INTESTINAL PERMEABILITY TO POLYETHYLENE GLYCOL 400 IN PATIENTS WITH CROHN'S DISEASE

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DECLARATION

I, David Ruttenberg, declare that the work upon which this dissertation is based is original (except where otherwise acknowledged). It is being submitted for the degree of Master of Science at the University of Cape Town. It has not been submitted before for any degree or examination at this or any other University.

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ABSTRACT

An altered small intestinal permeability has been proposed as an important aetiological factor in the pathogenesis of inflammatory bowel disease. The relevant literature was reviewed. Intestinal permeability to Polyethylene glycol 400 in patients with Crohn's disease, their relatives and healthy controls was examined and the data compared with studies of small bowel permeability to other similar sized probes. A new technique of analysis of urinary Polythylene Glycol 400 by High Performance Liquid Chromatography was described and compared with a previously established HPLC method. No evidence of an altered bowel permeability could be found using Polyethylene glycol 400, but the possibility that this may have been related to probe size and characteristics can not be excluded.

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LIST OF ABBREVIATIONS

PEG 400:	Polyethylene glycol 400.
^{51}Cr-EDTA:	Chromium 51-labelled-Ethylenediamenetetraacetic Acid
DTPA:	Diethylenetriaminopentaacetic Acid
HPLC:	High pressure liquid chromatography.
GLC:	Gas liquid chromatography.
mw:	Molecular Weight
d:	Diameter
NCCD	National Cooperative Crohn's Disease
Prox:	Proximal
Asc:	Ascending
Desc:	Descending
Transv:	Transverse

Permeability as used in this dissertation is defined as the recovery from urine within a specified time of an orally ingested dose of a specific oral probe.

CHAPTER ONE: LITERATURE REVIEW.

INTRODUCTION

An altered permeability of the small intestine has been invoked in the pathogenesis of various disorders. In terms of this hypothesis antigens gain entry through an abnormal bowel mucosal wall. A basic knowledge of the anatomy of the bowel wall and the physiology of uptake mechanisms is essential to the understanding of any dissertation on permeability, and this will be briefly discussed.

ANATOMY

The human small bowel is formed within 2 weeks of fertilisation, and is a derivation of both foregut and hindgut (Arey 1974). It is about 6 meters (20 feet) long, and extends anatomically from the duodenum to the terminal ileum. The four coats of its cylindrically shaped wall are, from within outwards, mucosa, submucosa, muscularis, subserosal and serosal layers.

Intestinal villi (finger-like projections) cover the convoluted mucosal folds. These villi are more numerous, thicker and taller in the jejunum than either the duodenum or the ileum, and the jejunum is thus the site of maximal absorption of all ingested foodstuffs (Kalser 1985). The villi in turn are lined by a single layer of columnar cells, the latter being best developed in the middle third of the villus. These cells are ultimately shed from the tip of the villus. Tightly packed micro-villi, (approximately 3000 per cell), comprising the brush border, line the luminal cell surfaces and it is through here that nutrient absorption ultimately occurs. Each cell, including its microvilli, is lined in its entirety by 2 layers of plasma membranes (Shiner 1985).

Intercellular spaces separate the cells and are larger in the jejunum (0.75nm) than in the ileum (0.35nm) or colon (0.25nm). Aqueous channels on the lateral wall of the cells, connect these spaces with the internal cell area.

The Intercellular spaces are sealed on the lateral aspects of the cells by close apposition between adjacent plasma membranes. This area of cellular contact is termed the tight (occluding) junction (Martinez-Palomo and Erlij, 1975). The total mucosal thickness of the jejunum is on average 690 micrometres.

PHYSIOLOGY AND TRANSPORT MECHANISMS

The mode of transport of any entity is dependant on several factors including the type, concentration and ionicity of the substance being transported, and the region of bowel involved.

Active and passive transport mechanisms are involved in transport across the small bowel mucosal wall. In general, **passive** transport (that not involving cellular energy) occurs through the intercellular spaces, and is directly related to the luminal concentration of the substance. This form of transport is also dependent on the size of the intercellular pores and is thus greatest in the jejunum. Transport can also occur directly through the gut mucosal lining cells throughout the gastrointestinal tract. This pathway of **active** transport occurs against an electrochemical gradient, and is most efficient at low luminal concentrations.

In addition to these two pathways, a simultaneous bi-directional movement of water and solute occurs between the serosal layer and the bowel lumen. The process, whereby solutes diffuse through the pores of intestinal cells in a stream of water, has been termed "solvent drag" (Fordtran et al, 1965). There is a progressive fall in the bulk movement of water, and thus "solvent drag", from the upper to the more distal small bowel (Fordtran et al, 1965).

Under normal physiological conditions, the small intestinal mucosa displays a certain selectivity in its ability to absorb fluid, electrolytes and larger molecules. The precise route by which water soluble molecules, which are not selectively taken up by cell mediated intestinal uptake mechanisms, diffuse across the small intestinal mucosa is not known (Fordtran et al, 1965). Non-ionic water soluble particles, greater than 4nm in diameter are excluded. Previous studies have shown that non-lipid-soluble molecules penetrate cell membranes through water-filled pores (Hober and Hober, 1937; Solomon 1960). The effective pore radius decreases progressively from the jejunum to the ileum and is approximately twice the size in the jejunum compared to that in the ileum (Fordtran et al, 1965). The exact site of the porous barrier is not known, but a constant distribution of water filled pores, some large and many small, is thought to exist (Fordtran et al, 1965). While the distribution of pores is constant, the absolute number of pores of all sizes is thought to decrease distally through the intestinal tract (Loehry et al, 1970; Presnell et al, 1978). This porous route is thought to be one mechanism of permeation of small sized molecules.

An association between molecular size and the rate of permeation from the small bowel lumen to the plasma (or vice versa), has been found in studies in rabbits using water-soluble substances with a molecular weight range of 60-80000 (Loehry et al, 1970 and 1973). The inverse relationship of molecular size to quantity absorbed in the serum, (and ultimately excreted in the urine) has also been shown to occur with Polyethylene Glycol (Westrom et al, 1984). This relationship, however, is not found in the urine after intravenous injection and this confirms the sieving effect (related to molecular size) of the gut mucosal wall.

PERMEABILITY IN THE HEALTHY INTESTINE

Permeability may be comprehended as the facility of a membrane to allow unmediated movements of a solute across it (Cooper 1986). It is a function of the bowel luminal surface area, the concentration gradient across the gut and the permeability characteristics of the gut wall (Weaver et al, 1987).

The neonatal bowel wall is especially permeable to macromolecules during the 24 hour period after the first ingestion of colostrum. This process, which occurs in man and certain other mammalian species, is important for the acquisition of passive immunity from colostrum (Kraehenbuhl and Campiche, 1969). With maturation, a process of "closure" occurs, whereby larger sized molecules (mw > 1100 daltons) are no longer absorbed into the circulation (Westrom et al, 1984a and 1984b)(Westrom et al, 1989). Factors underlying this closure are still obscure, but could relate to the development of a differentiated columnar epithelium (Colony and Neutra, 1985), dietary factors (Loehry et al, 1978), or adrenal cortical hormones (Halliday 1959; Clark 1959).

The healthy adult mammalian small intestinal wall is thus relatively impermeable to large protein and carbohydrate molecules (Chadwick 1977b). Tight (occluding) junctions prevent the passage of whole protein and other potentially antigenic macromolecules paracellularly, and absorption through the gut wall is dependant on prior enzymatic degradation of any macromolecules. Small quantities of macromolecules are, however, absorbed through the healthy small bowel wall. Bockman and Winborn (1966) in studies with ligated loops of hamster small bowel, demonstrated the presence of ferritin in the intestinal absorptive cells following intraluminal administration. Uptake of endotoxin in rabbits (Ravin et al, 1960), and bacteria in dogs (Schatten 1954) and humans (Schatten et al, 1955) have also been found. Horse radish peroxidase (HRP) is a water soluble macromolecule with a molecular weight of 40000 and a diameter of 5nm. The recovery of trace amounts of this substance in the serum following direct instillation in the rat jejunum represented the first demonstration of a macromolecular presence between the intestinal absorptive cells and their subsequent quantitation in the mesenteric lymph and portal blood systems (Warshaw et al, 1971).

There is evidence that alternative routes of uptake exist for larger antigenic molecules, as opposed to smaller sized ones. Studies, using Polyethylene Glycol 600 as the probe marker, have shown permeation (at size dependant rates), across the bowel wall independent of the latters permeability to larger proteins such as ovalbumin (mw=43000), and bovine serum albumin (mw=69000) (Westrom et al, 1984b). This would suggest an alternative route of permeation for these macromolecules in the healthy bowel. Indeed, recent evidence suggests that, while this occurs by membrane bound vesicles and pinocytosis in certain mammals (Walker et al, 1972), it is associated, in man, with highly developed `M' cells associated with lymphoid follicles (Colony and Neutra, 1985; Keljo et al, 1985).

The quantity of macromolecules which pass through the adult mammalian bowel wall is, under normal circumstances, relatively small and, generally, of no importance physiologically, being cleared by the immune system. The barrier function of the healthy bowel is dependent on various factors including an absence of inflammation and ulceration (Worthington and Syrotuck, 1976; Grusky and Cooke, 1955). Any circumstance which alters the physical or functional integrity of the bowel mucosa may, however, lead to an increased permeation of macromolecules across the wall and into the intestinal blood stream (Worthington and Syrotuck, 1976).

PERMEABILITY IN THE DISEASED INTESTINE

A variety of agents can affect the functional and anatomical integrity of the small bowel wall. The permeability of the small intestinal mucosa to various probes is known to be affected by bile salts in rabbits (Chadwick et al 1979), guinea pigs (Talbot et al, 1984), and rats (Hollander et al, 1989). In man, too, bile acids have been shown to affect both the small (Budillon et al, 1980) and large bowel mucosal permeability (Chadwick et al, 1977b). Parasitic infestations in rats have also been shown to result in an increased permeability to larger antigenic protein molecules (Cobden et al, 1979; Bloch et al, 1979).

Although naturally occurring detergents like lysolecithin and lyso-phosphatidylcholine have also been shown to increase the permeability of the rat (Tagesson et al 1985; Bolin et al 1981) and guinea pig small bowel (Talbot et al, 1984) to macromolecules, this has not been found with cetrimide, another detergent (Turner et al, 1988). These differences may reflect differences in bowel regions studied or the nature of the insult on the bowel.

Diffusion of large molecules across the small intestinal mucosa of children has been shown to be greater in damaged or abnormal bowel mucosa when compared to normal small bowel mucosa (Jackson et al, 1982). The epithelial cell turnover rate is greater in abnormal mucosa and in this in vitro histopathological study, using ruthenium red (mw=1000) as the marker, the number of damaged and non-viable enterocytes penetrated was found to be greater in the abnormal mucosa. The degree of penetration was also independent of morphological evidence of any damage. Moreover, a large degree of penetration by horse radish peroxidase (mw=40000) was also found in the abnormal mucosa. This may reflect a greater proportion of abnormal mucosal enterocytes permeable to antigenic sized molecules and thus a greater potential for passive diffusion in the abnormal intestine.

While the mechanism of an increased bowel permeability to large protein antigens may be related to the possible breakdown in the junctional complexes between epithelial cells lining the villi, an altered histology has not been a uniform finding. An increased small bowel permeability to horseradish peroxidase has been documented in the absence of morphological evidence of mucosal damage in guinea pigs, following exposure to lysolecithicin (Talbot et al, 1984). The altered bowel permeability associated with bile acids, alcohol, parasitic infestations, radiation damage and certain drugs, however, is accompanied by histological damage (Talbot et al, 1984;Bloch et al, 1979; Walker and Porvaznik, 1978; Erikson et al, 1987). Nadia and co-workers studied the acute affects of EDTA, tetracycline and sodium laurylsulfate on the rat small bowel and found varying degrees of histological damage (Nadia et al, 1972).

It would seem that any altered permeability is dependent not only on the physical characteristics of the agent involved but, possibly, also on the environment in which it acts. Laker and Menzies (1977) found that the fraction of lactulose excreted in the urine of healthy subjects following the ingestion of a lactulose-containing solution, increases if it is made sufficiently hypertonic. Udopihille (1974) independently found evidence to suggest that the site of this altered permeability was in the small intestine. It is probable that hypertonicity *per se* alters permeability. Morphological alterations in the small bowel mucosa have been noted immediately following the application of the hypertonic solution (Norris 1973). Evidence would suggest that changes in permeability induced by a hypertonic environment are reversible (Laker and Menzies, 1977; Norris 1973).

An increased intestinal permeability to small sized molecules has also been found in abnormal small bowel. Love (1969), in early experiments in rabbits, demonstrated an increased small intestinal permeability to mannitol, erythritol, urea and sodium chloride, following infection with cholera organisms. Whether this was as a result of a direct effect on the lining cells, or as a result of changes in the local circulation is not clear.

In contrast, however, Rohde and Chen (1972) found that, while canine jejunal permeability to ^{14}C-urea and ^{3}H-arabinose was unchanged following experimentally induced cholera, administration of the drug amphotericin B, resulted in altered membrane permeability and selectivity to these smaller molecules. The differences between these studies of intestinal permeability, may be due to differences in the sensitivity of the techniques employed, or nonspecific changes that occur in ligated and distended intestinal loops (Scherer et al, 1974).

The available data supports a concept of different routes of permeation across the small intestinal mucosa for different sized molecules. Small molecules, about 0.4nm in diameter, permeate through water filled pores in the cell membrane and absorption is related to mucosal surface area. The larger macromolecules, greater than 0.4nm in diameter, either diffuse across extracellular pathways between cells or they are taken up by a process of endocytosis. In these instances, any increased absorption is related to mucosal or epithelial damage,

Since the mechanisms of permeation for large and smaller sized molecules would appear to differ, the question arises whether changes in permeability to smaller sized molecules reflect and parallel alterations in permeability to larger sized molecules. Turner et al (1988) found an altered permeability to macromolecules and the sugar probes lactulose and rhamnose in rats experiencing hypersensitivity reactions but no correlation between the permeability to large proteins (mw 45000) and that of the smaller carbohydrate probes (mw 400) was found. In contrast, however, Ramage et al (1988) found that an increased permeability to ^{51}Cr-EDTA (mw=360) correlated strongly with an increased permeability to ovalbumin (mw=45000) in a study in rats that had been infected with Nippostrongylus brasiliensis. The difference between these two studies may reflect either different pathways of uptake for carbohydrates and EDTA, or a different mechanism of damage when parasitically and immunologically induced.

