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pH STUDIES IN COLOSTOMY PATIENTS CONSUMING DIFFERENT DIETS

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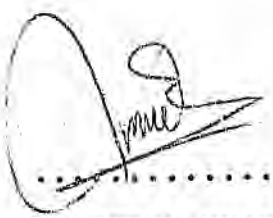
*

A dissertation submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in Medicine.

Johannesburg, July 1991.

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science (Medicine), in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.



..... 30/7/91.....

DEMETRIS CHARALAMBIDES DATE

The work reported in this dissertation was carried out in the Department of Surgery and the Gastroenterology Unit, Baragwanath Hospital, and the University of the Witwatersrand, Johannesburg, South Africa.

This project was approved by the Committe for Research on Human Subjects, University of the Witwatersrand (Protocol No 45/2/89).

This work is dedicated to -

* the memory of my late father

* my mother

* my wife

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It is with deep appreciation that the following persons are aknowledged -

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ABSTRACT

Colonic pH is important in the regulation of colonic cell growth, control of absorption and secretion, and bile acid degradation which may be a key step in the development of colon cancer.

The aim of this study was to determine differences in mucosal, luminal and faecal pH in the right and left colon of otherwise healthy patients, who underwent colostomies because of trauma.

The pH was evaluated while patients consumed different diets. In addition the mucosal, luminal and faecal ph in patients with colostomies carried out for colorectal cancer was also assessed. The ph was determined in 15 black patients, divided into three groups. Group A: 5 Patients with right sided colostomies; [colostomies performed after trauma to defunction the transverse or left colon]; Group B: 5 patients with left sided colostomies (sigmoid colostomies); [colostomies performed after trauma to defunction the sigmoid colon or rectum]; Group C: 5 patients with left sided colostomies (sigmoid colostomies); [colostomies performed for rectal cancer]. All patients consumed three consecutive different diets of 5 days duration, and the pH was evaluated on the last day of each diet. (i) A western diet, (ii) A high fibre diet and (iii) A high starch / low fibre diet. Breath methane was also determined in 12 of the above patients.

The results showed that mucosal pH was alkaline (pH 8) and was

similar on both sides of the colon of healthy colostomates and in colon cancer patients. Luminal pH (7.6) was the same in the 3 groups and was significantly lower than the mucosal pH. Faecal pH was significantly lower in right colostomates (5.8) compared to left (6.3) and in healthy colostomates compared to colon cancer patients (6.6). In addition faecal pH was significantly lower than mucosal and luminal pH. The breath methane results showed that methane production was significantly lower in the patients with right sided colostomies, compared to those with left sided colostomies ($p = <0.005$).

In conclusion it was found that the pH of the colon in absence of faeces is alkaline, and is approximately 8.0. In the presence of faeces the pH decreased but not significantly (pH 7.6). In 'healthy' individuals the faecal pH of the left colon is higher than the faecal pH of the right colon. The faecal pH of patients with colorectal cancer is higher than that the faecal pH of the left colon of 'healthy' patients. The faecal pH is significantly lower than the luminal and mucosal pH. The different diets did not have any significant effect on the faecal pH or the mucosal pH. However a high fibre, and a high starch/low fibre diet tended to result in a lower pH than a western diet. Methane production was significantly lower in patients with right sided colostomy as compared to left, but was not influenced by the different diets.

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- CHAPTER 1 -

INTRODUCTION

In contrast to Western populations colorectal cancer is reported to be rare in the South African black population. Previous studies from South Africa have described the low incidence of colorectal cancer in blacks. There is also a low incidence of other colonic diseases such as appendicitis, diverticulitis, and inflammatory bowel disease. (Walker et al., 1973; Bremmer and Ackerman 1970; Segal et al., 1977; Walker and Burkitt 1976). The incidence of carcinoma of the colon and rectum in Soweto was 1/100 000 per year; comparable values for whites are about 30 times higher. (Oettlé and Segal, 1988). It has also been shown that the anatomical and age distribution of neoplasms is the same as in high-risk populations (Segal et al., 1981). The observations that people of different cultures have profoundly different disease risks, and that incidences increase with environmental changes, has led to the hypothesis that dietary factors (which profoundly affect the milieu of the colon) are of paramount importance in colonic carcinogenesis (Wynder and Reddy, 1974; Walker and Burkitt 1976; Waterhouse et al., 1976) A similar hypothesis was suggested by Goligher (1984) in his book in the chapter 'Incidence and pathology of carcinoma of the colon and rectum'. He observed a high

incidence of bowel cancer in the black people of the USA compared with that in the black population of developing areas of Africa (World Health Organization data, 1981). Further epidemiological confirmation is illustrated by the much higher incidence of intestinal cancer in the first- and second- generation Japanese and Chinese immigrants in Hawaii than in these races when resident in their own lands (Haensel et al., 1973; Crowther et al., 1976).

More recent studies indicated that the consumption of a high fibre or a high starch diet, resulted in the decrease of the intracolonic pH of the faeces (Cummings and Bingham, 1987; Jacobs, 1988).

Previous studies in South African communities have shown that faecal pH was lower in blacks than in whites. It has been suggested that there is an association between the low faecal pH and the low incidence of colorectal cancer in the black population (Walker et al., 1986). Faecal pH however may not reflect intracolonic pH. No studies have been carried

out on colonic pH in South African populations. In fact only very few studies have been reported internationally on this important topic.

- CHAPTER 2 -

LITERATURE REVIEW

2.1. PHYSIOLOGY OF THE COLON

Recent studies indicate that the colon has major physiological functions and is an important metabolic organ. The major functions of the colon are:

1. Absorption
2. Secretion
3. Fermentation

These will be discussed in detail below.

2.1.1. ABSORPTION

The main function of the colon is absorption of water, Na^+, and other minerals. By removal of about 90% of the fluid, it converts the 1000-2000 mL of isotonic chyme that enters it each day from the ileum to about 200-250 ml of semisolid faeces.

It has been estimated (Turnberg, 1970) that 70 mmol of Na^+ and 400-500 ml of water are absorbed each day by the colon and rectum. It has also been shown (Devroede and Phillips, 1969) that the absorption of Na^+ is active with a concomitant passive movement of water.

Potassium is secreted into the lumen of the colon against a concentration gradient of up to 15 mmol/l. This seems to be an active secretion because the transmural electrical potential difference is not great enough for the movement of potassium to be due to passive transport down an electrochemical gradient (Edmonds and Godfrey, 1970). Comparisons of the right and left halves of the colon indicate that the caecum and ascending colon may have the greater absorptive capacity.

2.1.2. SECRETION

Mucus is secreted throughout the large bowel from the columnar cells known as Goblet cells. Mucin is synthesized in an area just above the nucleus of a cell and studies with the electromicroscope have shown that the droplets appear first in the membrane of the Golgi apparatus (Bierring, 1962). These have a life span of about three days in experimental animals.

It has been shown by autoradiography, to be sulphated and to be rapidly resynthesized within the goblet cell immediately after the cell has been stimulated to discharge. No proof is available of any nervous or humoral control of its release.

2.3. FERMENTATION

In animal physiology the term fermentation is conventionally used to describe the reaction in which carbohydrate, usually polysaccharide, is broken down by the concerted action of many species of anaerobic micro-organisms as shown in the figure of the following page (Figure 1). The end products of this process are short-chain (or volatile) fatty acids, principally acetic, propionic, and butyric acids, and the gases carbon dioxide, methane, and hydrogen.

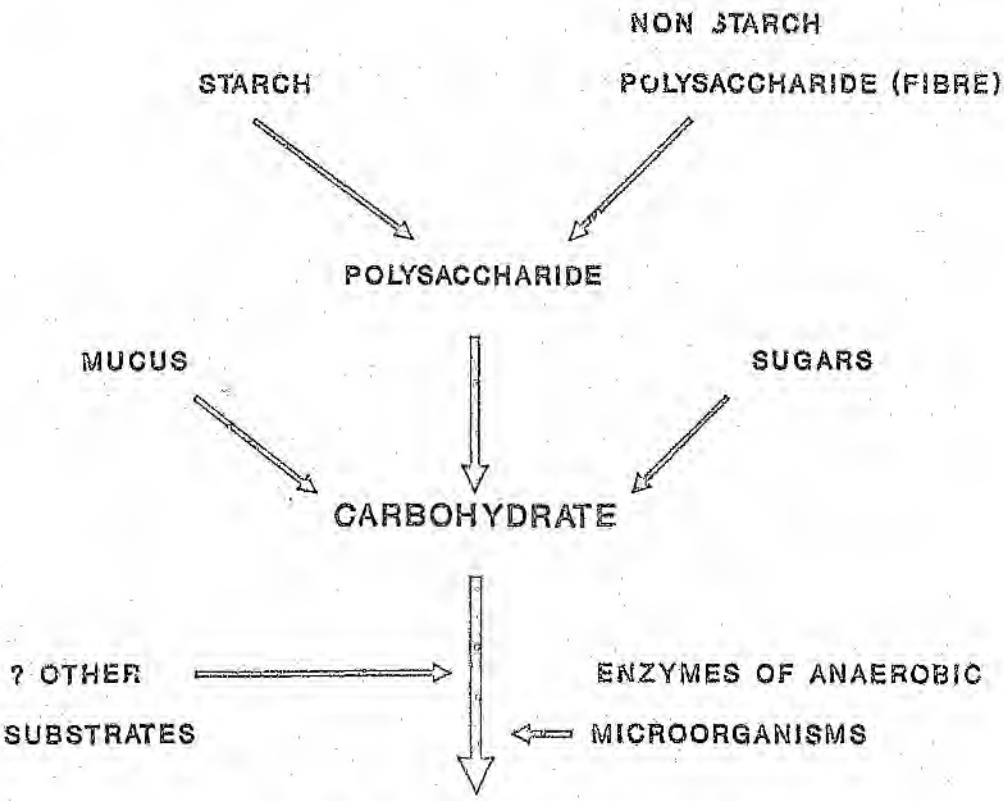


Figure 1
GENERAL EQUATION FOR FERMRNTATION
IN THE HUMAN COLON

This phenomenon has also been described by some investigators by the following equation, which is a reasonable approximation of what actually takes place in the ecosystem (Wolin, 1960):

$$57.5\ C_6H_{12}O_6 \rightarrow 65\ HAc + 20\ HPr + 15\ HBu + 60\ CO_2 + 35\ CH_4$$

Here, HAc, HPr, and HBu are acetic, propionic, and butyric acids, respectively. The substrate is represented as a monomeric unit of a hexose polymer.

