellulose 40%, cellulose 20%, lignin 15% and pectin 5%) with the effect being more pronounced in the distal colon. The distal colon also showed increased CCPR after cellulose and ispaghula mucilage were fed [598]. Wheat bran has been associated with increase proliferative activity in the proximal and distal colon [639]. This observation is in agreement with the finding that wheat bran produces high amounts of SCFA throughout the large bowel of the rat [640].

Jacobs and Lupton (1984) [628], tested the effects of pectin (10% w/w), guar (10% w/w) and oat bran (20% w/w). They did show changes in several mucosal growth parameters. The two highly fermentable dietary fibres (pectin and guar) showed their effects mainly in the cecum -guar with more startling results- although guar also produced significant changes further distally. The oat bran showed the majority of its effects mainly in the distal colon, but the net mucosal colonic growth (measured by mucosal wet weight, DNA and RNA content) was nil. Zhang (1994) [625], showed similar results in rats consuming pectin or oat bran at 6% w/w.

Similar results were obtained in miniature pigs when fed different types of fibre at 7% total dietary fibre w/w. The diets with higher soluble fibre (red kidney bean and oat bran) showed changes in the cecum proliferative parameters, while wheat bran and corn bran (at 13%w/w) produced changes in the distal colon [610].

On the other hand, other sources of fermentable fibre like fructo-oligosaccharide, xylo-oligosaccharide and gum arabic at approximately 5% of dietary intake had modest

effects on extent of proliferation zone (PZ), cell density and labelling index in the rat cecum and distal colon [599]. Reduced proliferative response to the supplementation of 1.5% w/w fructooligosaccharides compared to a control diet has also been reported in the dog [641].

Lastly, it is worthwhile considering the long term significance of the proliferative effect of fibre on the large intestinal epithelium. The importance of adaptation in reducing variability of fermentation of soluble fibres has been noted [642]. Adaptation in the long term to the stimuli that causes increase epithelial proliferation when consuming dietary fibre may decrease the response to dietary fibre supplementation. Edwards et al (1992) [602] found very little differences in colonic cellular proliferation labelling index in rats that had consumed diets with 1.7 or 13.3 % w/w dietary fibre (0 70% and 2.3% soluble fibre respectively) for 18 months [602]. Nonetheless, the acute and subacute effects of dietary fibre remain beyond doubt. Although many gastrointestinal functions depend on a healthy epithelium, how the proliferative response associated with the consumption of dietary fibre translates into improving gut physiology has not been studied.

Nutrient absorption and absorption of luminal compounds

The morphological changes produced in the small intestine by the consumption of dietary fibre suggest that nutrient absorption could be affected by virtue of the proliferative effects on the epithelium and the increase in mucosal surface area. However, other physiological effects of dietary fibre could also indirectly affect the absorption of luminal contents. Delayed gastric emptying and modified transit time [643], changes in the viscosity of the digesta [538], altered mucin production [644] or direct binding of nutrients by the dietary fibre [620] have all been implicated. In addition the volume, bile acid and enzymatic content

of biliary and pancreatic secretions increase with the consumption of viscous dietary fibre [645] but the cause of this increase and the functional implications of this change are not known.

An increased influx of nutrients has been observed in *in vitro* preparations of mid-jejunum after rats consumed a diet rich in fibre (10 % cellulose or 10 % pectin w/w) for four weeks [615]. However, not all nutrients (hexoses, beta-amino-butyric acid or sodium) showed the same magnitude of response. This suggests that the alterations in small intestine morphology were not solely responsible for the increased influx, unless the functional immaturity of the new enterocytes meant they did not possess a complete set of transport systems [646].

Schwartz and Levine (1980) [616] studied the effect of soluble and insoluble dietary fibre (10%w/w cellulose or 5%w/w pectin) on glucose absorption without adaptation (acute experiment) or after rats had been fed the fibre for 5 weeks (chronic experiment). In the acute experiment the viscosity of the mixtures of glucose and fibre was measured and a special control group with similar viscosity to the pectin/glucose mixture was included (gelatine/glucose). No differences in glucose absorption from the perfusate could be demonstrated between the rats fed the pectin, the gelatine and the fibre free solutions. Thus, viscosity did not impair absorption of glucose in this model. In the chronic experiment adaptive changes were ob served after consumption of pectin or cellulose that decreased the absorption of glucose compared to a fibre free diet. However, an improvement in glucose tolerance (curve of serum glucose concentration after glucose ingestion) occurred with pectin consumption regardless of the duration of fibre consumption. Cellulose, on the other

hand, produced increased tolerance to a glucose load in rats only after chronic consumption. Therefore it was proposed that the immediate effect of pectin may have been based on delayed gastric emptying or changes in transit time. An improvement of glucose tolerance with long term ingestion of cellulose or insoluble fibres has also been seen in diabetic cats and dogs [647,648].