The concept of an association between the permeation of a potentially antigenic macromolecule across the small bowel wall, and the subsequent initiation of a disease process was first mooted in 1958 by May et al with the Clostridium botulinum toxin A. Similar associations have also been noted with the Cholera toxin in animals (Kao et al, 1970; Serebro et al, 1968) and in man (Springer and Horton, 1969). An association of leukopenia and an altered intestinal permeability with an increased radiation induced-sensitivity to endotoxin has also been documented (Walker and Porvaznik, 1978).

CROHN'S DISEASE

Crohn's disease is a non-specific inflammatory disease of the gastrointestinal tract and is considered to be a generalised gastrointestinal disorder, affecting predominantly the small intestine. The disorder is characterised by the presence of ulcerative lesions in the bowel wall as well as extraintestinal manifestations such as joint, skin and ocular lesions. In Crohn's disease, the bowel mucosal ulceration is interspersed with normal intervening mucosa, giving rise to characteristic so called "skip lesions". Clinically, the condition is characterised by relapsing episodes of abdominal pain, accompanied at times by diarrhoea.

Treatment remains an often vexing problem, with medical therapy the mainstay of treatment. Treatment options here also include dietary manipulation, steroid therapy and powerful immunosuppressive agents. Surgery is currently reserved for those patients with surgically remediable indications or complications.

It should be stressed, however, that treatment is at best either symptomatic or palliative. The etiology and pathogenesis of Crohn's disease remains unknown, and treatment is thus seldom, if ever, curative.

The possibility of a multifactorial etiology can not be excluded. Factors that have been implicated in the causation of the disease include:

1. Genetic
2. Dietary
3. Infective (bacterial or viral)
4. Immunological
5. Environmental (smoking, external agents)

1. Genetic

Chromosomal abnormalities have yet to be demonstrated in Crohn's disease and the disorder is not currently considered to be genetically inherited. Multiple familial occurrences have, however, been documented in patients with inflammatory bowel disease (Farmer et al, 1980)(Tysk et al, 1988).

2. Diet

The role of diet in inflammatory bowel disease remains controversial. A large number of different food additives are present in a wide range of processed foodstuffs and there is evidence to suggest that the former may be an important factor. Carrageenans, which are found in various foods, have been implicated in the pathogenesis of colitis in guinea pigs (Watt and Marcus, 1975). In Crohn's patients a good clinical response to dietary manipulation and an elemental diet (O'Morain et al, 1984; Kushner et al, 1986) is further evidence for a dietary role. Dietary modifications of carbohydrate and fibre have, however, failed to alter the course of Crohn's disease (Ritchie et al, 1986).

3. Infective

Bacterial cultures of gut contents of patients with inflammatory bowel disease remain inconclusive for an infective aetiology. Bacterial overgrowth, however, seems to be a characteristic feature of Crohn's disease, along with high serum antibody titres to several anaerobes (Van der Merwe 1980). While a report of the isolation of an atypical mycobacterium from biopsy specimens from subjects with Crohn's disease, evoked much interest, (Hampson et al, 1988), this mycobacterium could only be isolated from a minority of patients, and specificity for Crohn's disease is also not proven. Evidence for a viral aetiology is not any clearer. Observations of granulomatous lesions in animals, following injection of homogenates of bowel or mesenteric lymphnodes from patients with Crohn's disease, have not been confirmed (Cave et al, 1977). Thus, while the infective theory is an attractive one, knowledge of the role of the gut flora, if any, in inflammatory disease remains obscure.

4. Immunological

A correlation between some of the extra-intestinal manifestations of Crohn's disease and circulating immune complexes isolated from patient sera has been noted (Jewell et al, 1972; Nielsen et al, 1978a & b). It is possible that these immune complexes are the direct mechanism of tissue injury. The relationship between mechanisms of inflammatory changes and diet may also be on the basis of food allergy or hypersensitivity to any, or all, food components. In terms of the food hypersensitivity hypothesis, the continued uptake of fragments of food proteins (albeit in minute quantities), may lead to sensitisation and a mucosal reaction on subsequent ingestion of the offending food (Bloch et al, 1988). Some investigators have found a raised antibody titre to milk proteins in patients with ulcerative colitis (Taylor and Truelove, 1961; Taylor et al, 1964). This has, however, not been a universal finding (Jewell and Truelove, 1972; Sewell et al, 1963; Dudek et al, 1965), and a poor correlation between the two different methods used by the various investigators has also been reported (Steele et al, 1964). Although inflammatory bowel disease has been associated with other immunologically mediated conditions, and a favourable clinical response is seen after corticosteroid therapy, there is currently no convincing evidence of an immune defect in the mucosal immune system of these patients (Elson 1988).

5. Environmental

While an association between inflammatory bowel disease and smoking has been noted and consistently reported, no explanation of its mechanism is forthcoming. A recent study found that smoking doubled the risk of acquiring Crohn's disease (Lindberg et al, 1988).

INTESTINAL PERMEABILITY AND CROHN'S DISEASE

There is a substantial body of evidence linking an altered intestinal permeability with the pathogenesis of inflammatory bowel disease (Olaison et al, 1990).

Whether the altered intestinal permeability is a primary or secondary phenomenon to the inflammatory changes, is not clear at present. The demonstration, in one study, of an increased small bowel permeability to Polyethyelene glycol 400 in healthy first degree relatives of patients with inflammatory bowel disease (Hollander et al, 1986), suggests that a permeability defect could indeed be a primary genetic phenomenon. In addition, the increased anticolon antibody titres noted previously in healthy first degree relatives of Crohn's patients, also implicates intestinal mucosal damage and an immunological response as the primary mechanism.

Crohn's disease is predominantly a disease of Western civilisation and Western diets. The latter is characterised by a conspicuous lack of fibre and this may also thus play a role in promoting an altered permeability of the small intestine. Bran has been shown to have a protective influence on the permeability-altering effects of bile acids (Gyory and Chang, 1983). In a recent study, Shiau and Chang (1986) found that rats fed a high fibre diet demonstrated a decreased intestinal permeability to the marker phenol red, when compared with rats that were fed a fibre free diet. While the mechanisms are not clear, protection could relate to the production of mucin, an important permeability barrier of the large bowel wall. Studies have shown an increased enzyme mucinase activity following fibre free diets (Shiau and Chang 1983). High fibre diets and a subsequent decreased mucinase producing bacterial population may thus also be the mechanism of the known protective role of the former, especially in relation to colonic carcinogenesis. Surface active food additives, (for example polyoxyethylene (20), sorbitan monostearate and sorbitan mono-oleate) which are also very prevalent in Western diets have been shown in rats to increase the permeability of the small bowel mucosa (Tagesson and Edling, 1984a). It has been hypothesised that inhibition of intestinal phospholipase activity by food additives and a resultant decrease in prostaglandins may be the mechanism. While the protective role of prostaglandins in the human stomach is well established (Hawkey and Rampton, 1985), it is only recently that this role has been found to extend to the small intestine. In a functional and morphological analysis of rat jejunum, using mannitol as the probe, pretreatment with 16,16-Dimethyl prostaglandin E_2 was found to decrease the mucosal injury caused by the bile acid chenodeoxycholate (Erikson et al, 1987).

Patients with Crohn's disease have also been noted to have decreased bowel luminal concentrations of lysophospholipase, an enzyme thought to prevent the accumulation of lysolecithin, a naturally occurring detergent (Bolin et al, 1984). As noted previously, certain detergents have been associated with an increased bowel permeability. In genetically susceptible individuals detergents may thus act as the external agent required to precipitate an immunological inflammatory reaction, via an altered bowel permeability.

TOXICITY OF POLYETHYLENE GLYCOL 400

The Polyethylene glycols are polymers of ethylene oxide and between molecular weights 200-600, are completely water soluble (Cox 1979). They vary in consistency from waxy solids to liquids, and are extensively used in the food, cosmetic and pharmaceutical industries. The metabolism and toxicity profile of ethylene glycol has been well documented (Parry and Wallach 1974; Cadnapaphornechai et al, 1981). Excretion of polyethylene glycol 400 is in a 2:1 ratio, urine to stool following oral ingestion. Approximately 55% of an oral ingested dose of PEG 400 is recovered in the urine over a 48 hour period (Chadwick et al, 1977a). Of this, 82% is excreted in the first 12 hours. While doubts have recently been expressed concerning potential toxicity and the possible topical absorption of large doses of PEG 400 from PEG containing burn creams (Bruns et al, 1982; FDA drug bulletin 1982; Herold et al, 1982), no causal relationship between burn creams and toxicity has yet been established (FDA Drug bull 1982). The reports (Bruns et al, 1982; Herold et al, 1982) are, however, flawed as no serum or urinary levels of PEG are given. The possibility of contamination of the creams by microorganisms with subsequent formation of ethylene glycol, has been mooted. Golytely, which contains PEG 3350 (formerly PEG 4000), is commonly used as a saline lavage prior to colonoscopic examination of the large bowel. Despite its use for more than 50 years, there are no reports of any side effects of this PEG when administered orally to patients with normal or diseased bowels (Goldman and Reichelderfer, 1982; DiPalma and Brady 1989; Brady et al, 1986).

The average dose of PEG obtained from 4 litres of Golytely is greater than 240g (Lifton 1984). No data is currently available for the LD_{50} of PEG 400 in man or animals. The LD_{50} of PEG 4000 in rats is greater than 50g/kg (Smyth et al, 1955). This is equivalent to 3500g in a 70kg man and is infinitely greater than the considerably smaller total oral dose of 5.6 grams given in the present study. The mean administered dose of PEG in Golytely is 2.0g/kg, and clinical studies have found it to be a safe and nontoxic method of colon cleansing with minimal absorption of polyethylene glycol (DiPalma et al, 1983; DiPalma and Brady, 1989; Brady et al, 1986; DiPiro et al, 1986). In addition, no side effects have been reported in studies with oral ingestion of 20 grams of PEG 400 (Chadwick et al, 1977a).

In view of the above data, it was felt that polyethylene glycol 400 would be a safe, non-toxic oral probe for use in permeability studies of the bowel.

PURPOSE OF STUDY

The pathogenesis of Crohn's disease may involve interaction between host, immunological responses and external agents (Shorter et al, 1972). In terms of this hypothesis, the external agent is an antigen of some type (bacterial, viral or dietary), which gains entry through the bowel wall in association with an altered gut mucosal permeability. The subsequent autoimmune reaction then precipitates the characteristic intestinal lesions seen macroscopically. In the presence of granulocytes, an increase in intestinal permeability to endotoxin, whether via discontinuities in the tight junctions or direct penetration through the lining cells, may lead to stimulation of the inflammatory response (Walker and Porvaznik, 1978).

A study by Hollander (1986) suggested a permeability defect in family members of patients with Crohn's Disease. The present study was undertaken in order to:

a) Validate the previously used methodology for determing the concentration of PEG 400 in urine.

b) Establish, in a larger group of subjects, the presence or absence of an altered intestinal permeability to PEG 400.

c) Examine the effect on intestinal permeability in Crohn's patients of: sex, age, extent of disease, effect of bowel resection, disease activity and treatment.

CHAPTER TWO: STATISTICAL ANALYSIS

Data are presented as urinary recovery of the orally administered dose of PEG 400, expressed as a percentage. Because results are expressed as a percentage, normal distribution cannot be assumed and results are expressed as median and range. As the data do not conform to Gaussian distribution, non-parametric tests of analysis have been used. Statistical differences between more than 2 groups were analysed using Kruskal-Wallis one way analysis of variance. The Mann-Witney test was used to calculate statistical significance when there were only 2 comparisons.

The Coefficient of Variation (CV) was calculated using the following formula:

$$\frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

The standard error of measurement (SEM) is calculated from the following formula:

$$\sqrt{\Sigma w^2/2n}$$

where w = difference between two results

and n = the number of duplicate samples

Results of comparisons were considered significant if $p < 0.05$.

CHAPTER THREE: CHOICE OF PROBE

Various probes have been used to examine small bowel permeability in the human. These include iron (Loehry et al, 1973), ^{51}Cr-EDTA (Peled et al, 1985; Ainsworth et al, 1989; Bjarnason et al, 1983), labelled-DTPA (Casellas et al, 1986; Resnik et al, 1990), the carbohydrates, lactulose and mannitol (Andre et al, 198; Cobden et al, 1978; Ukabam et al, 1983; Murphy et al, 1989), PEG 1000 (Tagesson et al, 1983; Olaison et al, 1987; Olaison et al, 1989), PEG 900 (Katz et al, 1987), PEG 600 (Olaison et al, 1988; Olaison et al, 1989) and PEG 400 (Hollander et al, 1986; Jenkins et al, 1986; Magnusson et al, 1983). Using these different probes, an altered small bowel permeability has been demonstrated in a variety of disease and physiological states in man eg. parasitic and bacterial infestations (Serrander et al, 1984; Serrander et al, 1986; Weaver et al, 1985; Bjarnason et al, 1985), atopic eczema (Bjarnason et al, 1985b; Jackson et al, 1981), coeliac disease (Bjarnason and Peters, 1984; Pearson et al, 1982), post-alcohol ingestion (Robinson et al, 1981), inflammatory bowel disease (Ukabam et al, 1983; Sanderson et al, 1987), and schizophrenia (Wood et al, 1987).
The methodology used in permeability tests of the small bowel all involves oral ingestion of a probe and subsequent recovery and quantitation of the probe in a timed urine collection. Recovery of the probe is expressed as a percentage of the orally ingested dose.