Fermentation also occurs in the human large intestine (Wolin and Miller, 1987; Cummings, 1983; Cummings and Bingham, 1987; Englyst and Macfarlane, 1986) but its place, both in the normal function of the bowel and in metabolic events outside the colon, remains largely unknown.

There is now substantial evidence that some dietary polysaccharides, notably dietary fibre, escape absorption in the small bowel and are then broken down in the large intestine of man (Promare et al., 1985).

2.1.4. FERMENTATION OF DIETARY FIBRE

Dietary fibre passes large y unchanged through the human stomach and small bowel to reach the caecum more or less unaltered (Englyst and Cummings, 1985, 1986, 1987). However, most fibre is extensively degraded in the intact intestine and there is now convincing evidence that this breakdown occurs in the large bowel (Cummings, 1984). The human colon is host to a large and diverse population of anaerobic bacteria many of which produce enzymes capable of breaking down a wide range of polysaccharides (Salyers and Leedle, 1983; Englyst and Macfarlane, 1986). An important feature of microbial metabolism in the large bowel is that it is anaerobic and therefore the end-products of dietary fibre breakdown are very different from carbohydrate digestion in the rest of the gut. Anaerobic breakdown of dietary fibre yields short chain fatty acids (acetic, propionic and butyric acids), various gases, such as methane, hydrogen and carbon dioxide, and energy, which the flora use for growth and maintenance of cellular function. This process, which is known as

fermentation, holds the key to understanding how dietary fibre may influence large bowel function to make large bowel cancer less likely to develop.

The potential mechanisms whereby fibre may protect against large bowel cancer are listed in the following table (Table 1). (Cummings and Bingham, 1987)

Table 1. Potential mechanisms by which dietary fibre may prevent large bowel cancer.

1.	Physical dilution of gut contents and more rapid transit time.
2.	Protective effect of butyrate on colon epithelium.
3.	Reduced availability of N-compounds through simulation of bacterial growth.
4.	Altered bile acid metabolism
5.	Lower pH

2.1.4.1 PHYSICAL DILUTION OF GUT CONTENTS AND MORE RAPID TRANSIT TIME

The pioneering work of Burkitt (1971) and Walker (1978) indicated that high-fibre diets are associated with increased faecal bulk and faster rates of intestinal transit. These observations prompted the hypothesis (Burkitt 1971) that these changes in colonic function would dilute out any carcinogens or tumour promoters present within the intestinal lumen, while also reducing the time available for their interaction with the intestinal epithelium. However, human and animal studies do not support the concept that transit time alone is important in the prevention of colon cancer, whereas faecal bulk does show an inverse correlation (Cummings et al., 1982; Hoff et al., 1986) implying possible protection. A number of in-vitro studies have demonstrated that different fibres are able to bind carcinogens, (Gulliver et al., 1983; Smith-Barbaro et al., 1981) and potential tumour promoters (Kritchevsky and Story, 1974), however, the relative importance of these effects in vivo, either in humans or animals, has not been fully determined.

2.1.4.2. PROTECTIVE EFFECT OF BUTYRATE ON COLON EPITHELIUM

Fermentation yields a mixture of short chain fatty acids usually in the ratio 60% acetate: 20% propionate: 20% butyrate (Cummings et al., 1987). The concentration of these acids found in the human caecum and sigmoid colon is shown in Table 2.

Table 2. Short chain fatty acids concentrations in human caecum and sigmoid/rectum (Cummings et al. 1987)

	Caecum (mmol/Kg contents (±1 SEM))	Sigmoid/rectum (mmol/Kg contents (±1 SEM))
Acetate	69.1 (5.0)	50.1 (16.2)
Propionate	25.3 (3.7)	19.5 (6.7)
Isobutyrate	2.1 (0.4)	1.9 (0.8)
Butyrate	26.1 (3.8)	17.9 (5.6)
Isovalerate	2.7 (0.5)	3.7 (0.9)
Valerate	4.[illegible] (0.5)	4.3 (0.9)
pH	5.6 (0.2)	6.3 (0.2)
No. of cases	6	5

Butyrate is both an important fuel for the colon epithelial cells and a powerful differentiating agent. In isolated human colonocytes Roediger (Roediger, 1980) has shown that butyrate is metabolized to CO_2 and ketone bodies. Butyrate suppresses glucose oxidation by these cells and accounts for 80% oxygen uptake in isolated cell preparations. Butyrate may be the preferred fuel of the large bowel epithelial cells, especially in the descending / sigmoid colon.

Butyrate is also known from cell biology studies to affect cell growth. It will reversibly change the in vitro properties of human colorectal cancer cell lines by prolonging doubling time and generally slowing down growth rate (Kim et al., 1982). It affects a wide range of cellular enzymes (Prasad, 1980), inhibits histone deacetylase and so induces the accumulation of acetylated histones in cell culture and may stabilize chromatin structure during cell division (Kruh, 1982; Smith, 1986). Low concentrations of butyrate have been shown to reduce DNA synthesis in vitro and suppress cell proliferation in a variety of cells (Hagopian et al., 1977). Virus induced cellular differentiation can be reversed by butyrate (Leder and Leder, 1975), while

in rat hepatoma cells butyrate leads to a reversal of the cell to more normal appearance and to their becoming anchorage-dependent for growth, a characteristic of non-transformed cells (Borenfreund et al., 1980). Experimental butyrate used in these studies is well within the range found in the human colon, of around 10 to 25 mmol/litre. Butyrate production, through fermentation, may therefore be critically important in maintaining the integrity of the colonic mucosa, and provides a ready link between dietary fibre and bowel cancer.

2.1.4.3. REDUCED AVAILABILITY OF N-COMPOUNDS THROUGH SIMULATION OF BACTERIAL GROWTH

A major controlling factor in nitrogen metabolism is the amount of carbohydrate available for fermentation. When dietary fibre intake is increased, faecal nitrogen excretion also increases. Both animal and human studies have shown that this is largely attributable to greater microbial mass in faeces (Mason, 1969; Mason and Palmer, 1973; Stephen and Cummings, 1979). Although bacteria are able to use free amino acids for protein

synthesis, the nitrogen available to them is mainly in the form of ammonia. This is generated by microbial breakdown of protein, urea and other sources. Ammonia produced in the large intestine may be used by the microflora for protein synthesis or it may be absorbed, transported to the liver, formed into urea and excreted in urine. The amount of nitrogen that bacteria require for growth and cell division will depend on the energy available to them. This energy is derived largely from carbohydrate fermentation, so the amount of fermentable substrate available to the microflora will directly influence nitrogen utilization by bacteria. Feeding dietary fibre stimulates microbial cell growth and increases microbial requirements for nitrogen. More ammonia is used for protein synthesis and so less should be absorbed. Animal studies have shown that portal blood ammonia levels fall in these circumstances (Demigne and Remesy, 1979). In man faecal nitrogen excretion increases with dietary fibre but ammonia concentration fall (Cummings et al., 1979).

In patients with hepatic cirrhosis Weber (Weber, 1979) observed that urea production fell and faecal nitrogen excretion rose when the subjects were given lactulose,

a non-absorbable disaccharide. This suggests a reduction in ammonia recycling from the portal system and an increase in protein synthesis by the flora. Active fermentation therefore routes ammonia into bacterial protein and in so doing lowers colon and portal venous blood levels.

Ammonia may affect the metabolism and morphology of colonic cells and may be a factor in tumour promotion (Visek, 1972, 1978; Topping and Visek, 1976; Visek et al., 1978). Low concentrations (5-10 mmol/Kg) alter intermediary metabolism and DNA synthesis, reduce the life span of intestinal cells and induce faster cell turnover. In general, ammonia is more toxic for healthy cells than for transformed cells and thus a high concentration of ammonia in the bowel lumen may select for neoplastic growth. By increasing cell turnover, ammonia increases the probability of genetic damage occurring in the presence of oncogenic agents. Dividing cell populations are more susceptible to chemical carcinogenesis (Warwick, 1979). Experimental evidence relating large bowel injury and dimethylhydrazine induced tumours shows that cell kinetics are of fundamental importance during chemical carcinogenesis.

Further evidence relating ammonia, cell turnover and cancer is seen in uretero - sigmoidostomy patients. These patients have very high luminal ammonia concentrations (up to 100 mmol/Kg) (McConnell et al., 1979) and a greatly increased risk of developing tumours distal to the site of the implantation (Tank et al., 1973). Fermentation by reducing ammonia levels may therefore have important implications for cell division in addition to the effects brought about by butyrate production.