Viscosity of chyme has been thought to prolong gastric emptying and retard mixing and diffusion of substrates and enzymes [561] but the role played by gastric emptying in the improved glucose tolerance is controversial [643]. It has been shown in humans that moderately fermentable [649] and fermentable fibres (ispaghula) increase the stationary activity of the jejunum, and the proportion of this type of activity correlates well with the area under the curve of plasma glucose concentration [650]. The increase in stationary activity would explain a reduction of stirring next to the mucosal surface and decreased access to the absorptive mucosa. Another possibility is that the polymer network of soluble gelling fibres acts as a molecular sieve trapping the glucose [651]. This seems unlikely because these fibres did not retard the movement of glucose from dialysis bags [650].

Increased secretion of luminal mucin may account for the delayed or decreased absorption of nutrients when dietary fibre is consumed. Sharma (1995) [652] showed that mucin secretion changes quantitatively and qualitatively with different diets. Although the nutritional analysis of diets was not disclosed, viscosity was thought by the authors to cause the differences seen. Rats fed 5 % citrus fibre (25% insoluble cellulose and lignin) or guar gum (viscous soluble fibre) for 4 weeks showed a statistically significant increase in luminal mucin in the small intestine with no change in the amount of tissue mucin, when

they consumed citrus fibre. This indicates that the insoluble fibre increased the rate of release of mucin that could affect the composition and resistance of the unstirred water layer (UWL) and its resistance to the transport of nutrients [644]. It has been reported that the thickness of the UWL is related to the amount of stirring produced by intestinal motility. The thickness of the UWL decreases when there is normal motility [653]. Therefore, insoluble fibres may affect the composition of the UWL, while soluble fibres may increase UWL thickness by reducing stirring. In both cases absorption of nutrients would be affected.

The same factors that affect glucose absorption in fibre-containing diets may influence when considering the digestion and absorption of fats. A decrease in the rate of digestion and/or absorption of fats was seen in rats that had been fed cellulose, guar and chitosan along with different types of dietary fats. In this study, guar and chitosan delayed the absorption of triglycerides and cholesterol when compared with cellulose [654]. Vahouny (1988) [655], showed similar results in rats adapted to dietary fibre. Dietary fibre has also been used to reduce cholesterol serum levels and to favourably modify lipid metabolism, although the results with different types of fibres have been quite variable [581]. These effects of fibre on cholesterol have been ascribed to bile acid binding properties In general, soluble viscous fibres and lignin (but not other insoluble fibres) bind bile acids and increase their faecal loss, sometimes decreasing cholesterol serum levels at the same time [538,581 558].

Lastly, dietary fibre has been observed to affect nitrogen elimination. Soluble fibres, such as pectin, guar and resistant starch, have been reported to increase net nitrogen elimination by increasing the absorption of ammonia from the large intestine, to a lesser extent than the urea flux from blood into the large intestine in rats [656]. The use of dietary

soluble fibre has been recommended in companion animals with hepatic disease to facilitate the elimination of urea and trapping of ammonia (mediated by a reduction in pH or by bacterial protein synthesis) [657].

Digestibility of macronutrients

When diets high in dietary fibre are consumed, a decreased digestibility of the macronutrients in the diet has been observed. However, the decrease in digestibility of the macronutrients does not seem to be due to a lack of digestive enzymes. The activity of pancreatic enzymes present when consuming a fibre rich diet does not decrease and there is conflicting data on the activity of intestinal brush border enzymes when consuming dietary fibre [645.658].

Rats fed pea fibre, oat bran and wheat showed lower *apparent digestibility* values of DM, energy and protein when compared with controls [590]. Protein digestibility was the lowest in the oat bran group. Nevertheless, the significant quantities of soluble dietary fibre present in some of these fibres will result in an increase of the bacterial faecal mass and of the protein of bacterial origin in faeces influencing the calculation of apparent digestibility.

On the other hand, pigs fed different fractions of oat bran (4.5 to 6.4 % dietary fibre) for 7 days showed decreased *true digestibility* (5-8 % for energy, 3-9 % for protein and 4-12 % for fat) of all nutrients at the terminal ileum, compared with pigs fed a control low dietary fibre diet. Starch, however, had been nearly always completely digested at the level of the ileum. Faecal digestibilities showed a change of lesser magnitude (1-3% for energy, 1-5% for protein and 3-6% for fat) but in the same direction [659]. Similar results were

obtained when pigs were fed pea fibre or pectin, although the differences in digestibilities were larger [605].