Chromium labelled ethylenediaminetetraacetic acid (^{51}Cr-EDTA) is a highly stable, water soluble, polar molecule with an average molecular weight of 380 daltons and no affinity for any particular organ. The normal human colon has, however, been found to absorb significant amounts of orally administered ^{51}Cr-EDTA in the absence of any intestinal inflammation (Ramage et al, 1987; Elia et al, 1987). As a single marker, urinary quantitation can be affected by incomplete urine collection and gastric emptying. It is apparently poorly absorbed from the gut lumen, its route of uptake being thought to be via the paracellular junctions.
^{51}Cr-EDTA has been extensively used as a marker of intestinal permeability, despite

the finding that ^{51}Cr-EDTA *per se* is associated with an altered bowel permeability (Windsor and Cronheim, 1961). The mechanism, though unclear, may be related to the chelation of calcium with a resultant weakening of the gut intercellular tight cell junctions (Windsor and Cronheim, 1961; Nadia et al, 1972). Indeed, EDTA has also been used as a promoter to aid in the absorption of sulphanilic acid in the rat jejunum (Yamashita et al, 1985).

Technetium^{99M}-diethylenetriamine pentacetate (^{99M}DTPA) has also been used as a probe of intestinal permeability. Its structure and clearance are similar to those of ^{51}Cr-EDTA (O'Morain et al, 1984). Indeed, studies by O'Morain et al and Resnick et al (1990) have found a positive correlation between these two probes when ingested simultaneously. In contrast to ^{51}Cr-EDTA, a considerably smaller oral dose of DTPA can be given. While the use of both probes involves radioactivity, it is in small and thus relatively safe quantities.

The double-sugar test involves ingestion of two sugars that have slightly different routes of permeation. The polar disaccharide molecules lactulose (mw=342, d=0.54nm) and cellobiose (mw=342, d=0.52nm) are restricted transmucosally, and are thought to cross the intestinal mucosa passively, through channels associated with the tight junctional complexes between cells (intercellularly) (Menzies et al 1979; Wheeler et al,1978). In contrast, the slightly smaller, relatively inert mannitol (mw=182, d=0.4nm) and rhamnose (mw=164, d=0.4nm), are thought to permeate directly through the cell membrane (transcellularly) by means of water filled pores (Menzies et al, 1979). Absorption of these markers would seem to be dependent on molecular volume, rather than molecular mass, molecular radius or area (Elia et al, 1987).

The carbohydrates mannitol, lactulose, cellobiose and rhamnose have been extensively used as markers of altered intestinal permeability. They are all of a similar size and, following ingestion of an oral dose, are fully excreted within 6 hours via the kidneys. A wider separation of normal and abnormal function is obtained when the percentage urinary recovery is expressed as a ratio than if the sugars are individually and separately considered. In addition, when expressed as ratio, absorption and excretion is less dependent on variables like gastric emptying, intestinal transit time and renal disease (Ukabam et al, 1983). The presence of intestinal disease affects the permeation of these markers differently. The passage of the transcellularly transported probes (like mannitol) seems to be reduced with villi shortening and loss of intestinal surface area. The carbohydrate probes transported paracellularly (like lactulose) seem to reflect actual morphological mucosal damage (Ukabam et al, 1983). For example, in patients with coeliac disease, the permeability to lactulose is increased, while that of mannitol and rhamnose is decreased (Menzies et al, 1979; Cobden et al, 1978).

A large number of healthy, as well as clinically ill, elderly people may have a significant degree of bacteruria, however, and urinary infection has recently been shown to possibly invalidate this double sugar test (Milnes et al, 1988).

Polyethylene glycols are hydrophilic, lipophilic polymers of ethylene glycol. They are mixtures of varying molecular weights and consist of populations of elongated molecules of differing sizes. PEG 400 is a colourless liquid consisting of nine separate polymeric units, with molecular weights ranging from 242 to 594 daltons. As the average weight is 400 it is called PEG 400.

Assessment of intestinal permeability involves oral ingestion of a probe and subsequent urinary quantitation in a given time period. Results, however, relate also to other factors such as gastric emptying, intestinal luminal dilution and renal function. The advantage of polyethylene glycol preparations in permeability studies is that, unlike a single test substance, they consist of a spectrum of molecular sizes. Absorption of the different molecular species can thus be expressed as a ratio. Permeability may thus be assessed independently of gastric emptying, intestinal transit and renal clearance.

Consequently, a comparison of ratios of absorption of the species is a reliable index of permeability.

While the site of maximal absorption of PEG 400 is not known, the absorption of the latter does seem to be independent of food intake (Chadwick et al, 1977a).

Initially, PEG 400 could be qualitatively assayed only by gas chromatography but a new method of analysis using High Pressure Liquid Chromatography (Delahunty and Hollander, 1986) has recently been described.

The selection of a probe is a contentious subject as each has its own advantages and disadvantages. This was addressed by Chadwick et al (1977a) when he published a list of properties the ideal permeability probe should possess.

a. It should be a non-toxic, biologically non-degradable substance

b. It should be a mixture of varying sized compounds which show decreasing mucosal transport with increasing molecular size.

c. Transport of each molecular species should follow first order kinetics.

d. The probe should not influence intestinal motility.

e. The probe should not be metabolised after absorption.

f. The probe should be able to be measured with precision, sensitivity, accuracy and ease.

g. The marker should be rapidly and completely excreted in urine, allowing quantitation, and thus be a measure of absorption.

The advantages of PEG 400 are that it is non-toxic and consists of a variety of differing molecular weight molecules that permit comparisons. The lack of radioactivity is important to subject and investigator alike. Methods for measurement have been established. Furthermore, the work that instigated this study was one in which PEG 400 was used to detect changes in permeability in family members of Crohn's patients.

CHAPTER FOUR: MATERIAL AND ANALYTICAL METHODS

CLINICAL MATERIAL

All subjects gave informed consent and the protocol was approved by the Ethics Committee of the University of Cape Town.

The Crohn's group: Forty five patients in whom the diagnosis of Crohn's disease had been clinically, endoscopically and radiologically established, and who were attending the outpatient department of the Gastrointestinal Clinic, were included in the study. Details of age and sex are shown in Table 1.

The family group: Twenty first degree relatives of 14 patients with Crohn's disease were studied. Six patients had two first degree relatives studied. All relatives were in good health and had no evidence of any gastrointestinal disease, either on history or examination. There was also no history of any drug or alcohol ingestion in this group in the 48 hour period immediately preceding the test. Their age and sex distribution are shown in Table 1.

The control group: This group consisted of 31 healthy subjects (Table 1). There were 6 males and 25 females. The youngest volunteer was 24 years old, and the oldest 72 years of age. All the control subjects were healthy, with no evidence, either on history or examination of any bowel disease. There was no history of ingestion of any drugs (specifically non-steroidal drugs) or alcohol in the 48 hours immediately preceding the test. The controls were of a similar socioeconomic background to the other groups studied.

Table 1. Age and sex distribution

Group	**All subjects**		**Males**		**Females**	
	n	Age (years) mean (range)	n	Age (range)	n	Age (range)
Crohn's	45	39.8 (19-72)	19	(20-72)	26	(19-60)
Family	20	33.3 (11-70)	8	(13-55)	12	(11-70)
Controls	31	42.2 (24-72)	6	(30-53)	25	(24-72)

INDICES OF DISEASE ACTIVITY IN PATIENTS WITH CROHN'S DISEASE

All patients with Crohn's disease underwent a clinical examination and had blood drawn for routine biochemical and haematological analysis. Using this data, two indices were calculated and used in the present study as reflections of inflammatory disease activity in the patient group viz. the van Hees index and the National Cooperative Crohn's Disease Index (NCCD). The van Hees activity index is based on the data derived from 63 patients (Van Hees et al, 1980). The following nine variables were found to correlate to the overall evaluation: serum albumin, erythrocyte sedimentation rate (ESR), sex, temperature, abdominal mass, body weight related to length, stool consistency, bowel resection and extraintestinal symptoms related to Crohn's disease. Index values below 100 are indicative of inactivity, values between 100 and 150 are associated with slight inflammatory activity, values between 150 and 210 as indicating moderate activity and values above 210 as indicating severe to very severe inflammation. The NCCD index is based on data collected prospectively from 112 patients with Crohn's disease (Best et al, 1976). It makes use of the following 8 variables: the number of liquid and soft stools in one week, the sum of 7 daily abdominal pain ratings, the sum of 7 daily ratings of general well-being, symptoms or signs related to Crohn's disease, use of lomotil or opiates for diarrhoea, presence of an abdominal mass, the haematocrit and body weight. Index values below 150 are associated with inactive inflammatory disease, while values above 150 have are regarded to be indicative of active inflammation.

SAMPLE COLLECTION.

After an overnight fast, subjects ingested 5.6 grams of PEG 400 with 200 ml of water. During the following 6 hours, the oral intake of water was unlimited, but no food was allowed. Any urine passed during this 6 hour period was collected, together with the urine passed at the end of the 6 hours. The total urine volume passed by each subject was measured and a 40ml aliquot stored in a sterile plastic container at -16^{o}C.

ANALYTICAL METHOD.

(I). Apparatus.

1) Freeze Dryer (Thermovac, Thermovac Corporation, USA)

2) Nitrogen Gas Manifold.

3) The HPLC system consisted of:

a) Chromatograph (model SP 8800, SpectraPhysics, San Jose, CA, USA).

b) Injection system (Rheodyne, Cotati, CA).

c) Interchangeable 20 and 200 microlitre sample loops.

d) Reversed phase column (styrene divinylbenzene column PLRP-S, 25cm (length), 4.6mm (id), Polymer Laboratories, Amherst MA, USA).

e) Refractive index detector (SpectraPhysics model SP 8430) f) Integrating recorder (SpectraPhysics model SP 4290).

(II) Chemicals.

Chloroform - Analar Grade

Methanol - HPLC Grade (Baxter Healthcare Corporation)

Deionized water - Milli-Q system Millipore Ltd, South Africa.

Polyethylene Glycol 400 - Lot 120F/0189, Sigma Chemical Corporation St Louis MO, USA.

HPLC TECHNIQUE

An isocratic mobile phase (equal volumes of methanol and water) was used to elute the PEG 400 from the column at a flow rate of 1ml/min. The mobile phase was degassed by continuous sparging with Helium.

PREPARATION AND ANALYSIS OF STANDARDS.

Standards were prepared by dissolving PEG 400 in water at concentrations of 0.2, 0.4, 0.8, and 1.6 grams per litre. Each standard was analysed in duplicate by injecting 20μl aliquots onto the chromatograph. A standard curve of total area for all peaks versus PEG 400 concentration, was plotted for each analytical run. A linear standard curve was obtained for concentrations varying from 0.2-1.6g/l (Fig 1). Nine peaks were readily separated and detected, within twenty minutes of injection, (Fig 2). These peaks corresponded, on the basis of retention times, to PEG 400-species with a molecular weight from 286-638 (Delahunty and Hollander, 1986). The six main components constituted about 90% of the total and could be measured with an acceptable degree of analytical precision throughout the range of concentrations (Table 2).

Table 2. Precision of PEG 400 Assay

Peak number	I	II	III	IV	V	VI
PEG 400 conc (g/l)	CV % for various PEG fractions					
0.2	8.8	8.2	6.4	3.3	6.4	6.7
0.4	8.5	3.4	4.5	5.2	6.1	6.9
0.6	3.8	2.7	2.6	6.4	7.2	7.0
0.8	4.0	3.8	3.0	5.0	4.7	5.0
1.6	1.3	1.5	1.5	1.9	2.2	1.4

PREPARATION AND ANALYSIS OF URINE SAMPLES

EXTRACTION METHOD

Initially the method of Delahunty and Hollander (1986) was used in analysis of the urinary PEG 400. Urine was thawed at room temperature. Duplicate 10ml aliquots of urine were lyophilized in a 100ml stoppered flask overnight. PEG 400 was extracted from the freeze-dried urine by addition of 10 ml of chloroform, followed by 10 minutes of vigorous shaking. The chloroform was transferred quantitatively to a 30ml test tube