2.1.4.4. ALTERED BILE ACID METABOLISM

There is good evidence which suggests that the carcinogen/s or co-carcinogen/s is a bacterially metabolized bile acid. Some colonic bacteria are able to degrade bile acids into recognised carcinogens (Hill, 1974) and degraded bile acids are co-carcinogens in animal models (Narisawa et al., 1974; Reddy et al., 1977;). Human populations at high risk of developing large bowel cancer have higher concentrations of degraded bile acids than low risk populations (Hill et al., 1971; Wynder and Reddy, 1974; Hill et al., 1975;

Crowther et al., 1976; Mower et al., 1979;), and individuals with colonic cancer have higher concentrations than controls (Reddy and Wynder, 1977). The initial, irreversible step in bacterial bile-acid degradation is the conversion of primary bile acids by 7-a-dehydroxylation. The activity of this reaction is greater in high-risk population (Hill et al., 1971) and also higher in cancer patients than in healthy controls (Mastromarino et al., 1978). The increase in the rate of bacterial 7a-dehydroxylation in healthy Dutch subjects with age is consistent with the age incidence of colorectal cancer (Van der Werf et al., 1980). Cholesterol metabolites have also been suggested as potential (co-)carcinogens, (Reddy and Wynder, 1977) and high levels of degradation of faecal cholesterol and bile acids are commonly associated (Hill et al., 1971; Wynder and Reddy, 1974; Reddy and Wynder, 1977; Mastromarino et al., 1978; Huang et al., 1976).

It is proposed that a high colonic pH promotes the degradation by the colonic bacteria of bile acids and cholesterol into (co-)carcinogens. 7a-dehydroxylation of bile acids (the major, initial degradation) is, in vitro, inhibited below pH 6.5 (Midtvedt and Norman,

1968; Aries and Hill, 1970), and recent work suggests that this may also be the case in vivo (Thornton and Heaton, 1981). Thus fermentation by lowering the pH of the colon, may inhibit this reaction and the production of the (co-) carcinogens (Thornton, 1981).

2.1.4.5. LOWER pH

Epidemiological data suggest that the increased risk of colonic cancer is correlated with a higher faecal pH (MacDonald et al., 1978; Malhotra 1982; Walker et al., 1986). Although some experimental studies have shown a protective effect against experimentally induced colonic cancer by acidifying colonic contents (Samuelson et al., 1985), others have shown that a more acidified colonic content is associated with increase cell proliferation (Lupton et al., 1985 and 1988; Jacobs and Lupton, 1986) and enhanced tumorigenesis (Jacobs and Lupton, 1986). It is now clear that simply acidifying colonic contents will not consistently decrease tumorigenesis. Perhaps the key is in how colonic contents are acidified - a decrease in base production or an increase in acid production. Or, more

important than luminal pH itself, may be factors affected by pH: the composition and metabolic activity of the microflora, the absorption of a particular luminal constituent, or enzyme activity (Newmark and Lupton, 1990).

2.2. pH OF THE COLON

2.2.1. DETERMINANTS OF COLONIC LUMINAL pH

Colonic luminal pH is determined by relative concentrations of acids and bases.

2.2.1.1. BASES

The main bases which are found in the large bowel, and are responsible for the determination of the luminal pH are Ammonia and Bicarbonate.

2.2.1.1.1. AMMONIA

Ammonia (NH_3 & NH_4) is the chief alkaline component that tends to raise the pH. Normally, ammonia ranges from 15-50 mmol/Kg of whole faeces or about 3-44 mmol/l of luminal fluid (Geigy Scientific Tables, 1981). Faecal ammonia is derived from bacterial degradation in the large bowel of endogenous urea from tissue fluid and also of proteins and amino acids that escape digestion and absorption in the small intestine (Geigy Scientific Tables, 1981; Demigne and Remsey, 1979). The urea concentration in blood depends primarily on protein intake, urine volume, and the functional state of the kidney. In adults the urea level rises with increasing age (Geigy Scientific Tables, 1984) and ranges from about 2.5 - 7.0 mmol/l and is over 100 fold higher than ammonia in blood (15-80 ?mol/l) (Geigy Scientific Tables, 1981). Some urea easily diffuses into the colonic lumen where the microflora rapidly convert into ammonia and CO_2 (Geigy Scientific Tables, 1981). In its ionic state, ammonia is not readily absorbed into the blood. The net result is primarily a one-way flow of urea into the gastrointestinal tract. Urea thus serves as one source of ammoniacal base in

the colon. The driving force of this passive diffusion process is the concentration gradient of urea between the blood (determined by dietary protein intake and metabolism) and the colonic lumen. To date there are no data on the rate of this process and its variation in different areas of the large bowel, but indirect evidence suggests that it occurs to some extent throughout the large bowel (Bown et al., 1974). Ureolysis releases 3-4 g of ammonia daily in human adults consuming western diets (Jones et al., 1969; Visek, 1981). Some of the ammonia is reabsorbed into the portal blood by passive diffusion and returned to the liver for conversion back to urea (Down et al., 1972; Wrong, 1971). Another portion of ammonia is incorporated into bacterial protein.

High dietary protein is considered to be a major contributor to the elevation of colonic and faecal pH. The higher the protein intake, the higher the blood urea nitrogen and the more urea that diffuses into the colon for hydrolysis. Also, dietary protein has a direct effect on the amount of protein and amino acids

entering the colon that are available for deamination because a certain percentage of dietary protein escapes absorption in the small intestine.

In addition to dietary protein, dietary fibre can also significantly affect the amount of free ammonia in the colon (Lupton and Marchant, 1989; Ehle et al., 1981; Shah et al., 1982). High-fibre diets greatly increase the numbers of micro-organisms in the colon, which influence hydrolysis of urea to ammonia. High dietary fibre also increases faecal nitrogen from both endogenous and dietary origin (Shah et al., 1982). Also, bacterial production of short-chain fatty acids (SCFAs) lowers colonic pH, helping to keep ammonia in its ionized form, thus "trapping" it in the colon. The ratio of protein to fibre in the diet may also be important. When fibre is limiting, facultative bacteria may begin fermenting proteins, thus releasing ammonia and raising pH (Jones et al., 1969). Although dietary fibre is thought to decrease the amount of ammonia or ammonium ion in the colon by "scavenging" ammonia nitrogen for colonic bacterial protein synthesis, which then appears in the faeces as protein (Visek, 1978), this only accounts for about one-sixth of faecal

nitrogen (Geigy Scientific Tables, 1981). A recent study shows that ammonia or ammonium actually increases severalfold in the colons of rats whose diets have been supplemented with fermentable fibre pectin (Lupton and Marchant, 1989).

2.2.1.1.2. BICARBONATE

Also contributing to colonic alkalinity is bicarbonate ion, which is secreted into the colonic lumen in exchange with SCFA uptake (Umesaki et al., 1980). A recent review (Hopfer and Leidtke, 1987) delineates the various mechanisms for proton and bicarbonate transport in the colon, including a system of Na^+ -H^+ and Cl-$HCO3^-$ double exchangers. Hopfer (Hopfer and Leidtke, 1987) and Newmark (Newmark and Lupton, 1990) suggest that maintaining a constant intracellular pH depends on the relative activities of the two types of exchangers and regional differences in net proton or net bicarbonate secretion. The absorption of H^+ from the colonic lumen is considered equivalent to the secretion of $HCO3^-$ into the lumen in part because of the presence

of carbonic anhydrase activity in the colon (Spicer et al., 1982). This functions according to the following reactions.

$$CO_2 + H_2O \xleftrightarrow{\text{(carbonic anhydrase)}} H_2CO_3 \xleftrightarrow{\text{(spontaneous)}} HCO_3^- + H^+$$

(Martin, 1988)

2.2.1.2. ACIDS

2.2.1.2.1. SHORT CHAIN FATTY ACIDS

The SCFAs, also called volatile fatty acids (principally acetic, propionic, and butyric), total 60-170 mmol/Kg in the faecal aqueous phase and represent about 75% of the a s present (Geigy Scientific Tables, 1981; Cummings, 1981). These acids are chiefly responsible for the neutralization of bases and the acidification of the colon contents and faeces. Essentially all SCFAs are derived from colonic microbial fermentation of undigested dietary

carbohydrate. One source of unabsorbed carbohydrate is the dietary fibre naturally present in foods of vegetable or fruit origin (Trowel et al., 1976).

Fermentation of the complex carbohydrate components of dietary fibre may supply 2-7% of the total energy of human subjects on an average western diet (Cummings, 1981) of 2.5 - 3.1 kcal/g fibre (Goranzon and Forsum, 1987). However, different components of dietary fibre can be processed differently in the colon. Therefore, dietary fibre should be considered in terms of the individual components or at least as semi-homogeneous chemical group types (Heaton, 1979; Spiller and Kay, 1980). The components of dietary fibre produce different amounts and types of SCFAs and may also be fermented at different anatomical sites. Not much is known about these variables in humans. Howeve , based on some animal data obtained in ruminants and a limited number of in vitro studies, a few assumptions can be made about these variables in humans.

* Pectins, including arabinans and galactans, are fermented primarily in the proximal colon. By analogy to rumen fermentation, rapid production of

SCFAs (particularly acetic acid) probably occurs. The effect on the acidity of the distal colon and faeces may be far less, unless large amounts are present in the diet (Goranzon and Forsum, 1987).

* Soluble gums (e.g., guar) include a great variety of beta - glucans, arabino - xylans, soluble pentosans, and gluco and galactomannans and are probably fermented in a similar fashion to pectins. However, the site of fermentation may be more diffuse over the colon rather than primarily in the proximal area because of a lower rate and extent of solubility.

* Sulfated polysaccharides (e.g., carrageenan) are also fermented in the colon, but details of area in the colon of the fermentation, the rate and proportion of SCFA products produced is not known. The liberated sulfate group would serve as a specialized acidulant of the distal colon, because it is not well absorbed (Bown et al., 1974; Albert, 1981).

* Hemicelluloses, roughly similar to the "noncellulosic polysaccharides" of Southgate (Heaton, 1979), are a major portion of cereal fibres (e.g., wheat, rye, oat). These are not readily solubilized until attacked by the hydrolytic enzymes of colonic microbial flora. Much of the actual fermentation, therefore, takes place in the central and distal colon. While acetic acid still predominates, higher levels of propionic and butyric acids are produced, than from the pectins.