In dogs the *apparent digestibilities* of dry matter, organic matter, protein and fat decreased with increasing dietary level of beet pulp fibre (0, 2.5, 5.0, 7.5, 10.0 or 12.5 %DM) [660]. However, beet pulp at 7.5 % decreased digestibility of dry matter and organic matter by 4 % while N and ether extract digestibilities were not very different from the control diet. Higher levels of inclusion of the same fibre though decreased the apparent digestibilities of N and lipids. Starch was always completely digested. All these differences were statistically significant but never very large. In another study several fibres (beet pulp, tomato pomace, peanut hulls, wheat bran and treated wheat straw at 11-13%DM) were tested in dogs to study the effects of fibre on digestibility of main nutrients. In this instance digestibilities were reduced compared to the control group but, although statistically significant, no large differences between diets were found [661]. The presence of oligosaccharides (raffinose and stachyose) in different by-products of soy at less than 1% w/w also caused a small reduction in the digestibility of dry matter, protein, fat and energy in dogs [662]. More recently the effects on digestibility of the dietary ratio of insoluble to soluble fibre have been studied in dogs [663]. This approach is sensible because most fibres have a mixture of both types of polysaccharides. It was reported that higher ileal digestibilities were observed when the ratio insoluble:soluble fibre was 1.9 or 7.2 (7.5% total dietary fibre content).

As a summary, a consistent decrease in apparent and true digestibility has been reported in different species when consuming different types of fibres. However, this

decrease has always been minor and does not justify avoiding the inclusion of fibre in the diet, especially when considering all the other potential benefits that dietary fibre can offer.

Fermentability

By definition dietary fibre is indigestible in the small intestine, and is the main substrate of colonic fermentation by the colonic flora [664]. Many of the biologic effects of dietary fibres depend on the degree to which they are fermented. Fermentability determines the utilization of the fibre (and its energy value), the amount of SCFA produced and hence modifies the degree of mucosal proliferation in the gastrointestinal tract, the microbiological environment, and the faecal bulking effects. In turn, many factors affect fibre fermentability, namely the extent of lignification of the cell wall polymers, the amount of associated silica and other indigestible substances, solubility, particle size, chemical composition, degree and type of processing, and the gut transit time [523]. Because of the number of factors that can affect fermentability in the colon, it is difficult to accurately predict the extent of fermentability *in vivo*.

In general, different types of fibres ferment to different extents according to their chemical structure. On average cellulose is fermented 30 to 50 %, hemicellulose 50 to 80 % and pectin and gums 90 to 100 % [665]. Variation in fermentability of poorly and highly fermentable fibres has been reported in rats [591]. In humans, individual variation caused up to a 30% difference in the fermentability of cellulose [666].

Livesey et al (1995a) [591] found that the fermentability of pectin, sugar-beet, soybean, corn bran and cellulose was not affected by the level of fibre included in the diet (5 to 10 %

w/w consumed for 2 weeks). Nyman and Asp (1985) [642] reported similar findings in addition to the finding that fermentability did not vary significantly with the length of the adaptation period or particle size of the fibre. However, these variables were found to affect the interindividual variability and therefore they do affect fermentation to certain extent. The content of protein in the diet seemed to be a limiting factor for fibre fermentation when the dietary content protein is lower than 10%w/w. However, increases in protein level above 10% do not produce further increases in fermentability [642].

Faecal inoculum has been used in several species to gain insight on the fermentability of dietary fibre. Bourquin et al (1992) [667] found a high degree of interindividual variation in the extent of substrate fermentation and products of fermentation when using faeces from different individuals. Sunvold et al (1995 and 1995a) [613,668], compared the *in vitro* fermentation of isolated dietary fibres and fibre blends by feline and canine faecal inoculum with the apparent digestibility *in vivo* of the same fibres. It was reported that *in vitro* fermentation was a reasonable predictor of *in vivo* fermentation but not a very accurate predictor of the digestibility of fermentable fibres.

Microbial colonic flora

The colonic flora is composed of a vast array of species that require different substrates for their subsistence. Dietary fibre influences bacterial mass, but this does not result necessarily in a change of the types of bacteria present [561,659]. Any species changes that occur with diet tend to be short-lived [247]. Changes in enzymatic activity of individual bacterial species do occur and they determine the fermentative activity of the colon [522]. The type of enzymatic mechanism used by a bacterial species to ferment a particular

polysaccharide usually depends upon the type of polysaccharide being fermented (substrate) rather than the isolated bacterial species present in the colon [666,669]. However, variation in the ability of microbial fermentation of different individuals has also been reported [666]. Unfortunately the methods to study bacterial flora are imperfect [270] and this has hindered our understanding of the relationship between gastrointestinal flora and dietary fibre.