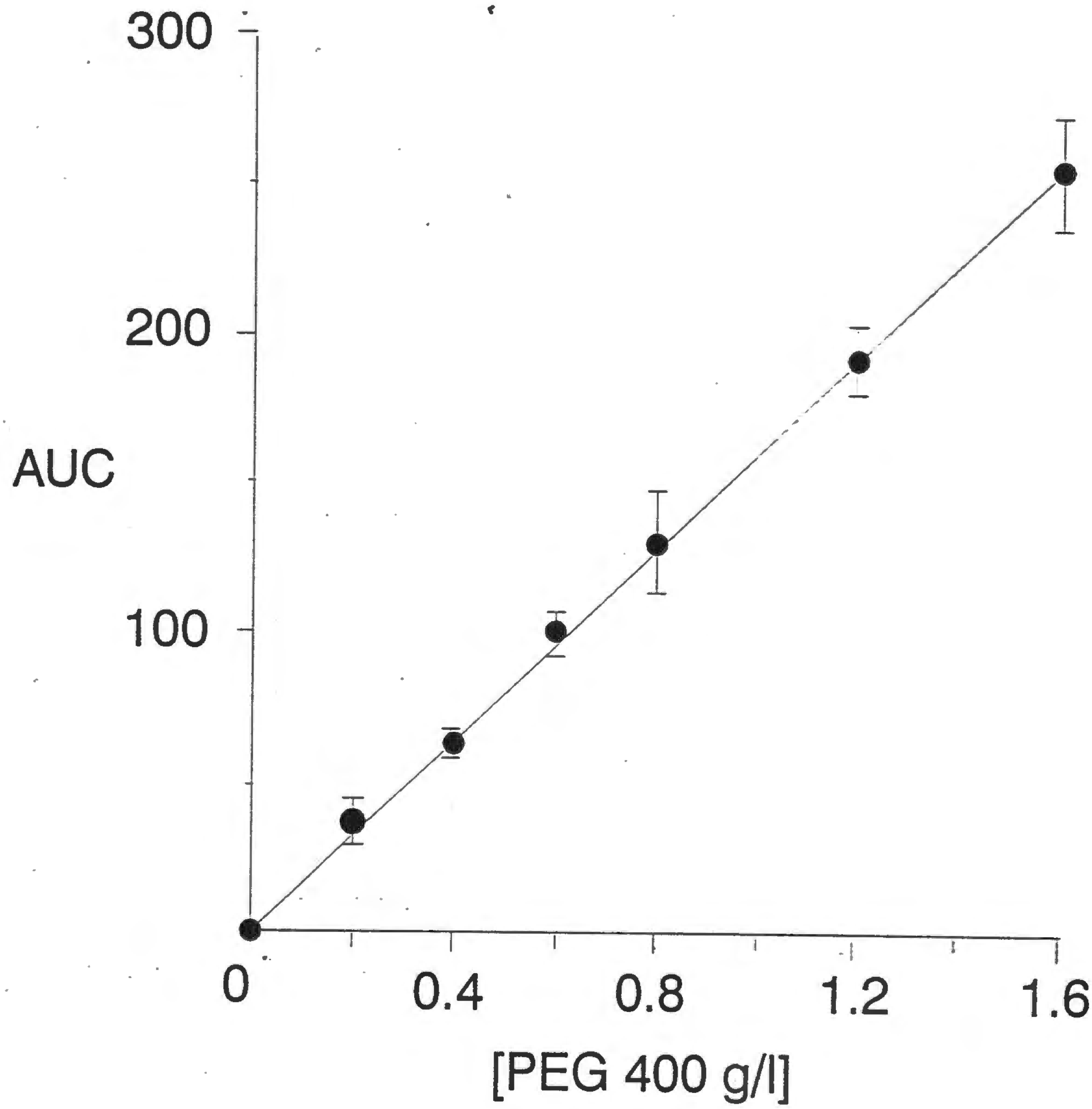

Fig. 1. Standard curve obtained by plotting total area of all peaks (AUC) against concentration of PEG 400.
Mean $\pm$ SD (10 separate analyses) is shown at each concentration.

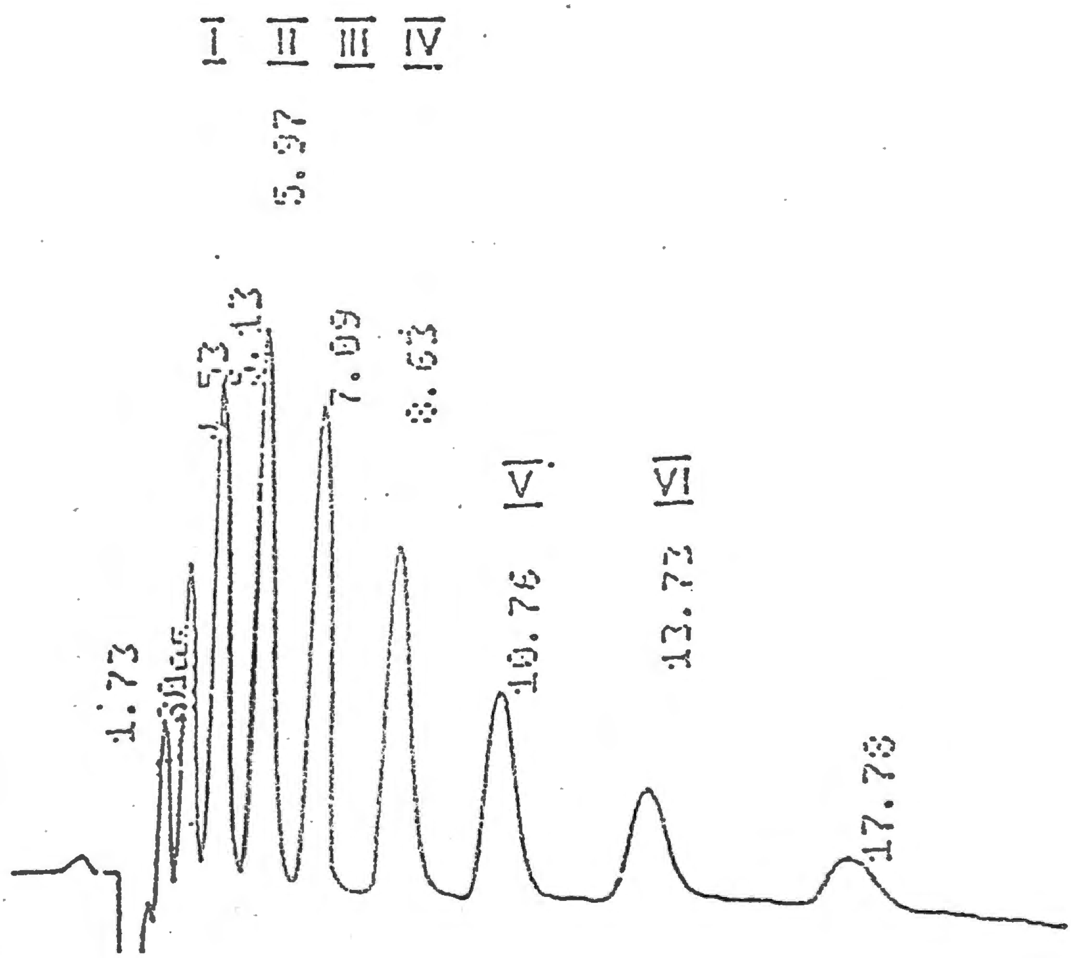

Fig.2. Typical HPLC chromatogram of PEG 400 (0.8g/L).
Retention times (minutes) are shown for each of the six peaks that were individually analyzed.

and evaporated to dryness under a stream of nitrogen. Two millilitres of the mobile phase for HPLC (methanol:water, 1:1, v:v) were used to reconstitute the sample. The sample was then aspirated with a 5ml disposable syringe and filtered through a 0.22 micron filter (Millipore Ltd, South Africa). Twenty microlitres of the filtrate was injected onto the chromatograph. HPLC analysis was performed in duplicate for each sample. The concentration of PEG 400 in the urine was obtained by reading off the standard graph. Results are expressed as the percentage of the ingested dose which is recovered in urine.

Recovery of PEG 400 in urine

Recovery of PEG 400 in urine was determined by the addition of PEG 400 to aliquots of pooled normal urine, to give concentrations varying from 0.2 to 1.6 grams per litre (Table 3).

Table 3. Recovery of added PEG 400

PEG 400 added (g/l)	0.2	0.4	0.8	1.6
% recovered (Mean)(n=7)	86.2	88.4	91.6	90.4
(SD)	4.1	5.3	4.6	5.7

Recovery varied from 82.1% to 96.2%.

Reproducibility

Reproducibility was determined by including aliquots of a single urine specimen in 10 analytical runs, performed on 10 separate days. The Coefficient of Variation (CV) was found to be 13.3%.

Limit of detection

The lower limit of detection in a urine sample was found to be at a concentration of 0.5g/l.

The above extraction method was used to analyse PEG 400 excretion in patients with Crohn's disease, their healthy relatives and a group of controls (Ruttenberg et al, 1989). A wide scatter within, and no difference between, any of the groups were found. The results were also characterised by poor reproducibility (CV=13%) and variable recovery (82-96%). The extraction method involves the numerous steps:- pipetting of a 10ml aliquot of urine, lyophilisation overnight, extraction with chloroform, evaporation under a stream of nitrogen and reconstitution of the sample prior to injection onto the HPLC column. Variable loss of sample can occur at any one or several of these steps. Technical imperfections in this extraction method might explain the wide scatter and lack of differences and a new more accurate method of direct injection HPLC was therefore developed.

DIRECT INJECTION METHOD

Urine specimens were thawed at room temperature and analysis performed in duplicate for each sample. Two millilitres of urine was diluted with 2ml of deionized water. The sample was mixed and, using a 5ml disposable syringe, filtered through a 0.22 micron filter. Two hundred microlitres of the filtrate was injected onto the chromatograph, and analysed in the same manner as the standards.

The percentage distribution of the six main peaks was similar in standards, PEG 400-supplemented urine and urine from the subjects studied (Fig 3).

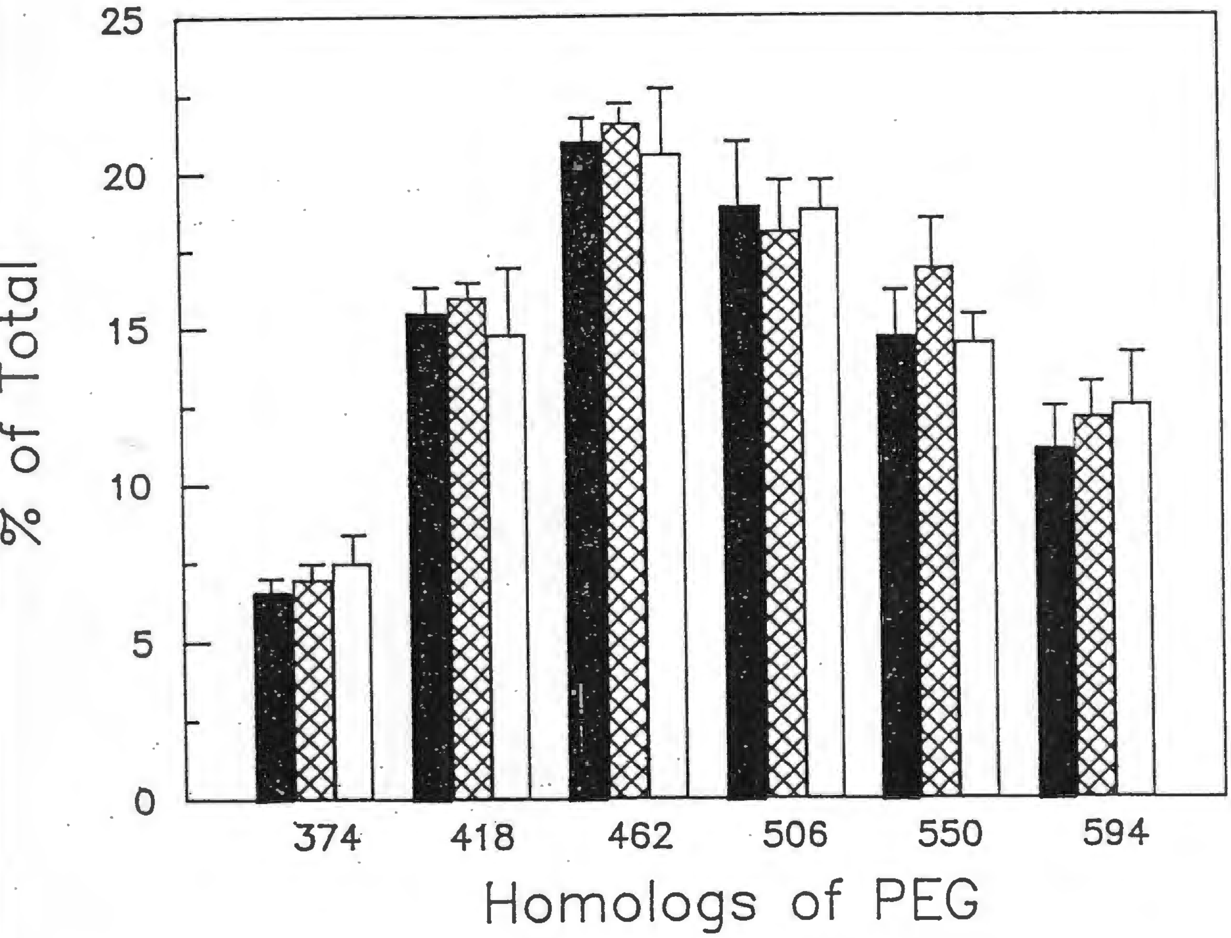

Fig.3. Relative abundance of each M_r fraction of PEG 400 as determined by HPLC.
The three bars for each fraction correspond to PEG standards, PEG-supplemented urines, or urines from subjects (left to right, respectively), mean $\pm$ SD.

Recovery of PEG 400 in urine

Polyethylene glycol recovery in urine was determined by the addition of PEG 400 to aliquots of pooled normal urine, to give concentrations varying from 0.2 to 1.6 grams per litre. A consistently high recovery of added PEG 400 was obtained at concentrations from 0.2-1.6g/l (Table 4).

Table 4. Recovery of added PEG 400

PEG 400 added (g/l)	0.2	0.4	0.8	1.6
% recovered (Mean)(n=9)	97.2	94.1	101.8	99.1
(SD)	3.9	2.7	5.4	5.4

Reproducibility of method

The standard error of measurement (SEM) between duplicate determinations was 0.87 (n=100). An aliquot of a single urine specimen was included in 10 analytical runs, over 10 separate days, to determine the reproducibility of the method. The Coefficient of Variation (CV) was found to be 3.3%.

Detectability

The lower limit of detection in a urine sample was at a concentration of 0.05g/l.

Stability of samples

The effect of freezing and thawing at -16^{0}C on PEG 400 in urine was examined. Ten samples were assayed before and after 3 months storage. The percentage excretion initially was 22.3$\pm$ 2.1% and after 3 months 21.5$\pm$ 3.1%.

COMPARISON OF THE TWO ANALYTICAL METHODS

All samples were re-analysed and results obtained with the new "direct injection" method were compared with results using the older extraction method of Delahunty and Hollander (1986). Both direct injection and extraction methods showed a similar percentage distribution of the six main peaks in standards, PEG 400-supplemented urine and urine from the subjects studied. The excretion values of PEG 400 for the `direct' injection method were generally higher than those obtained with the `extraction' method especially at the extreme range of excretory PEG 400 values (Figure 4). A significant correlation between the two analytical methods was, however, found in the midrange of PEG 400 excretory values.