* Heat-processed food components, such as those produced in breadmaking, act as fibres if the polymerization produced is sufficient to prevent digestion by mammalian endogenous enzymes. Some of these polymers are Maillard products, resulting from the reaction of aldehyde groups of saccharides with amino groups of proteins, often noted as a darkening of the food product (i.e., "toasting" of bread or production of the dark "crust" on breads or other foods during cooking). Little is known about the nature of their colonic

fermentation products, sites of maximum fermentation, or extent of fermentation of these "fibres".

* Age - polymerized starches, and other polysaccharides produced as a result of reactions occurring during storage (e.g., cross linking), may also be resistant to mammalian enzymes, thus resulting in different types of dietary "fibre" available for colonic microflora fermentation. Little is known of this group; however, heat - polymerized and age - polymerized starches are probably more important than previously thought (Cummings et al., 1986)

2.2.1.2.2. LACTATE AND SUCCINATE

Almost all intestinal anaerobes use the Embden-Meyerhof pathway to form pyruvate, which in turn forms SCFA and some lactate and succinate as shown in Figure 2 (Miller and Wolin, 1979). Although it is thought that little lactate and succinate are produced in the colon except under conditions of malabsorption (Cummings, 1981), a

reducing environment, nevertheless, favours their synthesis. Unlike SCFAs, lactate and succinate are not readily absorbed and do not elicit bicarbonate exchange. Thus, if they were produced during fermentation, they should lower luminal pH. High amounts of luminal lactate were found in subjects with poor digestion and absorption of lactose because of a lactase deficiency. When lactose reaches the colon, it is apparently fermented to lactic acid and much of it appears in the faeces (Durphy et al., 1965).

Production of SCFAs from Pyruvate

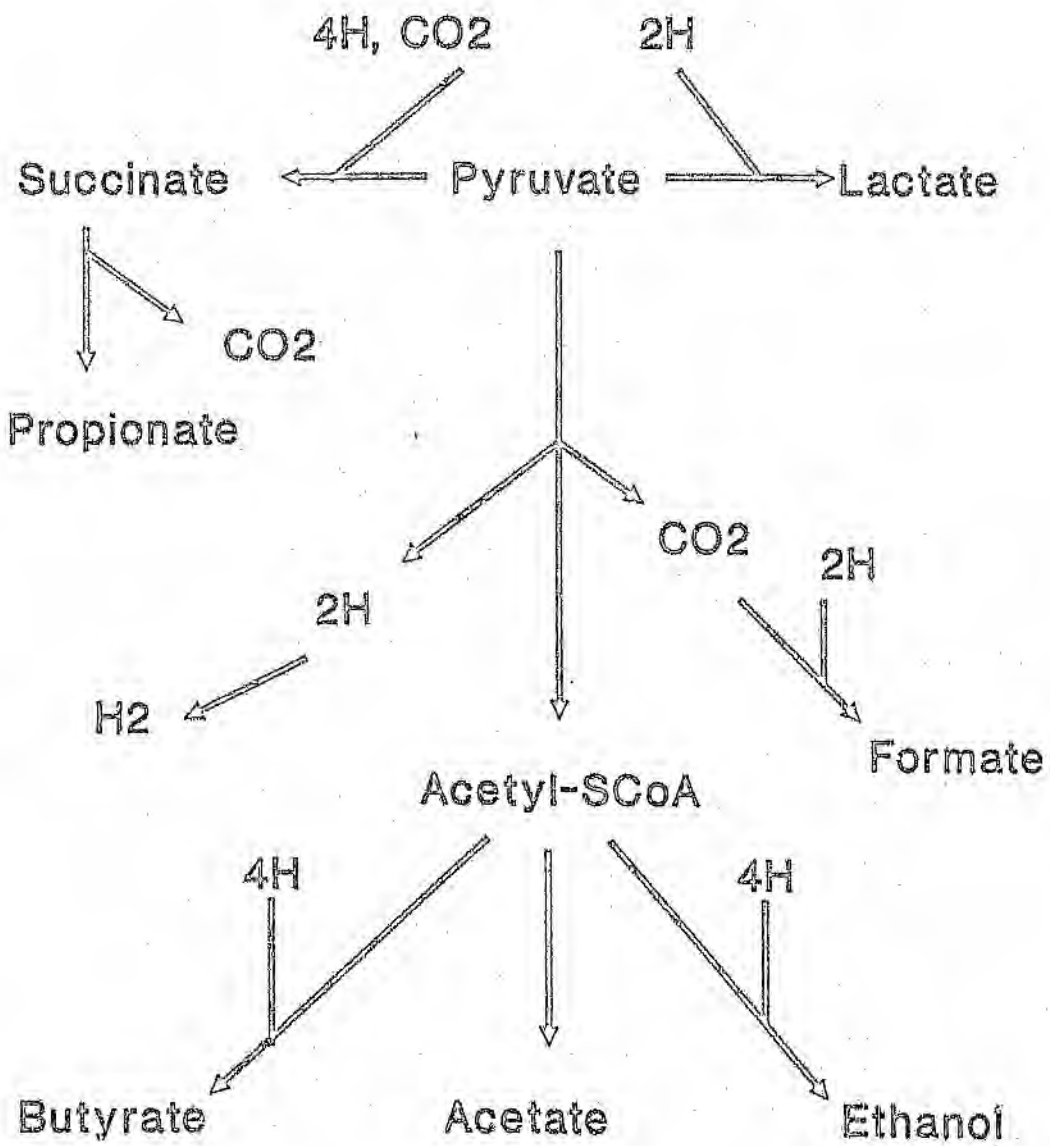


Figure 2

2.2.1.2.3. SULPHATE AND TARTRATE

Because diet-derived sulfate and tartrate are poorly absorbed, their contribution to faecal pH is determined by the absorption of their companion cations (Albert, 1981). Thus when sodium sulfate is given orally to humans, distal colonic contents are acidified (Down et al., 1972), possibly due to the absorption of the sodium ion in the proximal colon, which leaves the sulfate ion as the acidulating agent.

2.2.1.2.4. LACTULOSE

Lactulose fermentative products produce a marked acidification of the proximal colonic contents, but this effect is not consistently maintained into the distal colon (Bown et al., 1974) and thus will not reliably lower faecal pH. Either the lactulose is rapidly fermented and utilized by bacteria on entry into the proximal colon or the acidification it produces alters the microbial flora to inhibit

absorption of ammonia. Thus, lactulose produces quite a different acidification profile than does sulfate in the colon (Bown et al., 1974).

2.2.1.2.5. FAECAL BILE ACIDS

Faecal bile acids are the result of the escape of a small amount of conjugated bile acids from the normal enterohepatic circulation to the colon where the conjugates are rapidly hydrolyzed to the free bile acids (FBA) by the colonic flora (Newmark et al., 1984). The amount of FBA in faeces varies from 0.2 to 0.6 g/day (Geigy Scientific Tables, 1981), increasing with dietary fat, and is about 0.5 g/day (almost 1 mmol/Kg) in people on a western diet (Jacobson et al., 1984; Yang and Newmark, 1987).

2.2.1.2.6. LONG CHAIN FATTY ACIDS

Faecal long-chain free fatty acids (LCFAs; C14, 16 and 18) including myristic, palmitic, stearic, oleic, and even the polyunsaturated linoleic acid are excreted at a rate of about 4-10g/day and represent 10-20% of the dry weight of the faeces (Newmark et al., 1984; Jacobson et al., 1984; Yang and Newmark, 1987). However, they are very poorly soluble in the aqueous phase, except as alkaline salts (soaps) at high pH. At neutral or acidic pH, the LCFAs tend to be insoluble lipids and do not enter into acidification reactions.

It is clear that the principal dietary substrate for acid formation in the lemon is dietary fibre. With the exception of lignin (a phenolic polymer that is the primary structural component of cells in woody plants) and some forms of pure cellulose, the components of dietary fibre are fermentable by the colonic microflora. Also of importance are non-fiber polysaccharides-heat-processed food components and age-polymerized starches. Lactose, for lactose-intolerant individuals, acts much like a dietary fibre and also serves to acidify colonic contents. The

products of microbial fermentation include the soluble acids (SCFA, lactate, and succinate) and gases (CO_2, H_2, and methane). The SCFAs, acetic, propionic, and butyric acids represent the primary naturally occurring acids in the colon; they neutralize ammonia and bicarbonate and acidify the colonic contents and faeces (Cummings, 1981; Roediger, 1982; Roediger and Moore, 1981). The conversion by fermentation of different fibre components to different types and amounts of SCFA, in different areas of the colon, probably varies considerably with: a) the individuals, b) the remainder of the diet, and c) the nature of the microflora of the individual. The unfermented portion of fibre is available to dilute the other luminal constituents (Gazzaniga and Lupton, 1987). In addition to the soluble acids and gases produced from microbial fermentation, sulfates and tartrates act as acidifiers as do the fermentation products of the synthetic disaccharide lactulose. Finally, diet-derived LCFAs tend to acidify colonic contents. Because they are poorly soluble in water, they cause changes in colonic pH that are negligible, except at higher pH ranges where solubility and ionization increase for the LCFAs.