Another effect of dietary fibre on the bacteria-host relationship is the ability for non-fermentable, bulk forming fibres to reduce bacterial translocation from the gut to the splanchnic viscera, while fermentable fibres have no effect [670]. Since there is considerable debate on the significance of bacterial translocation in humans [279,671] and companion animals [672] and the mechanism for this effect of dietary fibre is unknown, the value of dietary fibre in situations of gastrointestinal injury that could increase bacterial translocation is uncertain.

Fermentation products - SCFA

The main products of large bowel bacterial fermentation of dietary fibre are short chain fatty acids (SCFA), mainly acetate, propionate and butyrate. Some branched-chain SCFAs are also found in the colon but they originate from the fermentation of protein [523,567]. These organic acids, especially butyrate, are thought to mediate at least partially the proliferative effects of dietary fibre in the colon [622]. Epithelial cells of the colonic mucosa obtain 60 - 70 % of their energy from bacterial fermentation products generated in the colon [523,627]. Butyrate is considered the preferred fuel for the colonocyte, although acetate and propionate can also be utilized. Because of their importance as a metabolic fuel and because they increase colon blood flow [641,673], SCFA have been used therapeutically in

humans. Butyrate enemas did improve experimental colitis in rats [674] and humans [675]. SCFAs also promote water and sodium absorption from the colonic lumen [523,627,676] possibly by activation of sodium transporters stimulated by intracellular acidification [677]. This effect of SCFA on colonic absorption of water and sodium is one of the reasons dietary fibre supplementation is thought to reduce diarrhoea during enteral feeding in human patients [678]. However, SCFA absorption is not solely responsible for this effect as more recently another mechanism of dietary fibre improving absorptive function in the small intestine has been discovered. Gum arabic shows a pro-absorptive action based on scavenging NO and reversing its pro-secretory activity [679].

The source of fermentable material and the rate of hydrolysis modify the molar ratios of SCFA within the colon [680]. The typical SCFA molar ratio produced by colonic fermentation is 60:25:15 for acetate, propionate and butyrate respectively [681]. However, it has been shown that resistant starch produces a mixture of organic acids with more butyrate and less acetate whereas lactulose produces more acetate than other fermentable substrates [567,584]. The concentrations of SCFA at different sites of the colon have been examined in rats [602,640] and pigs [659,682]. The highest concentrations of SCFA are always found in the cecum where the bulk of fermentative substrate first arrives. SCFA concentrations decrease progressively along the colon. Slowly fermented fibres have more influence in the distal segments because they increase SCFA production throughout the colon. Unfortunately, no correlation has been found between the concentration of SCFA in cecal contents and faeces [640]. The efficient absorption of SCFA in the colon (90 - 95 % of SCFAs produced) [627], prevents the use of SCFA concentration in faeces as a marker of colonic fermentation.

Butyrate has been shown to regulate the expression of tumour repressor genes and hence its provision by adding dietary fibre has been considered desirable [683,684] especially to decrease the risk of colon cancer. However, the paradoxical effects of butyrate *in vitro* and *in vivo* have complicated the understanding of how butyrate affects colonic epithelial proliferation and differentiation [683,685].

Fermentation Products - Breath Hydrogen Production

Dietary fibre fermentation results in the production of gases in addition to SCFA. These gases include hydrogen, methane and carbon dioxide [567]. However, hydrogen gas does not seem an important by-product of dietary fibre fermentation. For example, administration of dietary fibres (cellulose, raffinose, pectin, hemicellulose) of different fermentability to human volunteers produced very small amounts of H_2 when compared with that produced by administering lactulose [686]. In contrast, in another study (in which three weeks of adaptation to the fibre-containing diet was provided) an increase in hydrogen production was observed with gum arabic and raw carrot but not with wheat bran, gum tragacanth, gum caraya or potato fibre [558]. Similarly, rats fed gum arabic needed 28 days before they began to produce some hydrogen gas and methane after consumption of gum arabic [687]. As mentioned before adaptation is important when assessing fibre fermentation [642].

Motility

Dietary fibre affects gastrointestinal motility and transit time, but the effect varies with the fibre and contradictory studies have been reported. The influence of fibre on motility varies in different regions of the gastrointestinal tract and the effects of fibre in

colonic motility may counteract the effects of fibre in the proximal sections of the gastrointestinal tract. As a result, total gut transit time (a summation of the motility in the different regions of the gut) gives little indication of the effects of fibre on the motility of the gastrointestinal tract.

Several physical characteristics of dietary fibre are responsible for its effects on motility (i.e. viscosity, water holding capacity) but it has been reported that fermentable material and type of fermentation can also affect colonic motility [688]. While SCFA maintained colonic motility (duration of contraction during the observation period), acid fermentation to succinic (and possibly lactic) acid, which occur with an increase rate of colonic entry of fermentable substrates at a low pH, reduced colonic motility. Further evidence on the effects of SCFA on the contraction of smooth muscle in the canine colon has been also recently reported [689]. Butyrate, but to a lesser extent propionate and acetate, elicited contractions of longitudinal colonic smooth muscle in an *in vitro* preparation.