The direct injection method was found to have several advantages, when compared to the previously described extraction method. The analysis of urine samples using the extraction method involves several preparative steps, prior to HPLC analysis. Loss of sample at any of these stages, with resultant `underestimation' of PEG 400 in urine is therefore a real possibility. This is reflected by a higher recovery of PEG 400 in urine using the `direct' injection method and also possibly the lower interassay Coefficient of Variation for the `direct' method (3.3%), when compared to the `extraction' method (13.3%).

The lower limit of detection (0.05g/l) obtained using the `direct ' injection method is similar to that found by Chadwick using gas chromatography (Chadwick et al, 1977a). It is notably more sensitive than the 0.5g/L for the `extraction' method.

Extraction, evaporation and reconstitution are unnecessary and thus a considerable saving of time was found with the direct injection method. Dilution and filtration of a sample prior to injection takes about 5 minutes and thus analytical time taken is mostly dependant on HPLC analysis time. In view of the good reproducibility, and low SEM,

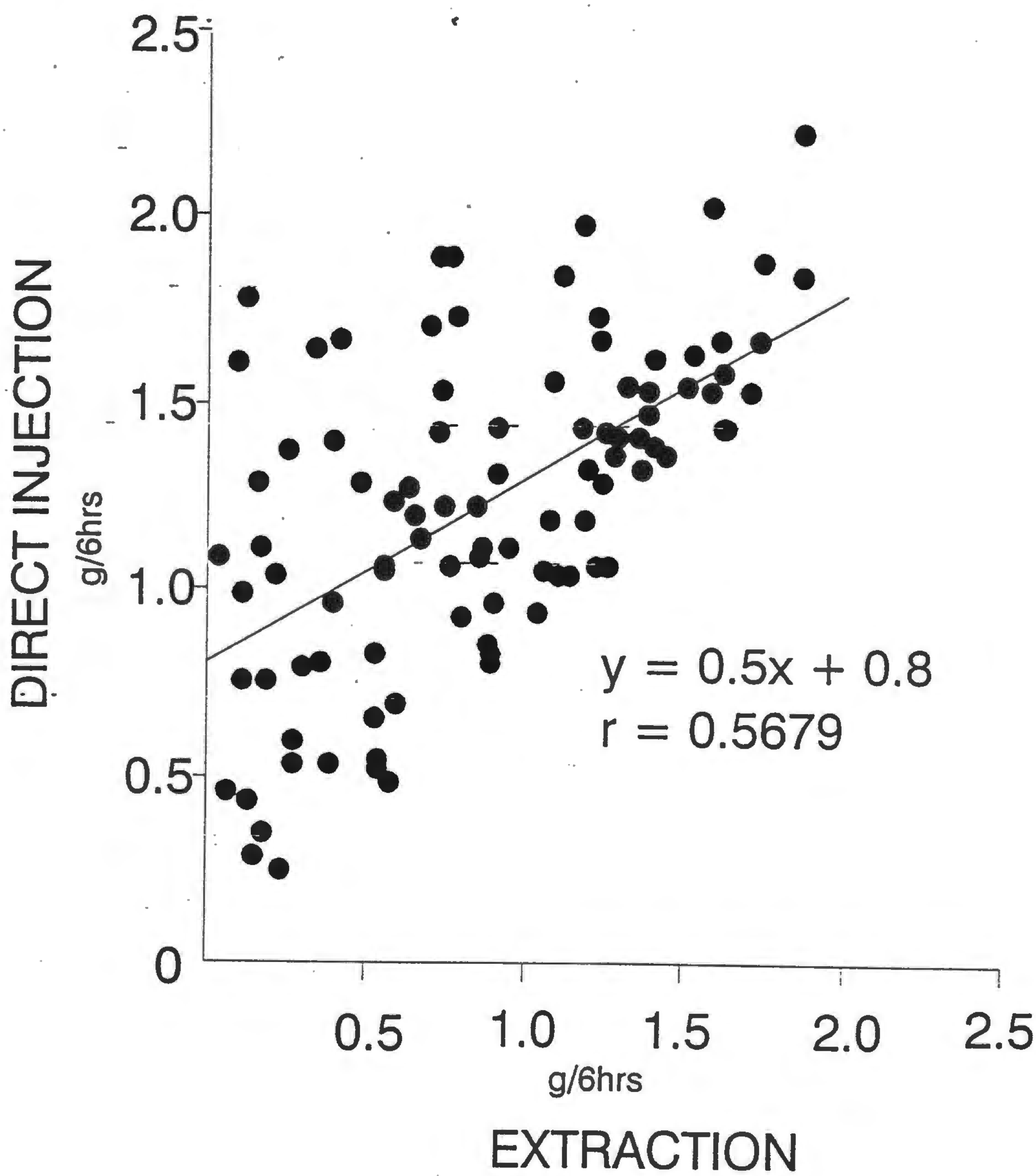

Fig.4. Comparison of PEG 400 excretion (g/6h) in 100 subjects as determined by direct injection and extraction methods.

duplicate analyses may be unnecessary: thus, with the use of an automated sampler, an analysis rate well exceeding 60 samples per day is possible. The time taken, however, to analyse any sample utilising the extraction technique, is in excess of 12 hours. Lyophilisation is a lengthy procedure, but was usually performed overnight. Furthermore, the extraction process and evaporation and reconstitution take about 40 minutes per sample.

As less sample is needed for analysis using the direct injection method, storage requirements are less.

Cost containment is also possible as fewer chemicals and less equipment is needed in analysis. The extraction technique involves the use of substantial quantities of chloroform for the extraction and nitrogen for evaporation.

The direct injection technique thus has the advantages of better sensitivity, precision and reproducibility. Furthermore, it is more cost-effective in terms of material and time.

REPRODUCIBILITY OF THE PEG-400 ORAL ABSORPTION TEST IN INDIVIDUAL SUBJECTS.

The oral PEG-400 absorption test was repeated in nine subjects at an interval of 5 months. The test-retest correlation coefficient was 0.74. The standard error of estimate between repeat determinations was 3.1. Individual values are shown in Figure 5.

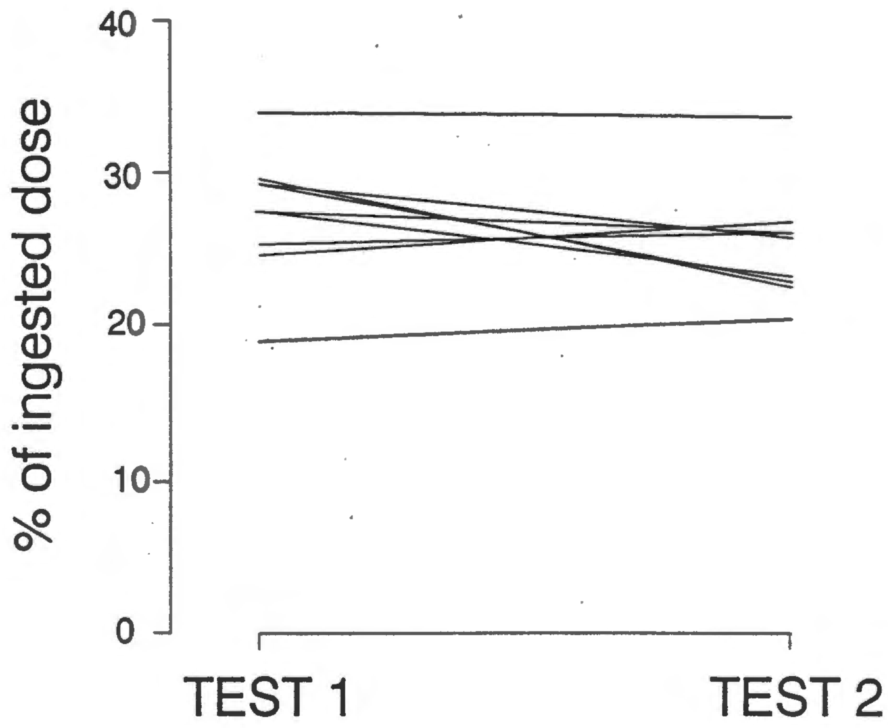

Fig.5. Reproducibility of the oral PEG 400 test. Values obtained on two separate occasions with an interval of 5 months are shown for individual subjects.

CHAPTER FIVE: RESULTS

Statistical analysis of all results showed a non-parametric distribution. Results are therefore expressed throughout as median and range.

The urinary excretion of PEG 400 as a percentage of the ingested dose is shown for all three groups in Table 7. There was no correlation between age (r=0.08) and urinary PEG 400 excretion within the group with Crohn's disease, the relatives, or the control group (Figure 6). No difference in urinary PEG 400 excretion was found between the males and females in all the groups (Tables 5 & 6, Figure 7). Furthermore, there was no difference in PEG 400 excretion between either men with Crohn's disease and healthy male controls, or between women with Crohn's disease and healthy female controls.

Table 5. Urinary PEG 400 recovery in all subjects

	n	Age	Median %	Range
Crohn's				
Males	19	20-72	23.2	7.6-33.4
Females	26	19-60	21.5	6.1-39.9
Family				
Males	8	13-55	22.9	18.8-39.9
Females	12	11-70	23.1	4.8-28.6
Controls				
Males	6	30-53	24.7	19.7-39.7
Females	25	24-72	25.0	4.5-36.2

Table 6. Gender of subjects and urinary PEG 400 recovery

	n	Median %	Range
Males	33	23.5	7.6 - 39.9
Females	63	23.1	4.5 - 39.9

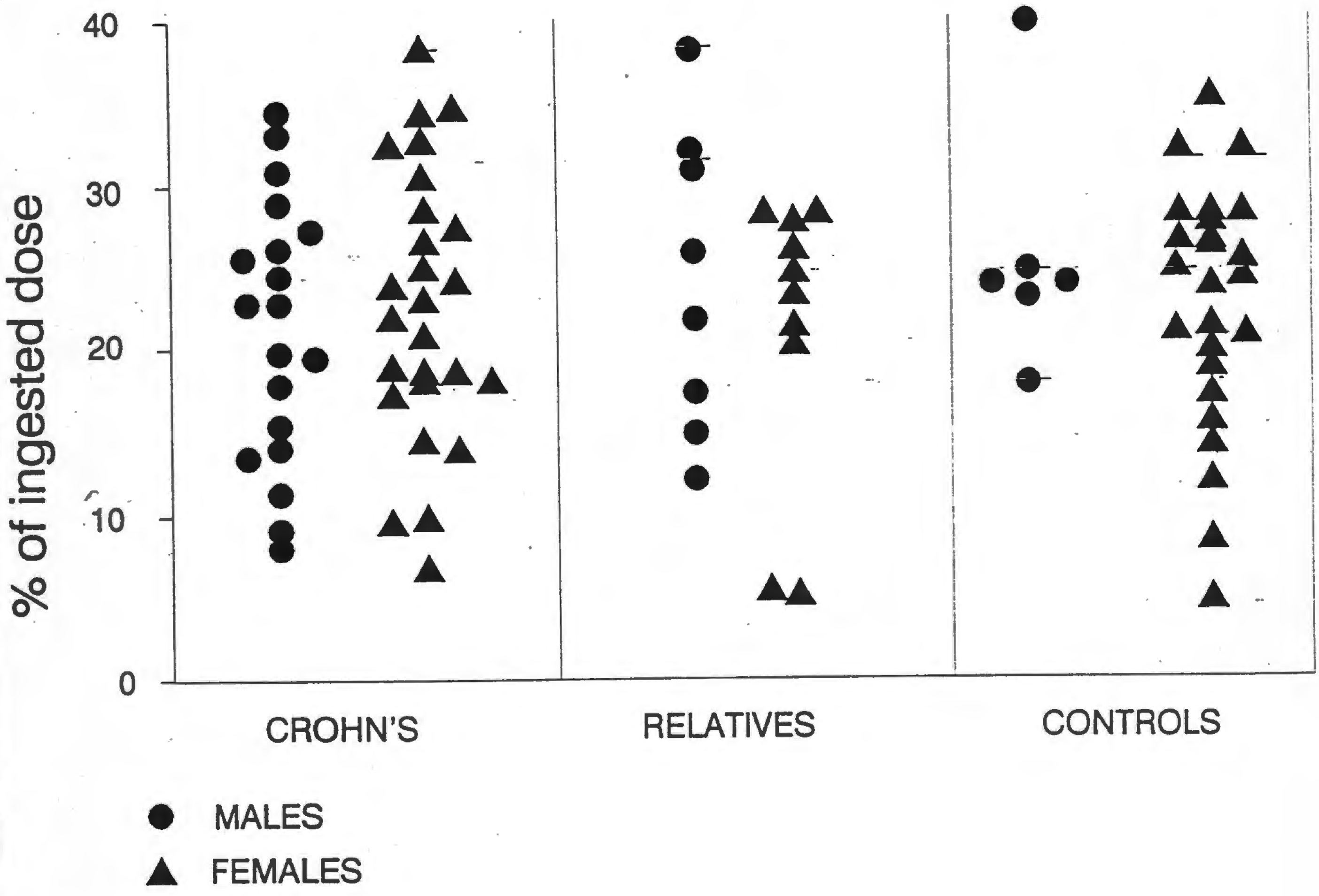

Fig.7. Relationship between gender and urinary PEG 400 excretion in patients with Crohn's disease, their relatives and controls.

The 45 patients with Crohn's disease excreted 21.9% (6.1-39.9%), the 20 relatives 23.7% (4.9-39.9%) and the 31 controls 25.0%_(4.5-39.7%) (Table 7). A wide scatter was evident and excretion for all three groups ranged from 4.55-39.9%.

Table 7. Urinary PEG 400 recovery

	n	Median %	Range
Crohn's	45	21.9	6.1-39.9
Family	20	23.7	4.9-39.9
Controls	31	25.0	4.5-39.7

No preferential absorption and excretion of individual PEG 400 polymers was found in any of the three groups studied (Figure 8).

On the basis of disease extent patients were further subdivided into groups viz:- those with ileal, ileocolonic or colonic disease (Table 8). Disease extent did not influence urinary PEG 400 recovery, but the numbers in each group were relatively small. Patients with ileal disease excreted 23.8% (7.7-30.6%), those with ileocolonic disease 22.6% (14.4-33.8%) and those patients with colonic disease 27.8% (9.5-33.5%).