2.3. ESTABLISHMENT OF COLONIC LUMINAL pH

The process of establishing the luminal pH is complex and dynamic. As fibre and undigested carbohydrate are fermented to SCFAs, the pH drops. At a lower pH, SCFAs are better absorbed thereby decreasing some of the excess acidity (Roediger, 1982). In addition, when SCFAs are absorbed, bicarbonate, a weaker acid [pK_a of 6.3 (Geigy Scientific Tables, 1981)], is secreted into the colonic lumen. The net effect of this exchange of SCFAs of moderate acidity (pK_a 4.76) for the weaker acid bicarbonate (pK_a 6.3) is a reduction in acidity. The SCFAs are weak acids, with a single carboxyl group and a pK_a of 4.76. The SCFAs in solution are ionized at a geometric rate as the pH rises - being more than 99% ionized at pH 7. Unlike SCFAs, the soluble acids lactate and succinate are not readily absorbed and do not elicit bicarbonate exchange. In addition, they are somewhat stronger acids (pK_a 3.08 for lactate and 4.16 for the first pK_a for succinate). Thus, they should have a greater acidifying effect than SCFAs. LCFAs (e.g., myristic, palmitic, oleic) have similar pK_a values to the SCFAs. However, the SCFAs are water soluble in both the ionized and un-ionized states; this

is in contrast to the long-chain acids, which can form soluble ion pairs or soaps only in the ionized form. The solubility of LCFAs is severely limited by the tendency for the ionized LCFAs to shift back to the insoluble unionized form, unless the pH is 6 or greater. Medium-chain fatty acids (C6-C12) are essentially ompletely absorbed in the upper gastrointestinal tract and do not appear in significant quantities in the colon.

The pH of intestinal contents is raised by the secretion of bicarbonate in response to SCFA production, the release of ammonia by deamination of protein, and the hydrolysis of urea (Down et al., 1972). Ammonia is better absorbed at higher pH values, again tending to stabilize luminal hydrogen ion concentration against excess alkalinity. Ammonia has a pK_a of 9.26. The formation of ammonium ion increases, geometrically, as the pH decreases. At a pH of 7, it is over 99% ionized.

Equimolecular mixtures of ammonia and one or more SCFAs have a pH of about 7, which is midway between the pK_as of ammonia and those of the SCFAs. The buffering effect

of such solutions at a pH of 7 would be minimal but would increase rapidly as the pH deviated above or below 7. As the pH changes above or below 7, there is an increased tendency to absorb H^+ ions to form undissociated acid in a solution below pH 7 or to absorb OH^- ions to form unionized NH_3 above a pH of 7. Faecal pH, which is largely dependent on the balance between ammonia and SCFAs, would thus tend to drift to a pH of 7 - on a 'physico-chemical basis'. In fact mean adult faecal pH is actually about 7, with a range of 5.6 - 9.4 (Geigy Scientific Tables, 1981; Wrong, 1971). This is expected given the fact that ammonia and the SCFAs comprise the major buffering base-acid components and the known reactions of weak bases and acids. The rate of production and removal of SCFAs and NH_3 at any site in the colon or faeces is a dynamic system that changes rapidly depending on the local availability of substrates, effects of other transient food components, and the local microflora. All are constantly changing and vary from subject to subject (Drasar and Hill, 1974).

2.4. METHANE

Although it has been known for more than 150 years that methane is produced in the human large intestine, nevertheless the physiology and importance of methanogenesis in man remains something of an enigma. Methane is one of the principal end products of fermentation in anaerobic systems such as the rumen, (Demeyer and Nevel, 1975) whilst in man active fermentation occurs in the large intestine (Cummings, 1983) and methanogenic bacteria such as Methanobrevibacter smithia have been found in faeces (Nottingham and Hungate, 1968; Miller et al., 1982; Miller and Wolin, 1983; Weaver et al., 1986; Misawa et al, 1986). Methane installed into the human colon rapidly appears in breath as do other fermentation gases such as hydrogen. In most published reports of population studies, however, only 35-61 % of healthy adult subjects have detectable methane in their breath (Bjorneklett and Jenssesn, 1982; Bond et al., 1971; Pitt et al., 1980; McKay et al., 1981; Hains et al., 1977; Pique et al., 1984; McKay et al., 1985; Flatz et al.. 1985; Peled et al., 1985) although two reports give higher values.(Calloway and Murphy, 1968; Drasar

et al., 1984) In detailed studies by Levitt and Ingelfinger of indivituals using intestinal perfusion with air or nitrogen, about half of the subjects tested showed no sign whatsoever of methane evolution in the colon.

A group of people with unexpectedly high breath methane levels are those with large bowel cancer. In two separate studies 80 and 91 % of these patients were methane producers. Methane production was also increased in patients with premalignant conditions of the bowel such as polyps (Haines et al., 1977; Pique et al., 1984). Physiologically methane production is associated with slow gut transit time, small faecal weight, and high faecal pH (Stephen et al., 1986) although these differences in faecal weight have not been found consistently (McKay et al., 1981 and 1985).

Breath methane was measured in four population groups in Southern Africa who show marked differences in bowel disease risk. The results showed that there were major differences in methane producer status amongst the populations, but these do not relate to the risk of bowel cancer (Segal et al., 1988).

2.5. HYPOTHESIS

As mentioned before, it is proposed that a high colonic pH promotes the degradation by the colonic bacteria of bile acids and cholesterol into (co-)carcinogens. 7a-dehydroxylation of bile acids (the major, initial degradation) is, in vitro, inhibited below pH 6.5 (Midtvedt and Norman, 1969; Aries and Hill, 1970; Thornton, 1981), and suggests that this may also be the case in vivo (Hill and Heaton, 1981). Black Africans eating a traditional high-fibre, low-fat diet had a faecal pH below 6.5, whereas White Africans eating a western diet (lower in fibre and higher in fat) had a faecal pH above this level (Walker, et al., 1979). Similarly, vegetarian Seventh Day Adventists, who have a reduced risk of large bowel cancer (Phillips, 1975) have a lower faecal pH than patients with colorectal cancer (MacDonald et al., 1978).

Burkitt suggested that dietary fibre protects against colorectal cancer by speeding colonic transit (so reducing the time a carcinogen is in contact with the bowel mucosa) and by increasing stool weight (so diluting the carcinogen concentration) (Burkitt, 1971).

Whilst these properties of dietary fibre are clearly important, its major action may be to lower colonic pH, so preventing or reducing carcinogen formation. Thus, increasing the fibre content of a diet may render the stools more acid (Walker et al., 1979; Hellendoorn, 1978), probably due to the substantial digestion by the colonic flora of many components of dietary fibre into short-chain fatty acids (Hellendoorn, 1978; Cummings et al., 1976). The consequent colonic acidification would be expected to reduce bile-acid degradation by 7a-dehydroxylation. This mechanism of the fibre's action is indirectly supported by the findings of a reduced proportion of degraded bile acids in bile after bran consumption. (Pomare et al., 1976; McDougall et al., 1978) A similar effect after the administration of lactulose (Thornton and Heaton, 1981) (a synthetic, non-absorbable, disaccharide known to lower colonic pH (Bown et al., 1974)) provides more direct evidence that colonic acidification inhibits 7a-dehydroxylation of bile acids. The negative correlation between British death rates for large bowel cancer and the pentose fraction of dietary fibre (Bingham et al., 1979) is of

interest, since bacterial digestion of this component of fibre produces short-chain fatty acids (Cummings and Stephen, 1980).

- CHAPTER 3 -

AIM

The aim of the study was to:

1) Assess whether there were any differences between
   i) mucosal
   ii) luminal and
   iii) faecal pH

2) Measure the effects of different diets on
   i) mucosal
   ii) luminal and
   iii) faecal pH
   in colostomies of 'healthy' patients (colostomies performed because of trauma) and colostomies in colorectal cancer patients

3) Compare pH in the right and left sides of the colon.

Additional aims of the study were to investigate:

4) Breath methane values of patients consuming different diets.

5) Breath methare values of patients with colostomies on different sides of the colon.

- CHAPTER 4 -

PATIENTS
AND
METHODS

4.1. SUBJECTS

The protocol was approved by the Ethics Committee of the University of the Witwatersrand.
(Protocol Number 45/2/89)

Patients were Black volunteers and were admitted to hospital for the entire period of the study.

Three groups of patients were studied.

Group A: 5 patients with right sided colostomies; [colostomies performed after trauma to defunction the transverse or left colon].
Patients were 4 males and 1 female.
Mean age 28 years.

Group B: 5 patients with left sided colostomies (sigmoid colostomies);
[colostomies performed after trauma to defunction the sigmoid colon or rectum].
All patients were males.
Mean age 32 years.

Group C: 5 patients with left sided colostomies (sigmoid colostomies);
[colostomies performed for rectal cancer].
All patients were males.
Mean age 54 years.

4.2. INCLUSION CRITERIA

1. The study of each individual was done at least 14 weeks after fashioning the colostomies (except for 1 trauma patient in whom the study was carried out after 8 weeks).
2. No sepsis was present in the operative field or anywhere else in the body.
3. All haematological and biochemical tests were normal.
4. The patients were not on any antibiotic therapy for at least 14 weeks before the study was commenced (except for the one patient mentioned above).
5. The patients did not have any previous or current gastrointestinal disease.

4.3. THE DIFFERENT DIETS

Three different diets were consumed as follows:

Diet A western diet

Diet B high fibre diet

Diet C high starch / low fibre diet

A 2 x 3 factorial design was used to randomise the diets. The patients received each diet for five days and the pH-level was monitored on the fifth day and the mornings of the 6th and 7th days. The order of the diets was different for each patient and a Latin square type of design was used.