Soluble fibres that form viscous solutions slow the emptying of liquids from the stomach [690]. In contrast, this type of fibre can speed the emptying of some solid foods from the stomach because the emulsification action of the fibre helps keep dense solid particles suspended in the flow of liquid chyme leaving the stomach [643]. In the proximal small intestine, viscous fibres do not change the rate of passage [643]. The reason for this lack of effect is thought to be because of dilution of gut contents with intestinal secretions (concentration is an important determinant of viscosity) [643]. Conversely, viscous dietary fibre slows down the passage of gut content along the caudal small intestine where the digesta concentrates because of fluid absorption in the previous segments [643,691].

Insoluble fibres do not seem to alter the gastric emptying of fluids but there is conflicting information about their effect on the emptying of solids [643,690]. In the small intestine, only insoluble fibres with high water holding capacity can change transit time by intestinal distension [650]. The distension caused by ispaghula or sugar beet (as compared to wheat bran) affects the ratio of propagatory to stationary contractions decreasing transit time, mixing and time for digestion and absorption [650]. In the dog cellulose increased small intestinal transit but wheat bran had only a very modest effect in altering transit time in the jejunum [691].

However, changes of contractile activity do not equal movement of digesta and the relationship between transit time and gastrointestinal contractile activity is complex [691]. Moreover, transit time is very variable in humans and dogs, varying in different days even when the same individual is consuming the same diet [692,693]. The transit time of dogs consuming beet pulp (11.2 %DM), or oat fibre (8.9, 10.0, 13.6 %DM) were not significantly different [588]. In humans fed for 3 weeks 14 g of cellulose and 6 g of pectin only the insoluble fibre cellulose induced a significant decrease in transit time [694]. The same results were obtained in people consuming dietary fibre in the form of vegetable, fruits and cereals when compared with a group consuming very low amounts of fibre in their natural diet. The group consuming more dietary fibre showed a significant reduction in total transit time [695]. Transit time in the dog decreased with cellulose supplementation at 11 and 14 %DM. [692].

Bueno et al (1981) [691] studied the relationship between contractile activity and digesta flow in the small intestine of the dog. The dogs were fed a standard ration with gum, wheat bran or cellulose added and the authors noted an increase in the duration of small intestine postprandial activity, especially with cellulose. The increase in postprandial activity was accompanied by a general increase in contractile activity. However, the digesta flow rate was reduced for cellulose and bran and increased 66% for gum, which can hold 3 to 4 times more water. Hence this water trapping can explain the considerable increase in flow when compared to the other fibres in the presence of similar increases in postprandial activity. In fact all fibres increased mixing activity and slowed propulsive activity. The motility index was only increased for gum but not for the other fibres (index of contractile force generated per time unit) and was based on low grade constant activity possibly due to the significant distension produced by this type of fibre. Somehow opposing results have been found in humans in which ispaghula and sugar beet decreased stationary activity and increased propagating activity, while wheat bran had no effect [650]. These subjects had an intraluminal probe as opposed as intramural chronically inserted transducers, which could have affected motility; but also had consumed a much lower dose of dietary fibre.

The study of the effects of cellulose on cranial colon motility in the dog [696] demonstrated a dose related effect reducing duration of spike activity and thus affecting the duration of the migrating spike bursts (MSB). The duration of MSB was negatively correlated with faecal weight, water output and defecation frequency and therefore a reduction in duration of contractile activity was associated with faecal bulk and distension of the colon. Results of myoelectrical activity in pigs are contradictory to these findings because there was an increase in spike activity when bran was added to milk, transit time

was decreased and faecal water content increased [697]. The nature of the diet and the different gastrointestinal tract of pigs may have made the difference.

Motility abnormalities have been considered central to the pathophysiology of ulcerative colitis and alterations in the ultrastructure of the interstitial cells of Cajal (colonic pacemakers) have been reported in severe ulcerative colitis [698]. It is uncertain if this damage is primary or secondary but decreased motility has been reported in cats and dogs during colitis [699,700]. Daniel (1975) [701] reported a lack of phase locking during the production of control potentials in the colon of cats with spontaneous diarrhoea with no change in velocity or frequency or spiking. Humans with active ulcerative colitis showed no changes in gastric emptying, but a reduced small bowel transit and cranial colonic transit. It was only in the caudal colon and rectum that a rapid transit occurred possibly due to increased irritability [702]. Burrows (1982) [692] mentioned a 'normalizing' effect of cellulose in dogs transit time of healthy dogs. This 'normalizing' effect may also prove beneficial in addressing the dysmotilities of colitis but this benefit remains unproven.