Table 8. Urinary PEG 400 recovery in Crohn's patients according to disease extent*

		% of ingested dose	
	n	median	range
Ileal	14	23.8	7.7-30.6%
Ileocolonic	9	22.6	14.4-33.8%
Colonic	10	27.8	9.5-33.5%
Controls	31	25.0	4.5-39.7%

*resections not included.

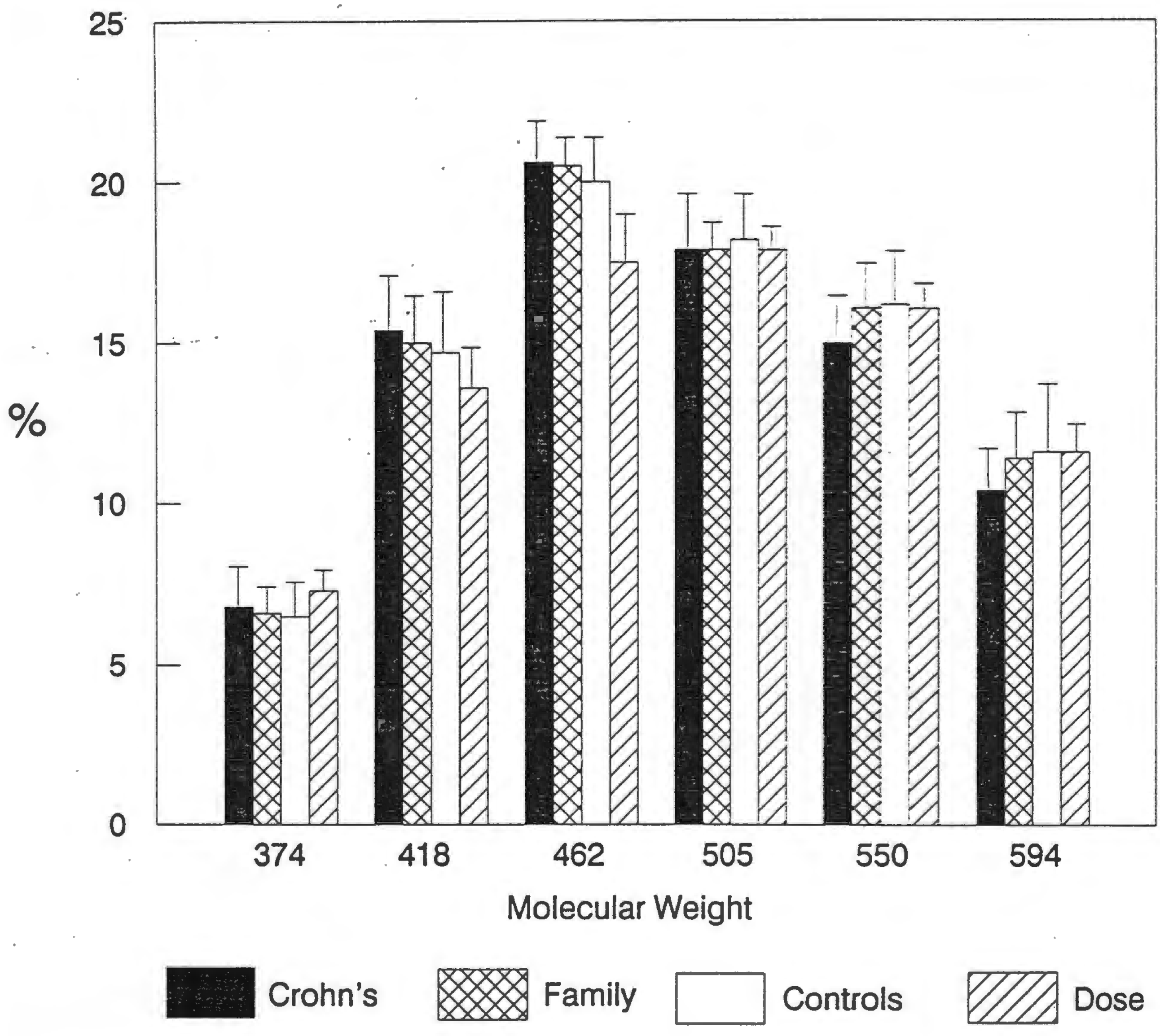

Fig. 8. Relative abundance of each M_r fraction of PEG 400 as determined by HPLC. The mean and standard deviation are shown

Twelve patients had undergone previous bowel surgery of varying length (Table 9). The median urinary PEG 400 excretion in this group was 18.8% (8.1-39.9%). While no difference in PEG 400 excretion was apparent between this group and the controls or the family, a significant difference was found between them and the non-resected Crohn's patients (23.5%, Quartile Deviation=5.8%, 6.1-33.8% $p<0.02$).

Table 9. Length of bowel resection and urinary PEG 400 recovery

Length of resection	Patient no.	% of ingested dose
Less than 5cm	1	39.9
	2	23.7
5 to 20cm	3	13.4
	4	19.4
	5	17.2
	6	15.2
	7	9.4
> 20cm	8	18.9
	9	8.1
	10	19.0

The non-resected group of patients was further subdivided according to disease activity using the van Hees and NCCD indices (Table 10). Patients with a score of less than 150 with either index were considered to be well. As adjudged by the van Hees Index, 10 patients were well. The median PEG 400 excretion in this group was 21.6% (14.2-39.9%). Twenty nine patients had significant disease activity and their median PEG 400 excretion was 22.2% (6.1-33.8%). On the basis of the NCCD index, 36 patients were well and had a median PEG 400 excretion of 22.7% (6.1-33.8%). Four patients who were ill excreted a median PEG 400 of 18.4% (13.5-25.2%).

Table 10. Disease activity and urinary PEG 400 recovery

Index	n	Status	% of ingested dose Median	Range
NCCD	36	Well	22.7	6.1-33.8
NCCD	4	Ill	18.4	13.5-25.2
V.Heese	10	Well	21.6	14.2-39.9
V.Heese	29	Ill	22.2	6.1-33.8

The effect of treatment alone (irrespective of clinical disease activity) was examined in the non-resected patients. There were 11 in the treatment group and 22 patients off treatment (Table 11). Patients on treatment excreted 24.4% (9.5-33.8%) of the ingested dose as compared to 23.3% (6.1-33.5%) in the untreated group.

Table 11. The effect of treatment on urinary PEG 400 recovery

		% of ingested dose	
	n	Median	Range
On Medication	11	24.4	9.5-33.8
Off Medication	22	23.3	6.1-33.5

CHAPTER SIX: DISCUSSION

CLINICAL PROTOCOL

The subjects in the study were allowed water only following the ingestion of polyethylene glycol 400. This is in accordance with all the other investigators using PEG 400. Previous work by Chadwick (1977a) using PEG 400, and more recent work using PEG 1000, has found no difference in urinary PEG excretion when ingested with or without food. In this latter study in 13 volunteers, using PEG 1000 as the probe, the protocol included the ingestion of a substantial breakfast consisting of rolls, coffee and tea followed some 3 hours later by three open sandwiches (Philipsen 1988). No significant difference in the 6 hour urinary PEG recovery was found between the fasting and non-fasting subjects.

In the present study, the dose of PEG 400 was mixed in 200ml of water. An unlimited quantity of water was allowed in the subsequent 6 hour urinary collection period. The osmolality of the ingested solution was 64 mOsmol (Normal 70-300). While hypertonic solutions are known to alter small bowel permeability *per se* (Norris 1973; Laker and Menzies, 1977), the ingested test solution would in any event have been diluted subsequently by the luminal bowel fluid. It was considered beyond the scope of this study to study the effects of differing osmolalities on the small bowel permeability.

A six hour urine collection period was chosen so as to permit comparative analysis with other investigators. It is highly improbable that a longer period of urine collection would have led to different conclusions. Chadwick (1977a) examined urinary excretion for a 48 hour period immediately after the ingestion of 10g of PEG 400. Twenty six percent of the ingested dose was excreted in the first 6 hours, a further 19% was found in the subsequent 6 to 12 hour period, 7.2% in the 12 to 24 hour period and 3.1% in the 24 to 48 hour collection period. Maxton (1986) examined a 24-hour time sequence after oral administration of 10g of PEG 400 and found that 16% of the ingested dose was excreted in the 5 hour period following ingestion. In view of the above, it was considered that any extension beyond a 6 hour collection period would not yield any significant information. Furthermore, twenty-four hour urine collections are cumbersome and subject compliance is a problem.

The test dose used in the present study was 5.6 grams. Absorption and excretion of PEG 400 polymers is proportional to the ingested dose and therefore follows first order kinetics. A larger test dose of PEG 400 would have not have yielded different results. In addition, the sensitivity of the direct injection methodology permits oral administration of a smaller dose of PEG. Furthermore, a 5.6 gram dose would also mean less possible sequestration of absolute amounts of PEG 400 in the tissues.

The effect of body mass on PEG 400 urinary excretion was not studied. While individual body mass was not measured, no subject was either particularly obese or emaciated, and therefore a constant oral dose of 5.6 grams of PEG 400 was used.

CLINICAL RESULTS

A large scatter in urinary excretory values was evident in all the groups. This is not easily explained. The reproducibility of the method was very good (CV=3.3%). Consequently, technical and methodological faults and inaccuracies are not likely to be responsible for the wide scatter. The standard error due to the intraindividual variation was 3.09, while the test-retest reliability coefficient was $r=0.74$. The latter value reflects the results of only nine subjects and a better correlation might be found with a larger sample. Alternatively, it may suggest that this scatter may not be biological, but rather, probe related, and probably reflects the inherent characteristics of the probe.

A large intragroup scatter has also been found by other investigators. Indeed, Jenkins et al (1986) had a range from 12.1 to 35.9% in their control group. Excretion of PEG 400 into the urine is not dependent solely on the gut mucosal permeability, but also its space of distribution in the body (Blatzinger et al, 1981). While data on the latter is not readily available, capillary filtration may play an important role in sequestration into the extravascular space (Tagesson and Sjodahl, 1984). While the effect of lean body mass on urinary PEG 400 recovery was not examined in the present study, all the controls were within the normal range of normality. The absolute amount of PEG 400 sequestrated in the tissues obviously differs from person to person and this may also be a contributing factor in the wide scatter.

A wide scatter has also been found by some investigators using ^{51}Cr-EDTA. Despite differences on a group basis, there was nevertheless, considerable overlap of the results for patients and controls in the studies of Peled et al (1985) and Ainsworth et al (1989). The control group in the study by Peled et al was not homogeneous as all the subjects had digestive pathology, albeit not of the small bowel. This may account for the considerable overlap with the Crohn's group. The finding of overlap found by Ainsworth et al may, however, reflect individual subject differences and is similar to that found in the present study. Overlap in urinary recovery values may also be a function of the probe. No overlap with normal control values has been found in patients with Crohn's disease using the probe DTPA (O'Morain et al, 1984).

RELATIONSHIP TO PERMEABILITY OF GENDER

No relationship was found between the gender of subjects and PEG 400 excretion in any of the groups (Table 5 & 6, Figure 7). There was also no difference in the 6-hour urinary PEG 400 excretion between the male patients with Crohn's disease and the control males, or between the Crohn's females and the control females. These findings are in agreement with the findings of Ainsworth et al (1989) using ^{51}Cr-EDTA.

RELATIONSHIP TO PERMEABILITY OF AGE

In the present study, while the youngest subject was 11 years old, the majority were mature adults. No correlation was found between age and PEG 400 excretion in any of the groups studied (Fig 6). No published data exists on the relationship between age and PEG 400 excretion.

Findings using the similar sized probe ^{51}Cr-EDTA have not proven any more conclusive. While a negative correlation was found between age and urinary ^{51}Cr-EDTA recovery by Aabakken (1989) no correlation between age and ^{51}Cr-EDTA excretion could be found by Ainsworth et al (1989).

There is, however, some evidence that ageing may affect permeability, particularly to larger molecules. An increase in bowel permeability to larger sized molecules has previously been documented in the neonate as compared to mature animals (Halliday, 1959; Clark, 1959; Westrom, 1984). As previously discussed, a process of closure occurs and the amounts of macromolecules subsequently absorbed through the healthy mammalian adult gut wall are insignificant. In a recent *in vivo* and *in vitro* study using PEG 900 however, an increased small bowel permeability was documented in senescent rats when compared to younger rats (Katz et al,1987). The difference between the smallest polymer of PEG 900 (mw=800) and the largest polymer of the PEG 400 used in the present study (mw=642) is not great. The possibility therefore exists that either the age spectrum in the present study was not wide enough, or that polyethylene glycol 400 may still, however, be too small a probe to detect an altered permeability within the age spectrum studied.

PERMEABILITY STUDIES IN NORMAL SUBJECTS

The PEG 400 excretory value for the control group (23.85%) is in agreement with those of other investigators. Chadwick (1977a) and Blatzinger et al (1981) using gas chromatography found an excretion of 22-25% of the ingested dose (10g) in controls. Jenkins et al (1986) found that the total 6-hour urinary PEG 400 excretion ranged from 12.1% to 35.9%. Robinson et al (1981) found a mean 6-hour urinary PEG 400 excretion of 21.2% following the oral ingestion of 10g of PEG 400. Magnusson found an average excretory value of 20% in a group of 24 healthy controls (Magnusson et al, 1983). Maxton et al (1986) found an average 5-hour urinary excretion of PEG 400 of 16% following the ingestion of 5 grams in 10 volunteers. In contrast, a mean excretion of 4% was found for the control group studied by Hollander (1986). This is at variance with the present study and other data in the literature. The methodology in this study involved lyophilisation of samples, and one can only speculate as study involved lyophilisation of samples, and one can only speculate as to whether the discrepancy could be explained

if several or, possibly, all the urine samples from the controls were freeze dried in a single batch, and that loss of sample occurred in this step.