Left Bowel		Right Bowel	
Patient	Order of diet	Patient	Order of diet
1	ABC	1	CAB
2	ACB	2	ABC
3	BAC	3	CBA
4	BCA	4	BAC
5	CAB	5	ACB

4.3.1. DIET A (WESTERN DIET) - (Amount eaten daily)

Energy	8400 KJ
Fat	40% --> 3360 KJ = 88.9g
Protein	12% --> 1008 KJ = 60g
CHO	48% --> 4032 KJ = 240g: starch = 20% -> 100g
*1,2 Fibre	12g

FOOD ITEM	AMOUNT (g)	CHO (g)	PROTEIN (g)	FAT (g)	FIBRE (g)	ENERGY (KJ)
Milk - whole	250	11.75	8.25	8.25	0	642.5
Fruit - apple	75	9.15	0.15	0.3	2.33	183.75
Vegetable A: (Green Be ns)	60	2.34	1.14	0.18	2.34	88.2
Vegetable B: (Carrots)	75	4.73	0.83	0.15	3.15	141
Maize meal porridge	200	27.2	3.2	1	1	548
Brown Bread	30	13.59	2.67	0.87	1.59	332.7
Rice	200	57	4	0.2	1.4	912
Meat: beef/chicken/ fish	180	0	43.5	22.38	0	1585.5
*3 Fat	65	0	0.13	52.65	0	2080
Sugar: White	60	59.7	-	-	-	967.2
Jam	15	10.34	0.09	0.02	0.17	170.85
Sweets	40	37.24	-	0.08	-	618
Totals:		233.29	63.96	86.08	11.98	8269.7
% of Energy		47.4%	12.9%	39.3%		
Starch % of Energy		21.6%				

(Gows and Langenhoven, 1986; NRIND food composition tables)

4.3.2. Diet B (HIGH FIBRE DIET) - (Amount eaten daily)

Energy	8400 KJ
Fat	40% --> 3360 KJ = 88.9g
Protein	12% --> 1008 KJ = 60g
CHO	48% --> 4032 KJ = 240g ; starch = 20% -> 100g
*1,2 Fibre	30g

FOOD ITEM	AMOUNT (g)	CHO (g)	PROTEIN (g)	FAT (g)	FIBRE (g)	ENERGY (KJ)
Milk - whole	250	11.75	8.25	8.25	0	642.5
Fruit - apple	75	9.15	0.15	0.3	2.33	183.75
Vegetable A:(tomato-spinach-squash-etc)	600	15.6	1.68	1.68	14.58	61.2
Maize meal porridge	150	20.4	2.4	0.75	0.75	411
*4 Bran - S biscuit	2 bisc.	22.6	3.2	-	5	660
Rice	200	57	4	0.2	1.4	912
Meat: beef/chicken/ fish	180	0	43.5	22.38	0	1585.5
*3 Fat	65	0	0.13	52.65	0	2080
Sugar: White	80	79.6	-	-	-	1299.6
Jam	15	10.34	0.09	0.02	0.17	170.85
Sweets	15	13.97	0.12	0.12	-	231.75
Fibre: (Unprocessed bran)	10	2.68	1.4	0.5	4.4	87.2
Totals:		243.09	64.83	86.83	28.63	8325.35
% of Energy		49.1%	13.0%	39.4%		
Starch % of Energy		20.2%				

(Gows and Langenhoven, 1986; NRIND food composition tables)

4.3.3. Diet C (LOW FIBRE / HIGH STARCH DIET) - (Amount eaten daily)

Energy	8400 KJ
Fat	40% --> 3360 KJ = 88.9g
Protein	12% --> 1008 KJ = 60g
CHO	48% --> 4032 KJ = 240g ; starch = 40% -> 200g
*1,2 Fibre	12g

FOOD ITEM	AMOUNT (g)	CHO (g)	PROTEIN (g)	FAT (g)	FIBRE (g)	ENERGY (KJ)
Milk - whole	125	5.88	4.13	4.13	0	321.25
Fruit - apple	75	9.15	0.15	0.3	2.33	183.75
Vegetable A: (carrots)	75	4.73	0.83	0.15	3.15	141
Maize meal porridge	250	34	4	1.25	1.25	685
White bread	90	44.9	7.29	2.25	2.52	1003.5
Rice	400	114	8	0.4	2.8	1824
Meat: beef/chicken/ fish	150	-	36.25	18.65	-	1321.25
*3 Fat	75	0	0.15	60.75	-	2400
Sugar: White	30	29.86	-	-	-	483.6
Totals:		242.52	60.8	87.88	12.05	8363.35
% of Energy		48.7%	12.2%	39.1%		
Starch % of Energy		38.7%				

(Gows and Langenhoven, 1986; NRIND food composition tables)

4.3.4. COMMENT ON DIETARY CONSTITUENTS

*1 Dietary fibre is defined as the sum of the polysaccharides and lignin which are not digested, and therefore not absorbed by the human gastrointestinal tract (Trowell et al., 1976; Paul and Southgate, 1978).

*2 The fibre content of the food is given as dietary fibre, g/100g. However when the dietary fibre content of a food was not known, the crude fibre content is given. The South African Food Tables use mainly U.K. and U.S.A. food composition tables. The fibre values thus reflect the methods of Southgate (Southgate, 1969) and Prosky (Prosky et al., 1984).

*3 Fat = the fat contained in the meat portions, spreading margarine, and oil for preparation of food.

*4 Bran S biscuit = a high fibre biscuit (2.5g dietary fibre / 18g biscuit).

Coffee and cigarette smoking were not permitted during the study.

A tupperware type of colostomy bag was used in order to faciliate frequent access to the colostomy site. (Figure 3). A Convatec stomahesive with a 57 mm flange was attached to the colostomy site, and a Convatec System 2 Opaque Closed Pouch was attached to the flange. After the colostomy was examined the pouch was discarded and replaced with a new one. This was very well accepted by the patients as there was no need for the replacement of the stomahesive which can occasionally be painful.

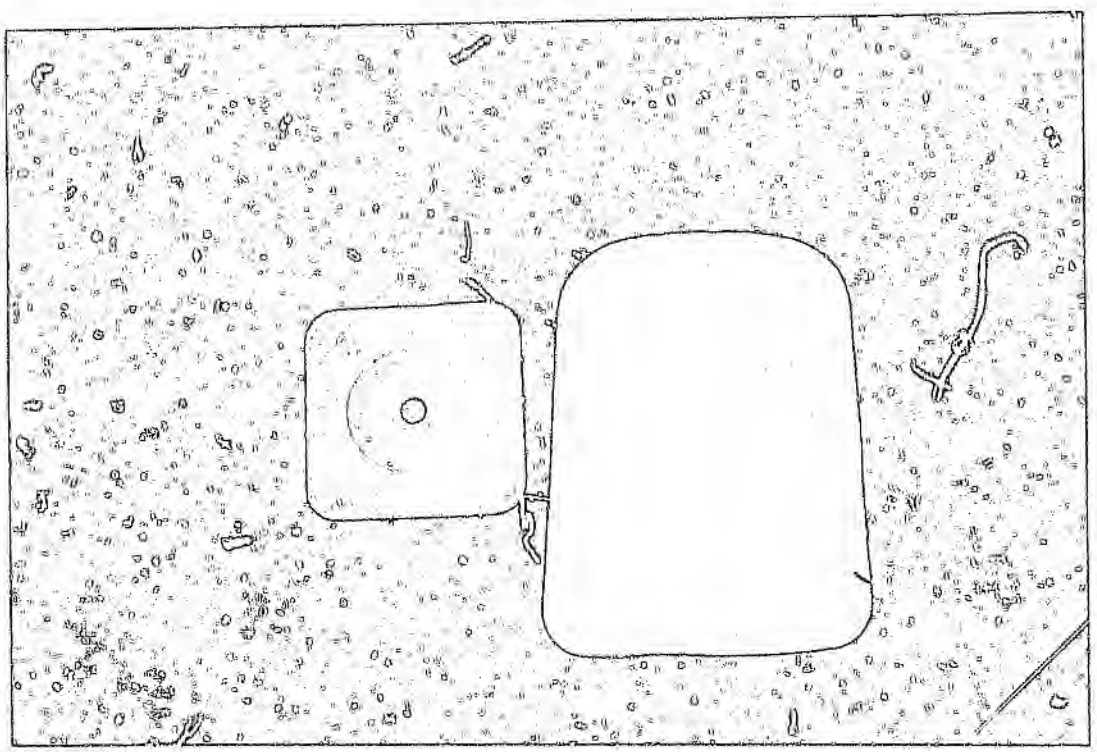


FIGURE 3

CONVATEC STOMAHESIVE WITH 57 mm FLANGE

AND SYSTEM 2 OPAQUE CLOSED POUCH

During the 5th, 6th and 7th day faeces were collected from the colostomy bag at the times specified below, and pH measurements of the faeces and the colon were done at the same intervals.

Day 5 of diet period.