Faecal bulking

Faecal bulking is the oldest known effect of fibre. A treatise on the characteristics of human excreta, and possibly the antecedent of a faecal grading system, was published in 1733 from which the link between the type of faeces, social class and diet could be inferred [562]. Of the dietary components, only dietary fibre has a significant effect on stool weight [561].

Insoluble fibre is poorly digested and survives passage through the colon. It carries water inside its cellular structure and as a result increases faecal volume. Soluble fibre, on the other hand is fermented by the microflora, stimulates bacterial growth and increases bacterial faecal mass. The conversion of substrate to bacteria in an anaerobic environment is only about 30 - 35 % w/w. In dogs fed beet pulp 7.5 % DM there was an increase in the amount of wet faeces compared with the control and the content of DM was decreased. The addition of 2.5, 5 or 7.5 %DM oat fibre (no soluble fraction) increased the weight of faeces but did not modify greatly the faecal DM content [588]. Some rapidly fermentable fibres such as pectin have very little effect on faecal weight [558,694,703]. Similarly, fruit and vegetables are unpredictable in their effects on bacterial mass and faecal weight [558]. The bulking activity of fruit and vegetables does not correlate with their water holding capacity. Cereal fibres on the other hand depend on this characteristic to be good bulking agents [592].

The effect of the products of fermentation on faecal mass is yet undefined. Slowly fermentable fibres may increase faecal bulk by production of SCFA in the lower colon that are not absorbed and retain water in the faecal mass [561,703].

In veterinary science and more specifically in the pet food industry, faecal characteristics are an important consideration when designing diets because of the impact this has on petfood purchasing preferences. Therefore, dietary fibre supplements and dietary fibre additives (gums mainly) have to be carefully screened in the species to which they will be fed for amount of faeces produced and faecal characteristics.

The cat and dietary fibre: what do we know so far?

Dietary fibre is not traditionally considered an important component of the diet of strict carnivores like the cat. However, in the wild, felids consume entire carcasses including the abdominal organs usually filled with semi-digested plant material. Acceptance of a diet of meat was high in cats even when wheat bran, cellulose, horn meal, feather meal, rumen content, grass meal, raw potato starch and raw corn starch were added at 5, 10 or 15 % total weight [704]. These are all highly indigestible fibres and materials that could potentially affect palatability and texture of the food, both of which are important determinants of food consumption in the cat [345]. Peat, dried apples, pectin and dried sugar beet pulp added at 5 % total weight were refused by cats [704].

Several sources of dietary fibre (cellulose, wheat bran, rumen content and grass meal) decreased apparent digestibility of organic matter and protein (except cellulose), fat and digestible energy which was in proportion to the amount of crude fibre added [704]. Decreased digestibilities had been observed before when studying different types of cat food [570]. Canned foods were found to have a lower digestibility than dried and semi-moist foods. It was suggested that this effect was due to the addition to the canned diets of carrageenans, carob gums and soy oligosaccharides as gelling agents.

Cat faeces have been shown to contain microorganisms that can ferment dietary fibre [613]. Strictly speaking the presence of these microorganisms does not prove that cats can derive the same benefits from dietary fibre utilization than other species. *In vitro* fermentation of fibres with faecal inoculum has not been validated. Intestinal microflora

changes along the colon in accordance to their capacity to metabolize different substrates and also in relation to the decrease in fermentable substrates along the colon [253,523]. Therefore, although in vitro testing indicates that cats potentially may have the ability to ferment dietary fibres, *in vivo* testing is required to confirm *in vitro* findings and establish the effects of dietary fibre.

Sunvold (1995) [705], demonstrated that cats can ferment different types of fibres, although the digestibility of soluble fibres was poor compared with other species, such as dogs and pigs [588,605]. Soluble fibres (mainly different types of gums) did cause higher faecal output and markedly reduced digestibility of nitrogen, lipids and energy. Interestingly there was a high correlation between the results of the *in vitro* fermentation and *in vivo* digestibility tests when fermentation of insoluble poorly fermentable or moderately fermentable fibres (beet pulp) were used, but not when soluble highly fermentable fibres were involved [613]. This supports the requirement for measuring fermentability of dietary fibre *in vivo*.