Table 12. Urinary PEG 400 recovery in normal subjects

Study	Year	Dose	n	% of ingested dose
Chadwick et al	1977	10g	5	22
Blatzinger et al	1981	10g	17	25
Robinson et a[l]	1981	10g	7	21
Magnusson et al	1983	10g	2	19
Hollander et al	1986	5.6g	17	4

* Time for urinary PEG recovery was 5 hours. All other studies time for urinary PEG recovery was 6 hours.

PERMEABILITY STUDIES: CROHN'S PATIENTS VERSUS CONTROLS

No difference in PEG 400 excretion could be found between the patients with Crohn's disease and the controls. These findings are in contrast with the findings of other investigators also using PEG 400 (Table 13). Jenkins et al (1986) and Magnusson et al (1983) found that patients with Crohn's Diseas. While the exact number is not given, most of the patients in Magnusson's study had previously undergone intestinal resections and a decreased uptake and excretion of PEG 400 would thus have been anticipated. Details of possible resections are not given for the fifteen patients comprising the Crohn's group studied by Jenkins' et al. Severity of disease may, however have been a factor, for all these patients had moderate to severe Crohn's disease activity. In contrast to these studies, the patients witt to these studies, the patients with Crohn's disease in the study of Hollander et al (1986), were found to excrete up to twice the amount of PEG 400 when compared with the controls. While the values for the Crohn's patients are comparable to those in the literature, the values for the control group as previously noted, were extremely low and not consistent with the literature. This may explain the apparent increased permeability in the patients, relative to the controls. Moreover, this finding is surprising in view of the fact that 7 of the 11 Crohn's patients in this study had previously undergone bowel resection and could have been expected to have a decreased rather than an increased excretion.

Table 13. Urinary PEG 400 recovery in Crohn's patients and controls

Investigators	Crohn's patients		Controls	
	n	%recovered	n	%recovered
Magnusson et al, 1983	24	14	24	20
Hollander et al, 1986	11	9	17	4
Jenkins et al, 1986	15	15	40	19

Several investigators have found decreased small bowel permeability to PEG 1000 in Crohn's patients as opposed to controls (Heuman et al, 1982) (Olaison et al, 1987;

Olaisson et al, 1989). All the patients in the first study and a substantial percentage of the patients in the last study had, however, undergone previous bowel resections. As the effect of resection was found to be unrelated to permeability in the second study, an alternative explanation is that the decreased excretion is also reflective of absorptive defects in apparently uninvolved areas of bowel.

Table 14. Summary of studies of intestinal permeability with Cr-EDTA in patients with Crohn's disease.

Investigator	**Crohn's** n	**Controls** n	**Crohn's vs Controls**
Bjarnason et al, 1983	32	28	INCREASED
Jenkins et al, 1985	54	48	INCREASED
Peled et al, 1985	6	27	INCREASED
Jenkins et al, 1987	100	80	INCREASED
Ainsworth et al, 1989	15	28	INCREASED

Studies in patients with Crohn's disease and controls, comparing urinary recovery, following ingestion of ^{51}Cr-EDTA, have yielded more consistent results (Table 14). Patients with Crohn's disease have been consistently found to excrete significantly more ^{51}Cr-EDTA than healthy controls. Differences in probe characteristics and size may account for the variance of the data between ^{51}Cr-EDTA and with that using PEG 400 and PEG 1000. While the route of uptake of both PEG and ^{51}Cr-EDTA is thought to be via the tight intercellular junctions, their physiochemical characteristics differ markedly.

The finding of an increased permeability in patients with Crohn's disease has also been demonstrated using the probe DTPA. In a study by Casellas et al (1986), urinary recovery of DTPA was greater in 16 patients with Crohn's disease than in 10 healthy controls. This is in agreement with the findings of O'Morain et al (1984), where patients with Crohn's disease of the small bowel (n=18) had a significantly higher urinary excretion than controls (n=15).

Tests using carbohydrates as probes have consistently shown an altered permeability as measured by the excretory ratios of sugars (Table 15). Analysis of the individual sugars of the ratio, however, show that, while in general, absorption of mannitol is decreased and that of lactulose increased in patients with Crohn's disease as compared with controls, this is not always the case. Pearson et al (1982) found a large increase in permeability in 8 patients with Crohn's disease when compared with 31 controls. Analysis of individual sugars reveals that while permeability to lactulose was increased in the patients relative to the control group, that of mannitol was unchanged. Furthermore, the test solution administered was hypertonic. In the study of Murphy et al (1989) and Katz et al (1989), while the urinary excretion of lactulose was increased, that of mannitol and rhamnose was unchanged in the patients with Crohn's disease relative to controls.

Table 15. Summary of studies of intestinal permeability using carbohydrate probes.

Author	Year	Sugars	Permeability in Crohn'S Versus Controls
Pearson et al	1982	lactulose/ mannitol	INCREASED
Ukabam et al	1983	lactulose/ mannitol	INCREASED
Sanderson et al	1987	lactulose/ rhamnose	INCREASED
Andre et al	1988	lactulose/ mannitol	INCREASED
Murphy et al	1989	lactulose/ mannitol	INCREASED
Katz et al	1989	lactulose/ rhamnose/mannitol	INCREASED

PERMEABILITY STUDIES IN RELATIVES

No evidence of an altered small bowel permeability to PEG 400 could be found in patient relatives in the present study. This is in contrast with the only other study in the literature examining patient relatives using PEG 400 (Hollander et al, 1986) and consequently at variance with the concept of family members having occult inflammation or predisposing permeability disorder. Two other studies using different probe-markers, have examined small bowel permeability in relatives of patients with Crohn's disease (Table 16).

Table 16. Permeability in relatives compared with controls

Investigator	**Year**	**Probe**	**Relatives vs Controls**
Ruttenberg et al	1989	PEG 400	NO DIFFERENCE
Hollander et al	1986	PEG 400	INCREASED
Ainsworth et al	1989	^{51}Cr-EDTA	NO DIFFERENCE
Katz et al	1989	lactulose/ rhamnose/mannitol	NO DIFFERENCE

All the probe-markers used, although of differing chemical characteristics, are of a similar size. As previously noted, PEG 400, ^{51}Cr-EDTA and lactulose are thought to permeate via the paracellular route, while rhamnose and mannitol permeate via the transcellular route. In spite of this, no evidence of an altered permeability has been found in patient relatives. In the study using carbohydrate probes in relatives of patients with Crohn's disease (Katz et al, 1989), neither lactulose, rhammnose nor mannitol could detect evidence of an altered permeability in family members. The above data would therefore suggest that a primary defect is not, in fact, present in patients with inflammatory bowel disease.

SELECTIVE PERMEABILITY

The composition of the PEG 400 in urine from all subjects was similar to that of the ingested dose. There was no evidence of preferential uptake and excretion of any individual PEG 400 polymers in the patients with Crohn's disease, their relatives or the control group (Fig 8). This is in agreement with the findings of Jenkins et al (1986) and Blatzinger et al (1981) where no evidence of selective permeability to PEG 400 was found in either the Crohn's patients or the controls. In addition, no evidence of selective permeability could be found in patients' relatives in a recent study by Hollander et al (1986). This absence of selective permeability is in contrast with the findings of Maxton (1986) and Magnusson (1983) where considerably more of the smaller molecular weight species of PEG 400 were found in the urine of patients and controls.

Table 17. Summary of studies using different sized PEG probes

Investigator	**Year**	**PEG Size**	**Selective permeability**		
			Crohn's	**Controls**	**Family**
Blatzinger et al	1981	400	-	NO	-
Magnusson et al	1983	400	YES	YES	-
Hollander et al	1986	400	NO	NO	NO
Jenkins et al	1986	400	NO	NO	-
Maxton et al	1986	400	-	YES	-
Olaisson et al	1988	600	NO	-	-
Olaisson et al	1989	600	NO	NO	
Tagesson et al	1983	1000	-	YES	-
Olaisson et al	1987	1000	YES	YES	-
Olaisson et al	1989	1000	YES	YES	-

While not comparable to oral absorption studies, Olaison et al (1988, 1989), could find no evidence of preferential uptake of the slightly larger PEG 600 (mw, 590-942) in a recent *in vivo* study, examining regional absorption in ligated loops of small bowel (Table 17). The contradictory evidence in the literature would suggest that selective permeability may not, however, be consistently apparent using lower molecular weight species of PEG. Indeed, the data for a selective permeability is more consistent with the higher molecular weight PEG 1000. Tagesson et al (1983) and Olaisson et al (1987 and 1989) found that urinary recovery of orally ingested PEG 1000 (mw, 634-1294) was inversely proportional to the individual molecular weights in both patients with Crohn's disease and controls. An interesting finding by Olaisson et al in 1989, was the loss of selective permeability to PEG 1000 following direct colonic instillation of the marker in patients with Crohn's disease. This would suggest that any filtering and preferential uptake occurs at the level of the small intestine.

Following intravenous administration of PEG 400 in pigs, urinary recovery of PEG 400 is directly proportional to molecular size for PEG 400, but not for PEG 1000 (Tagesson & Sjodahl 1984). Indeed, capillary filtration of the smaller molecular weight PEGs may play an important role in sequestration of the latter into the extravascular space (Tagesson & Sjodahl 1984). Evidence of preferential permeability following orally administered PEG 400 must therefore take into account non-intestinal factors.

EFFECT OF DISEASE SITE ON URINARY PEG RECOVERY

Polyethylene glycol is thought to be absorbed primarily in the proximal small bowel and absorption decreases distally (Chadwick et al, 1977). The finding by Magnusson et al (1983) of a decreased excretion of PEG 400 in a patient after a jejunal resection would seem to support this.

In accordance with current thinking permeability to PEG 400 could be either increased or decreased in patients with ileal disease. Some investigators have speculated about a tightening of the cellular tight junctions with any inflammation. Bowel permeability to PEG 400 would, under these circumstances, be decreased. If, however, one subscribes to the theory of a widening and disruption of the paracellular junctions with inflammation and ulceration, then an increased permeability to PEG 400 would have been expected. While an altered permeability might therefore have been expected in patients with ileal disease no difference in PEG 400 excretion between the patients with ileal disease and controls was found (Table 9). These findings are similar to those of Casellas et al (1986) using the similar sized probe, DTPA. The number of patients in the study of Resnick et al (1990) was too small to allow statistical analysis.

The lack of differences in the present study may be a reflection of the probe size rather the probe structure. Some support for this may be found in the study by Olaison et al (1989), where, using PEG 1000 as the probe, patients with ileal Crohn's disease had decreased absorption when compared with controls (Olaison et al, 1989).

Patients with colonic Crohn's disease excreted a similar amount of PEG 400 as patients with ileal or ileocolonic disease or controls in the present study (Table 9). While there is no comparable data for PEG 400, PEG 1000 was used as the probe in a recent study by Olaisson et al (1989). This showed that, in patients with colonic disease, urinary recovery following an oral load of PEG 1000 was less than that in patients with ileal disease or controls (Olaisson et al, 1989). This would be compatible with the theory of increased permeability due to inflammation and swelling of the tight junctions in the site of predominant PEG absorption. In attempting to reconcile the differences, the authors, however, suggested that the impaired colonic absorption relative to the controls may denote a more severe variant of Crohn's disease. Similar results (of impaired colonic permeability relative to ileal absorption) have also been found using the probe ^{51}Cr-EDTA (Ainsworth et al, 1989). Direct colonic instillation of the PEG 1000 has been found to result in the reverse situation; i.e. urinary recovery of PEG 1000 is greater from the patients with colonic Crohn's disease than from the patients with ileal disease (Olaison et al, 1989). The methodology used in the intracolonic administration involved instillation and deposition of the probe just below the splenic flexure in the colon thus excluding ileal permeability. It may be speculated that while these results may have been anticipated, it may alternatively, also reflect more severe colonic disease.

There is conflicting evidence concerning the permeability to ^{51}Cr-EDTA and sugars in patients with colonic disease. Both an increased (Bjarnason et al, 1983) and a similar (Ainsworth et al, 1989) excretion viz a viz controls has been reported with ^{51}Cr-EDTA. Methodological differences may account for these disparate results using the same probe. An alternative explanation (Bjarnason et al, 1983) was that it may also represent the heterogenicity found in Crohn's colitis which is indistinguishable from ulcerative colitis in 10 to 15% of cases (Price 1978).

Patients with colonic Crohn's disease have been found to have either a decreased or an unaltered absorption of mannitol relative to controls (Ukabam et al, 1983; Andre et al, 1988; Katz et al, 1989). Interestingly, although the number of subjects in the former study is small, no difference in intestinal permeability was found between the 7 colonic Crohn's and 16 controls as measured by lactulose absorption. Katz et al (1989) could also find no difference in lactulose absorption and excretion between patients with colonic disease and controls. This is in agreement with the findings of the present study. Lactulose and PEG 400 are of a similar size and are thought to permeate via paracellular tight junctions. The large number of conflicting results using the same probes would suggest that no probe is at present site specific.

EFFECT OF PREVIOUS BOWEL RESECTION ON URINARY PEG RECOVERY.

A significantly lower PEG 400 excretion was evident in patients who had undergone previous resections, when compared with non-resected patients. As surface area may be related to absorption and permeability, these findings are compatible with an overall decreased uptake and excretion of PEG 400. No difference, however, in urinary PEG 400 excretion was evident between patients with Crohn's disease who had undergone a previous resection and the healthy controls. The possibility does, therefore, exist that this significance is due to statistical random chance. The findings of a lack of influence of intestinal resection (within the patient group) in at least two other studies, using carbohydrate probes, would seem to support this concept of statistical random chance (Andre et al, 1988; Katz et al, 1989).

While a tendency towards a decreased absorption and excretion of PEG 400 was found by Hollander (1986) in his 7 patients who had undergone intestinal resections, no statistically significant differences could be found between them and the non-resected patients or the controls. The resected group in the present study included patients with varying lengths of bowel resection (Table 9).