08:00

11:00

14:00

17:00

20:00

Day 6 of diet period

08:00

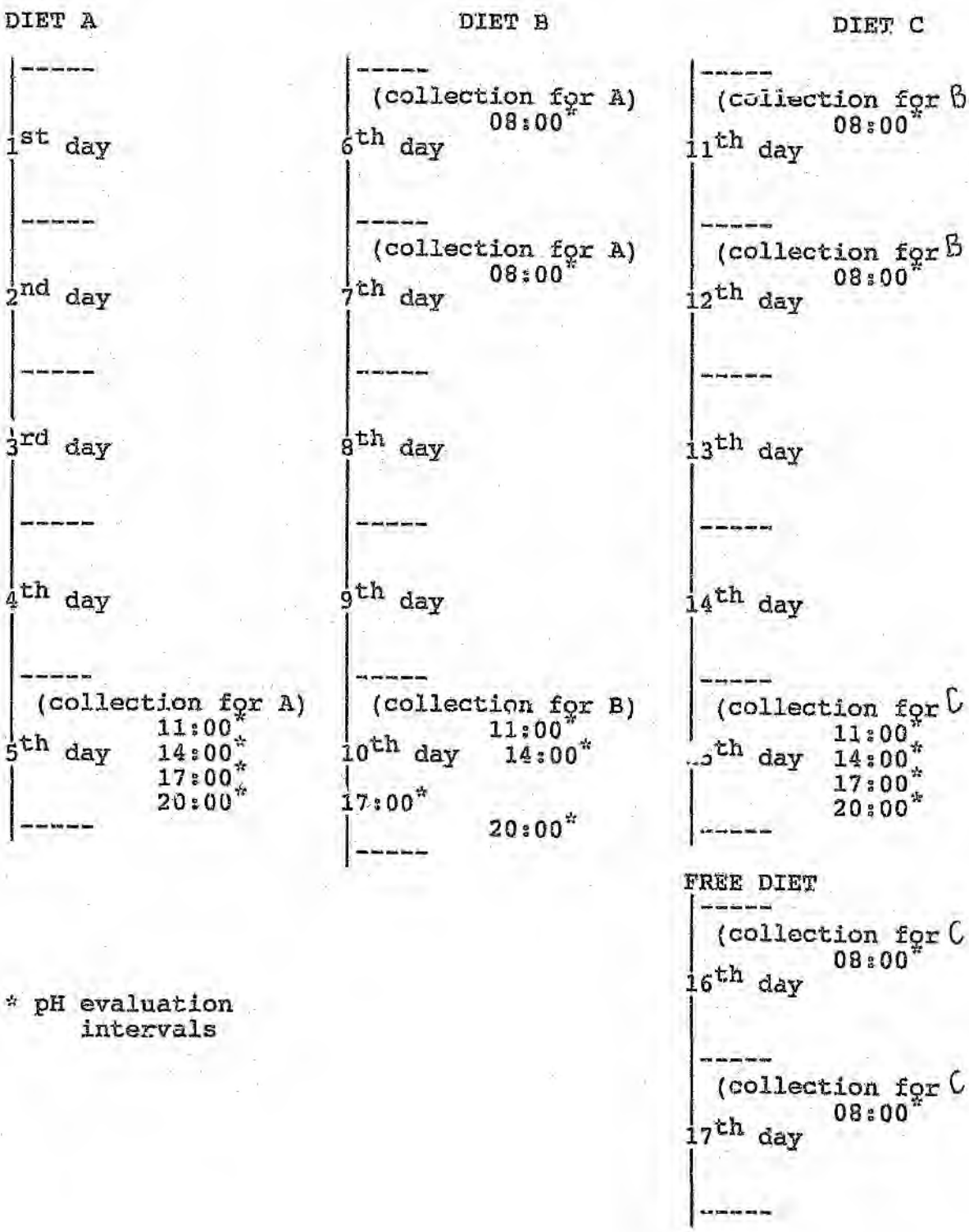
Day 7 of diet period

8:00

As shown in Figure 4 each patient was on a specific diet for 5 days. At 08:00 am of the fifth day and while the patient was on this diet (according to our example diet A), the pH determinations were carried out. Similar determinations were done at 11:00, 14:00, 17:00, and 20:00 of the fifth day. The same diet was given until 08:00 of the morning of the sixth day. At this time pH measurements were carried out and simultaneously the second diet was introduced. On the morning of the 7th day determinations of pH were done again, whilst the patient was on the next diet.

This second diet continued until 08:00 of the eleventh day, and exactly the same determinations at the same intervals were carried out, as for the first diet.

At 08:00 of the 11th day the third diet was introduced until 08:00 of the 16th day. Again all determinations, as before, at the same intervals were carried out.


DIET A
1st day
2nd day
3rd day
4th day
(collection for A)
11:00*
5th day 14:00*
17:00*
20:00*
DIET B
(collection for A)
08:00*
6th day
(collection for A)
08:00*
7th day
8th day
9th day
(collection for B)
11:00*
10th day 14:00*
17:00*
20:00*
DIET C
(collection for B
08:00*
11th day
(collection for B
08:00*
12th day
13th day
14th day
(collection for C
11:00*
th day 14:00*
17:00*
20:00*
FREE DIET
(collection for C
08:00*
16th day
(collection for C
08:00*
17th day
* pH evaluation intervals

TIME INTERVAL OF pH DETERMINATION

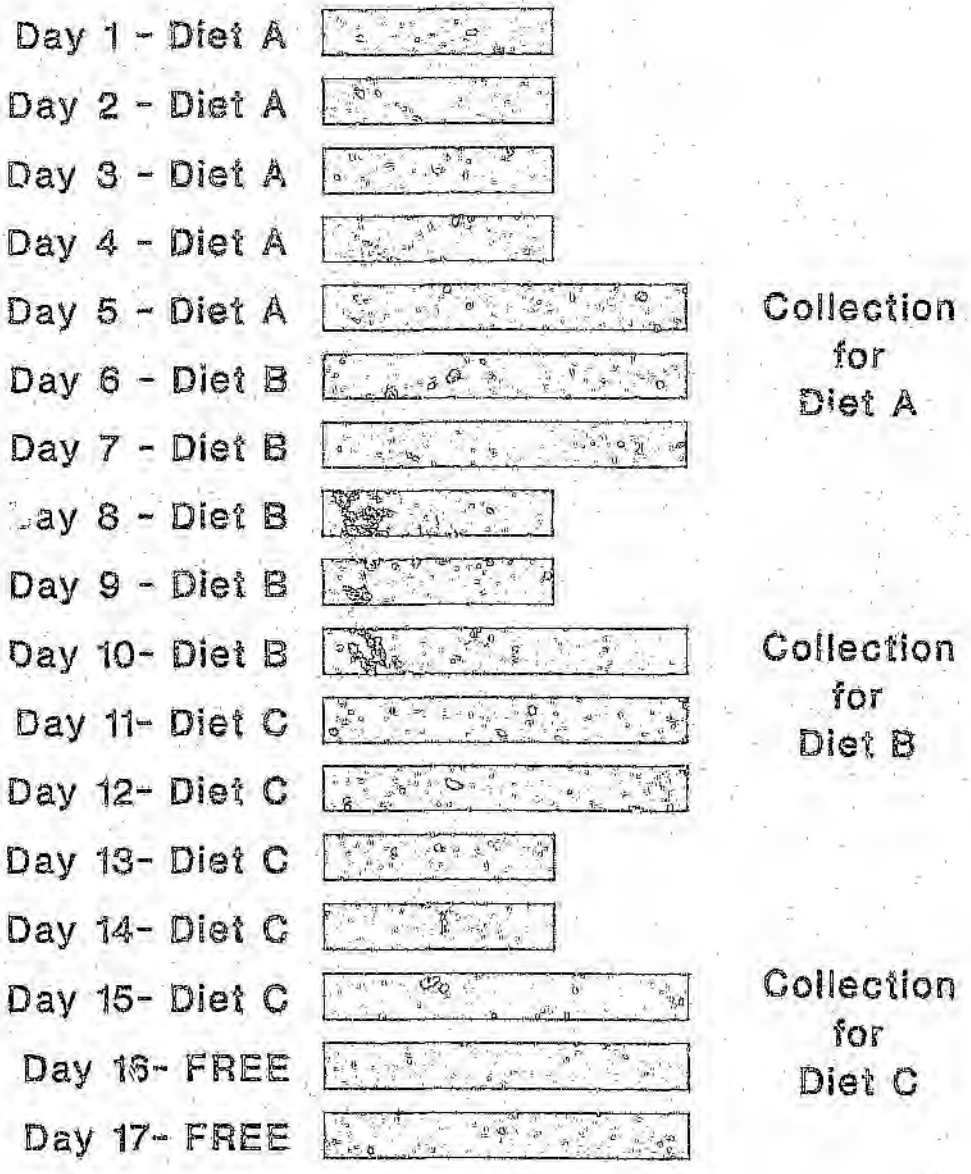


Figure 4

4.4. pH EVALUATION

All the above pH determinations were carried out using a portable Beckman, model 3500, pH digital meter (Figure 5).

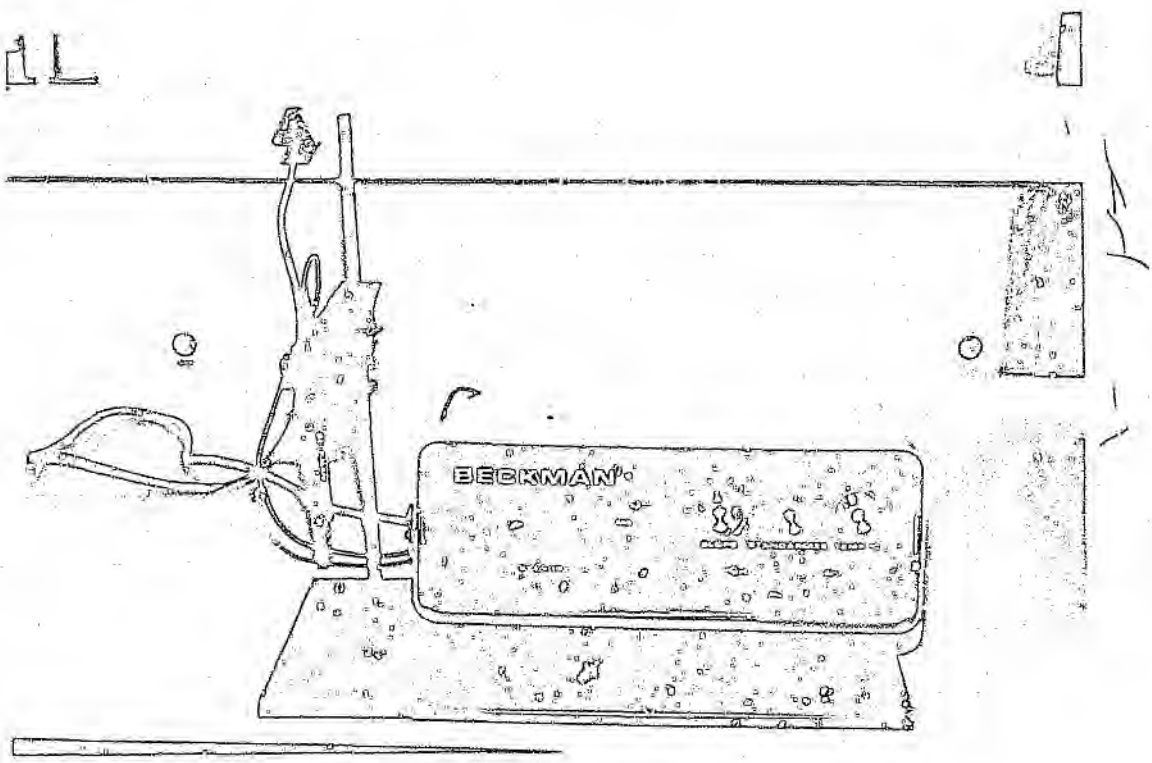


Figure 5

THE PORTABLE BECKMAN

MODEL 3500

pH DIGITAL METER

The pH determinations were done by probing either the lumen of the afferent and efferent loop (Figure 6), or the faeces in the colostomy bag.