No model of colonic fermentation is available for studying the processes that dietary fibre undergoes in the colonic environment. Colonic perfusion studies allow the study of the effects of dietary fibre in colonic structure and function but these studies are only available as a research technique since they are quite invasive and therefore not suitable for use in clinical cases of gastrointestinal feline disease. Bueno (2000a and 2000b) [706,707] used this technique to assess the effects of dietary fibres of different fermentabilities (cellulose, beet pulp and pectin/gum arabic at approximately 8.5% as fed) on the colon of healthy cats. There was a definitive difference between the control diet and all the diets with dietary fibre

added. All diets supplemented with dietary fibre increased colon weight and decreased surface:mass ratio of the colon. Unfortunately, no clear division between the different fibres was found regarding transport of SCFA, faecal bacterial counts, colonic weight per kilogram of body weight, electrolyte and water transport or colonic surface:mass ratio. Moreover, the control diet (no fibre) seemed to behave in a similar fashion to the highly soluble and fermentable pectin/gum arabic mixture regarding SCFA production and faecal bacterial count. Although the group of cats on the pectin/gum arabic mixture consumed less food and lost weight during the experimental period, they, on average, still consumed more dietary fibre than the control group. Mucosal DNA content was similar for all diets including the control diet with no fibre, but mucosal oxygen consumption was increased for most fibres, especially the pectin/gum arabic mixture. Morphological differences were non-apparent between any of the diets. Crypt depth was lower in the distal segment of the colon than in the proximal colon irrespective of diet but this finding has been reported as a normal feature of feline colon [16]. Unfortunately, statistical analysis is not well described, and it is uncertain if allowance for multiple comparisons has been considered. As a summary, from this study it can be said that dietary fibre has been shown to produce an increase in colonic weight in the cat most likely due to muscular hypertrophy since there was no increase in mucosal DNA content to support an increase in mucosal cells. The increase in mucosal oxygen consumption indicates an increase in metabolic activity. This may be due to the utilization of fermentation products like SCFA, which would be more abundant with any fibre (when compared to the control diet) but especially with highly fermentable fibres like pectin/gum arabic mixture. However, the functional significance of a fermentable substrate in the cat colon is still in question.

Earlier studies had shown that the addition of dietary fibre to the diet (beet pulp) causes a change in the capacity of feline faecal inoculum to ferment dietary fibre [705] possibly by changing the quality or quantity of the bacterial flora or their metabolic capacities. This was further confirmed in more specific studies in which feline faeces were cultured before and after the addition of lactosucrose [708] or fructo-oligosaccharides [534] to the diet. These studies showed increases in *Bifidobacteria* spp., *Bacteroides* spp. and *Lactobacilli* spp., and reductions of *E. coli* and *Clostridium perfringes* but these changes were not always statistically significant. The functional significance of these changes in bacterial flora in cats is unknown and controversial at best in other species. In addition, these cats may not have been a good model to study the gastrointestinal flora of common veterinary patients as some of them were Specific Pathogen Free (SPF) cats-barrier maintained. Itoh et al. (1984) [709], compared the flora of several groups of laboratory cats, some SPF and some conventionally kept. He did found differences in the bacterial species present in the faeces between the SPF and conventional cats. Similarly, it is possible that differences occur with different feeding habits, as it has been shown in rats and fowls [710], which could be an important difference between public owned cats and research cats (ad-libitum feeding vs. meal feeding). However, a more recent report found no difference between research cats and privately owned home cats [711].

The use of prebiotics in humans has been developed with the aim of increasing the number of *Bifidobacteria* in the colon to take advantage of the purported benefits of these bacteria. However, as in the dog [520], *Bifidobacteria* have not always been isolated from the feline gut [518,708,709,712]. Nevertheless, one report suggests that lactosucrose appears to be able to promote the proliferation of Bifidobacteria in the feline colon [708].

Sparkes et al. (1998b) [518] repeatedly sampled cats to obtain duodenal juice for culture before, during and after being on a diet enriched with fructo-oligosaccharides (FOS). No changes on the duodenal flora were found in spite of the fact that the cat harbours higher numbers of bacteria than other species in its small intestine [710,713,714]. Fermentation of FOS and selective growth of bacterial species in the small intestine is conceivable because the duodenal bacterial flora of cats more closely approximates large bowel flora than that of other species [714].

The studies described above show that diet affects the colonic and faecal flora of cats, but the situation in the small intestine is less certain. However, a high degree of variation exists in one individual at different times while consuming the same diet, and in between individual cats [518] that makes it difficult to reach clear-cut conclusions.

There is no experimental data on the effects of fibre on feline gastrointestinal motility and contractile activity. The cranial colonic motility of cats includes slow waves and migrating spike bursts as are seen in the dog [715]. Therefore, fibre may affect feline colonic motility in a similar manner to that of the dog (see earlier discussion). Small intestinal motility is different in the cat when compared to the dog [716,717] and hence, the effects of dietary fibres on the feline small intestine may be more unpredictable.

Gastric emptying has been measured in cats with barium impregnated polyethylene spheres (BIPS) when consuming a diet high in insoluble fibre (29.1 %DM). The gastric emptying of the BIPS was more rapid on the high fibre diet than when the cats consumed a

low fibre diet [718]. However, the diets were not directly comparable, specifically in their fat content, which is known to affect gastric emptying.