While the extent of surgery varied considerably between patients, there were too few patients in this group to correlate PEG 400 excretion and length of bowel resection. Only one study has thus far correlated length of intestinal resection and PEG excretion (Heumann et al, 1982). Here, a negative correlation was found between length of resection and 6 hour urinary recovery of PEG 1000. A tendency towards a negative correlation was also found by Olaison et al (1987).

Surprisingly, an increased small bowel permeability to PEG 400 immediately (6 weeks) following intestinal resection has also been documented (Magnusson et al, 1983).

This, the only study comparing urinary PEG recovery pre and post resection, was based on the findings in only two patients.

Some support for these interesting findings can be found in animal studies. Changes are known to occur in the remnant small bowel of the rat following intestinal resection. Mucosal hyperplasia occurs and is usually considered maximal at about 4 weeks post resection (Williamson et al, 1978). An increased mucosal cell turnover in the remnant small bowel may manifest as a consequent functional immaturity with an associated increased permeability. In a recent study in rats, using ^{14}C-erythritol, the effect of extent of small bowel resection on erythritol clearance was studied in the small and large bowel, 4 weeks post resection (Vacquez et al, 1988). It is noteworthy that a significantly increased clearance of erythritol per unit small bowel mass was found only when 80% of the small bowel had been resected. Erythritol and PEG 400 are thought to have similar routes of permeation via the paracellular junctions. While the increase in permeability in the above animal study may be compatible with the increase found by Magnusson (1983), it is highly unlikely that his patients had had an 80% small bowel resection.

The increased permeability documented in the above animal studies after surgical small bowel resections may be related to the probe size. Olaison et al (1989) could find no differences in 6 hour urinary recovery of PEG 1000 between patients with and without ileostomy and between patients with and without previous colectomy. Uban et al (1983) could also find no evidence of an altered permeability to the even larger PEG 4000 in the small bowel of resected and sham operated rats (Urban et al, 1983).

EFFECT OF DISEASE ACTIVITY ON URINARY PEG RECOVERY.

Disease activity as assessed by clinical and laboratory indices did not seem to influence permeability to PEG 400. Both the NCCD (Best et al, 1976) and van Hees (Van Hees et al, 1980) activity indices were used as more objective assessments of disease activity. With either index, a score of less than 150 is indicative of disease remission. Most of the patients with Crohn's disease were clinically well according to the NCCD index (Table 10). If intestinal permeability to PEG 400 in any way reflects inflammatory change and mucosal damage, either an increased, or a decreased permeability to PEG 400 should have been evident in the patients who were ill. This was not so in this study where no difference in urinary PEG 400 excretion was found between the patients who were well and the patients with more active disease. This is in agreement with the data of Olaison using the larger sized PEG 1000 where no differences in urinary PEG 1000 recovery could be found between non-resected inactive patients (n=11), and previously resected patients with active (n=14) and inactive (n=19) disease (Olaison et al, 1987).

Results of permeability studies and disease activity are contradictory (Table 18).

Table 18. Influence of disease activity on intestinal permeability

Investigator	Year	Probe	Influence of disease activity on permeability
Present study	1989	PEG 400	NO INFLUENCE
Olaison et al	1987	PEG 1000	NO INFLUENCE
Jenkins et al	1987	^{51}Cr-EDTA	INCREASED
Ainsworth et al	1989	^{51}Cr-EDTA	NO INFLUENCE
Bjarnason et al	1983	^{51}Cr-EDTA	NO INFLUENCE
Casellas et al	1986	DTPA	INCREASED
Resnick et al	1990	DTPA	INCREASED
Ukabam et al	1983	lactulose/ mannitol	NO INFLUENCE
Gomes et al	1983	lactulose/ rhamnose	INCREASED
Sanderson et al	1987	lactulose/ rhamnose	INCREASED
Murphy et al	1989	lactulose/ mannitol	INCREASED

Several groups have studied the relationship between disease activity and permeability to ^{51}Cr-EDTA. While Jenkins et al (1987) found a significant increase in patients with active disease, Ainsworth et al (1989) and Bjarnason et al (1983) found no relationship between disease activity and permeability to ^{51}Cr-EDTA. The latter findings are in agreement with those of the present study.

Casellas et al (1986) and Resnick et al (1990) both found a direct relationship between disease activity and permeability to DTPA.

Contradictory findings have also been made in studies using carbohydrates as probes. While the findings of the present study are in agreement with those of Ukabam et al (1983) who could find no correlation between disease activity and permeability to these sugars in 20 patients with Crohn's disease, they are in contrast with those of Gomes et al (1983), Sanderson et al (1987) and Murphy et al (1989). The use of different disease activity indices may account for these discrepancies. Permeability to mannitol and lactulose has more recently been found to correlate with CDAI as predictors of disease relapse (Valpiani et al, 1990).

EFFECT OF TREATMENT ON URINARY PEG 400 RECOVERY.

Overall, treatment did not influence urinary PEG 400 excretion. Indeed most of the patients with Crohn's disease were not on medication of any kind. In view of their mode of therapeutic action, a decreased absorption of PEG 400 might have been anticipated, in patients on oral steroids due to the reduction in inflammation. No conclusions, however, could be drawn from individual treatment regimes in the present study because of the relatively small numbers of patients involved.

These findings are also in agreement with the findings of Jenkins et al (1987), where urinary recovery of ^{51}Cr-EDTA was independent of medication.

The use of an elemental diet in the management of patients with Crohn's disease has been found to result in an improvement in the altered permeability as reflected by the excretory ratio of mannitol and rhamnose (Sanderson et al, 1987). This data would support the concept of the inflammatory changes being a secondary rather than a primary phenomenon.

CHAPTER SEVEN: COMPARISON BETWEEN PEG 400, ^{51}CR-EDTA, DTPA AND CARBOHYDRATE PROBES.

The molecular size spectrum that comprises PEG 400 coincides with rhamnose and mannitol at one end, and lactulose and ^{51}Cr-EDTA at the other end. In only two studies has PEG 400 been compared with these similar sized probes. These studies have involved the simultaneous ingestion of lactulose, mannitol, ^{51}Cr-EDTA and PEG 400. In the study by Maxton et al (1986), 10 volunteers ingested lactulose (mw=342), rhamnose (mw=164), PEG 400 (mw=400) and ^{51}Cr-EDTA (mw=340), simultaneously in the form of an isotonic solution. Paper, thin-layer and gas chromatography were used in urinary analysis of probes collected over a 24 hour period, following oral and intravenous administration. The effect of a hyperosmolar solution on urinary recovery was also examined.

Following intravenous administration, the percentage urinary recovery of all the markers with the exception of PEG 400, was almost complete. While the overall urinary recovery of PEG 400 was much lower (40%), a linear relationship between the individual polymer size of PEG 400 and percentage urinary recovery was observed. This supports the concept of urinary underestimation of PEG 400 following oral administration and furthermore, as renal clearance of PEG 400 is similar to that of creatinine (Robinson et al, 1981), also suggests sequestration into the tissues (Maxton et al, 1986).

Following oral ingestion of all the markers, intestinal permeation was greatest in the first 6 hours of urinary collection. Quantitatively, however, permeation of individual polymers of PEG 400 was markedly greater than the other markers. Maxton found that at the lower end of the spectrum, permeation by the PEG 400 polymer with a molecular weight of 194 was 3 times greater than the similar sized rhamnose. Permeation of the PEG 400 polymer with a molecular weight of 326 was over 100 times greater than the similar sized lactulose and ^{51}Cr-EDTA (Maxton et al, 1986). The response to the hyperosmolar stress by PEG 400 also differed to that shown by the other probe-markers. While increased permeation was found to all probe-markers, no correlation could be found between PEG 400 and the others.

All the above data support the concept of two pathways of permeation in the small intestine. Particles with a radius of less than 0.4nm pass mainly through numerous small pores. Molecules with a larger radius like lactulose (radius greater than 0.5nm) would seem to pass through a few larger sized pores. While the exact route of PEG 400 uptake is unclear at present, it would seem to involve partition through the lipid cell membranes as well as via pores (Maxton et al, 1986).

No correlation has thus been found between the permeation of PEG 400 and lactulose, ^{51}Cr-EDTA, L-rhamnose or D-mannitol (Maxton et al, 1986). In a more recent study in smokers and non-smokers by Prytz et al (1989), no correlation between urinary recoveries of ^{51}Cr-EDTA and the urine recoveries of PEG 400 could be found. Intestinal pathways for PEG 400 thus do not relate specifically to other similar sized molecules.

While the carbohydrates have been used as markers of an altered intestinal permeability, their value is best seen when urinary recovery is expressed as a ratio. Thus, patients with Crohn's disease have an elevated ratio when compared with controls. From the literature, it would seem that results of individual sugar quantitation are not consistent between patients and controls. While a marker of disease presence and, maybe, disease activity, their role in association with disease site needs further evaluation in view of the discrepancies already found between investigators. Furthermore, a morphologically abnormal small bowel mucosa does not seem to be a prerequisite for an abnormality in permeability, as measured by sugar excretory ratios. Strobel et al (1984) found a group of patients with normal jejunal histology associated with abnormal permeability to the carbohydrates mannitol, cellobiose, sucrose and lactose. As noted previously, hypertonicity *per se* may alter intestinal permeability (Laker and Menzies, 1977)(Wheeler et al, 1978). The increase in small intestinal permeability to lactulose appears to be especially marked when the lactulose is given in a hypertonic solution (Budillon et al, 1982). Leaky and disrupted tight junctions as a result of the hypertonicity *per se* could explain this (Laker and Menzies, 1977).

While a useful probe, the use of ^{51}Cr-EDTA, as a screening test for patients with Crohn's disease, would seem to be limited by its large intra-individual variation in normal subjects and the fact that significant quantities are absorbed in the normal colon. In addition, it does not seem to be a useful marker for site or degree of inflammatory disease.

[^{99}mTc]DTPA would seem to have distinct advantages as a probe of intestinal permeability over either PEG or ^{51}Cr- EDTA. Further studies are needed, however, to evaluate its potential role as a marker of site of disease and to evaluate the effect of previous bowel resection on urinary recovery.

Small quantities of PEG 400 are absorbed under normal circumstances. The use of entirely non-absorbable larger PEGs would thus be preferable. Indeed, certain polyethlyene glycols are virtually non-absorbed (Shaffer 1947). Golytely, a colonic lavage preparation, consists largely of PEG 3350 and its absorption has been extensively studied. Only 0.06% of an oral dose could be recovered in the urine of 10 volunteers over 2 days. Schedl et al (1966), using turbidimetric methods, were unable to demonstrate any urinary PEG 400 excretion, following steady state intestinal perfusion with the probe. It is not known, however, whether complete recovery of the intraluminal solution was possible in these early experiments. Shields et al (1968), also showed complete recovery of the larger molecular weight PEG 4000 in rectal effluent, following perfusion of the human colon with this marker.

Other workers have shown that the quantity of polyethylene glycol 4000 that can be recovered in the stools following oral ingestion, varies from 75% (Beeken 1967) to 97% (Hyden 1956). In this latter study, however, more PEG was found to be excreted in the urine than had been orally ingested in some of the subjects. Methodological differences could account for discrepancies between these and later studies.

The use of polyethylene glycol 400 as a permeability probe has been questioned. In addition to its size, excretion of PEG 400 into the urine is not solely dependent on the gut mucosal permeability, but also its space of distribution in the body and permeability profile by the kidney (Blatzinger et al, 1981). Studies have shown, however, no relationship between urinary volume and renal clearance and PEG 400 excretion (Robinson et al, 1981). Nevertheless, data on sites and routes of sequestration of PEG 400 is not available at present.

CHAPTER EIGHT: SUMMARY

The literature is replete with conflicting and confusing data regarding altered small bowel permeability in patients with Crohn's Disease. An increased and a decreased permeability have been documented to PEG 400 and other similar sized probes .
A new more sensitive and accurate HPLC technique for the analysis of PEG 400 was developed and compared with the previously described extraction method. There was good analytical recovery (94-102%) and reproducibility (CV=3.3%). Direct injection of dilute urine samples onto the analytical column proved to be more economical in time and chemicals and also allowed a rapid rate of analysis.

The direct injection method was subsequently used to examine intestinal permeability in a group of patients with Crohn's disease, some of their first degree relatives and a group of healthy controls. A wide scatter in urinary recovery was evident in all the subjects studied. The wide scatter of results found in the present study was, however, not related to technical or methodological reasons.

There was no evidence of an altered permeability to PEG 400 between patients with Crohn's disease, their relatives or healthy controls. The 6 hour urinary recovery of orally ingested PEG 400 was found to be independent of the following variables: age, sex, site of disease, bowel resection, inflammatory disease activity and medication.
The results found in the present study are in accord with those of the literature with respect to the lack of association between age, sex, disease activity and medication and intestinal permeability as measured by the urinary recovery of orally ingested PEG 400. There does not, however, seem to be agreement on the influence of the presence and site of disease, and the effect of previous bowel resection.

CHAPTER NINE: CONCLUSION

In spite of the examination of a larger group of subjects than has previously been studied, and the use of refined, more sensitive and accurate technology, no evidence of an altered bowel permeability to PEG 400 could be found between subjects with Crohn's disease and healthy controls or in relatives of patients with Crohn's disease. The wide scatter in results, the test-retest reliability coefficient of 0.74, together with the lack of concurrence in the literature, would suggest that, either no altered intestinal permeability difference exists in patients with Crohn's disease, or that PEG 400 is not the ideal probe with which to examine this.

The pathways of uptake and physiochemical characteristics of smaller sized molecules differ. The findings and presence of an altered permeability to any one molecule cannot be extrapolated and do not necessarily correlate with an altered permeability to another similar sized molecule. The only conclusion that can be drawn is that an altered permeability exists with respect to that particular probe.

The existence of an altered bowel permeability to such a molecule may not, in any event, be of clinical relevance, as most antigenic molecules are up to 50 times larger. As most studies have confined themselves to the smaller sized probes without antigenic properties, this question remains largely unanswered.

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