Faecal characteristics have also been reported to change when dietary fibre is added to cats' diets. The use of wheat bran, horn meal, feather meal, rumen content, and grass meal decreased dry matter content in the faeces and increased the frequency of defecation while cellulose and raw starch did not [704]. In another study, cats consuming different fibres (8 –12.5% DM) increased wet faecal output, but especially so when soluble fibres were added [613]. The worse faecal characteristics (softer faeces) and higher number of defecations occurred as a result of the addition of a mixture of gums and pectin. Cellulose increased the faecal dry matter to equal proportions than a mixture of cellulose and gum arabic. This latter combination maintained good faecal characteristics with the added benefits of fermentable fibres. Beet pulp performance was similar to this combination but produced slightly wetter faeces.

In summary, dietary fibre has shown to influence several parameters of gastrointestinal structure and function in the cat including digestibility of other nutrients, colonic weight, bacterial flora and faecal characteristics. However, more research is needed, especially to determine if dietary fibre has beneficial effects on colonic injury, colonic electrolyte and fluid absorption and colonic motility in cats.

CONCLUSIONS

Diet before, during and after IBD. Does it matter?

A great deal of research has been carried out in the last few decades on the response of the gastrointestinal tract to diet and disease. Some of the encouragement for this work came from the increase in the prevalence of idiopathic inflammatory conditions in the human population and the challenge of finding a cause and better treatments for diseases that can affect quality of life as markedly as Crohn's disease and ulcerative colitis. The interest in clinical nutrition in veterinary science has been fuelled by the commercial interests of companies to provide better diets than their competitors in a pet food industry that has far exceeded the economic success expected of it.

As we have seen from the epidemiology discussion, it has not been easy to marry certain foods with the presence of IBD although we have gained a great deal of understanding as to how some nutritional and non-nutritional factors may change the risk of these gastrointestinal diseases. The results of the epidemiological studies have not always been that rewarding. Nevertheless, the gains from these and other studies have been significant even if they have not identified the aetiology of IBD and other gastrointestinal diseases. Thus, we have deepened our understanding of gastrointestinal and mucosal immunity, of the close relationship we enjoy (or otherwise) with our own bacterial flora, the effects of different foods in the development and health of our gut and how dietary advice can be used to change the course of disease.

Human IBD in its main forms of expression, Crohn's Disease and ulcerative colitis, has been well characterized in its course, clinical signs, histopathological specific lesions and its pathophysiology has become clearer so that treatment has been much improved even though we still do not know the causal agent. Veterinarians are not that fortunate. There is a lack of specificity in the diagnosis of IBD of companion animals and our diagnostic methods are still crude. Recent attempts to study the gastrointestinal functions of dogs and cats are a step in the right direction. Nevertheless, as discussed, a variety of effects of nutrients on the structure and function of the gastrointestinal tract have been reported and hence optimizing the diet fed to patients with IBD should provide a therapeutic avenue that decreases treatment failure in the management of inflammatory bowel disease in companion animals. However, the current theory on the aetiopathogenesis of idiopathic inflammatory disease involves the interaction of host and environment and therefore it is not easy task to work out its intricacies

In veterinary science there is an urgent need to improve our ability to make a more definitive diagnosis of IBD. There is also a pressing need to explore the role of diet in the maintenance of gastrointestinal inflammation and the use of a number of nutritional therapeutic strategies including different types of proteins or polypeptides, specific fatty acids, antioxidants and dietary fibre. Added to this is the potential to unravel the interaction of diet with the bacterial flora and their interplay with gastrointestinal disease. These goals will not be realized until better tools become available to document the influence of diet on gastrointestinal structure and function, such as better techniques to study peptide handling by the enterocyte and the immune system of the gut, better techniques to characterize and quantify normal bacterial flora; more reliable methods to

measure gastrointestinal motility and its interaction with gastrointestinal secretion and absorption, and more specific markers of gastrointestinal health. It is possible that future work using such tools will eventually demonstrate that the syndrome of IBD is made up of a group of diseases with similar clinical and pathologic manifestations, but perhaps requiring different dietary management.

The following chapters of this thesis include a multicentre epidemiological study of feline IBD to identify risks factors for the presence of disease; a study of carbohydrate tolerance in cats with IBD and healthy cats; a study of the effects of inulin on the proliferation of the gastrointestinal epithelium, colonic motility, macronutrient digestibility and faecal characteristics in healthy cats and a small number of cats with a history of colitis; and lastly the development of a novel device to collect rectal mucosal fluid for future studies in feline IBD characterization or diagnosis. Through the work described in the chapters of this thesis an attempt has been made to contribute to the enormous gaps in our knowledge of feline IBD and to shed some light on its potential causes, pathophysiology, diagnosis and nutritional management.

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