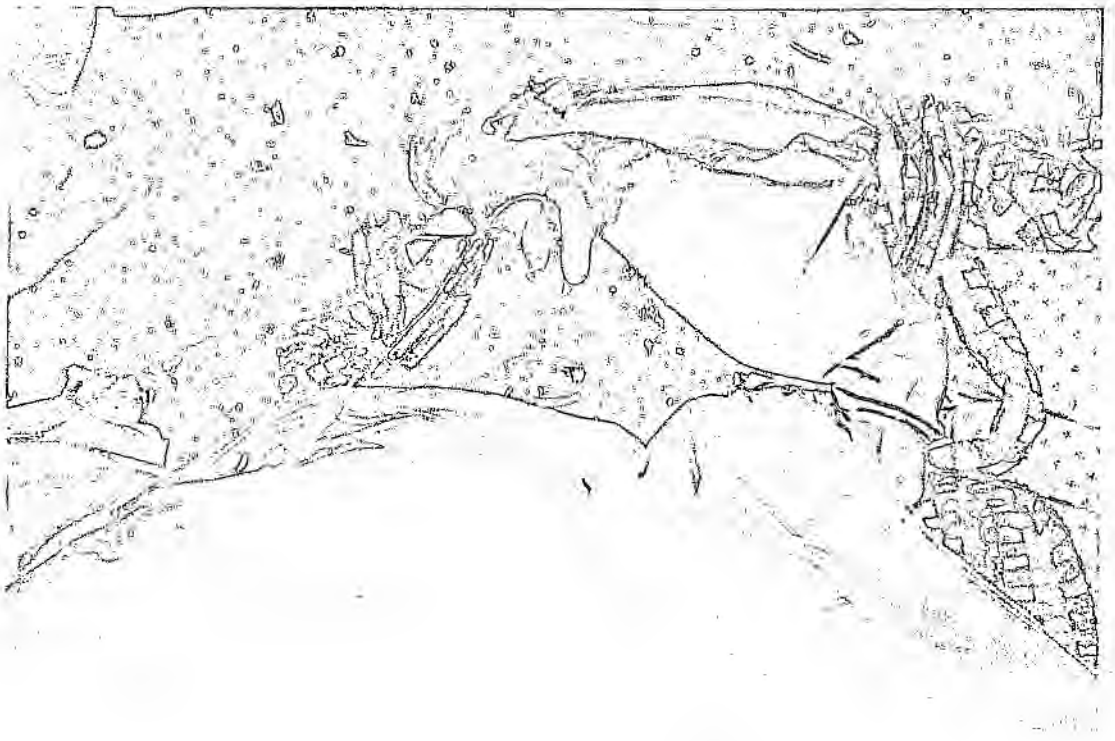


Figure 6

PROBE IN COLOSTOMY

The electrode used was a futura plus combination electrode (Epoxy body, AgCl, 12mm x 130mm, gel-filled electrode). (Figure 7)

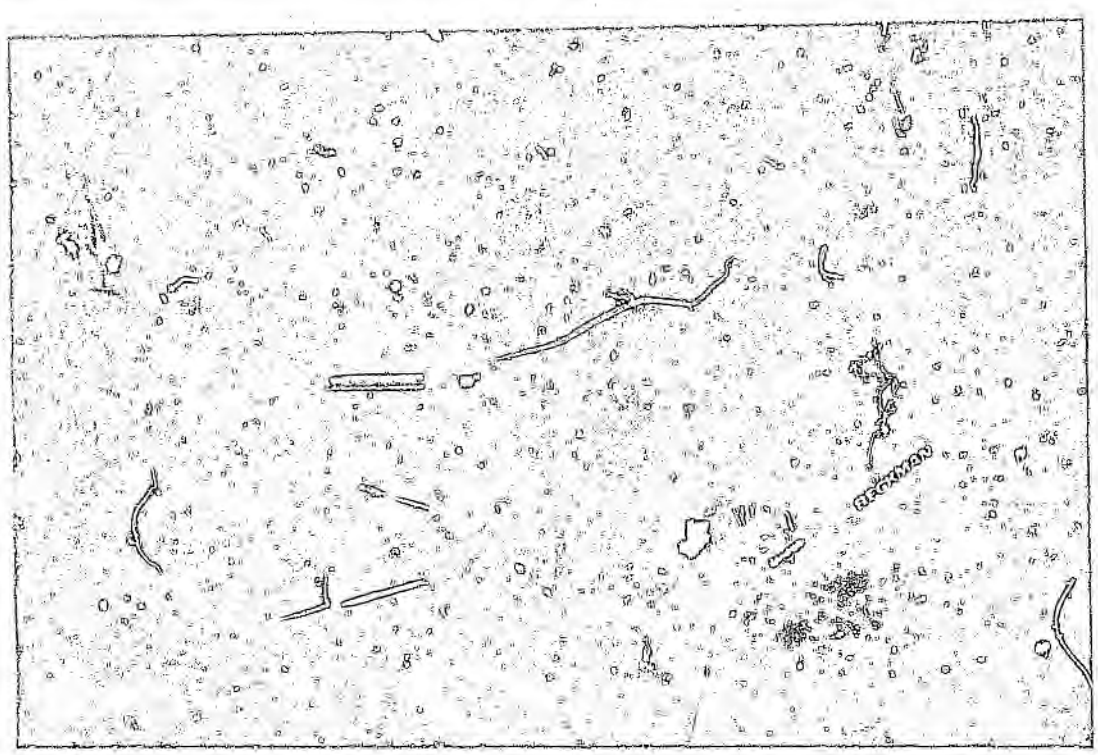


Figure 7

EPOXY BODY

AgCl, 12mm x 130mm

GEL-FILLED ELECTRODE

Before any of the above measurements were taken the reading of the apparatus was checked against a buffer solution of pH 7.00 and pH 4.00. (Figure 8)

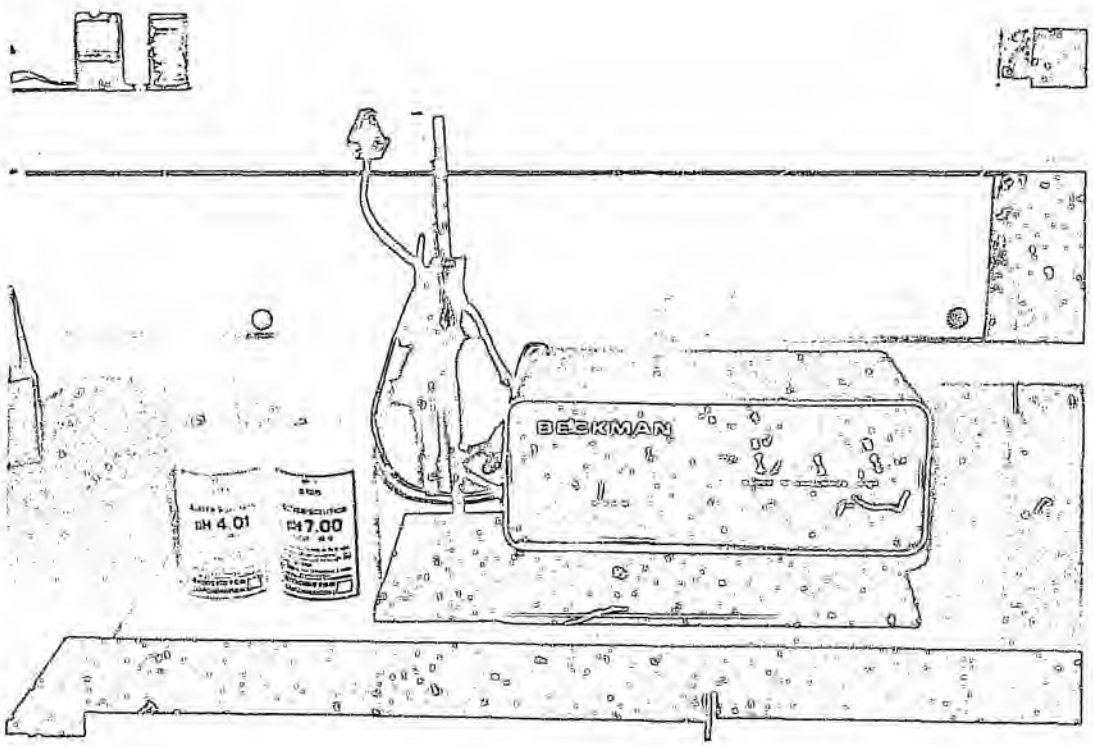


Figure 8

BUFFERS pH 4.0 & pH 7.0

4.4.1. pH OF THE COLONIC MUCOSA

With regard to colonic mucosa, two readings were carried out:

A. One where the probe was covered with faeces; This was recorded and regarded as the luminal pH.

B. For the second reading, either there was no faeces on the probe, or the faeces were manually removed so that the colon was cleared of faeces. This was regarded as mucosal pH.

The proximal and the distal limbs of the colostomy were examined in a similar way. Obviously as the distal limb is a defunctioned loop, no faeces was present there.

4.4.2. pH OF THE FAECES

With regard to the measurement of faecal pH this was performed by mixing the faeces in the bag with a wooden spatula and immersing the electrode into the mixed faeces. (Figure 9)

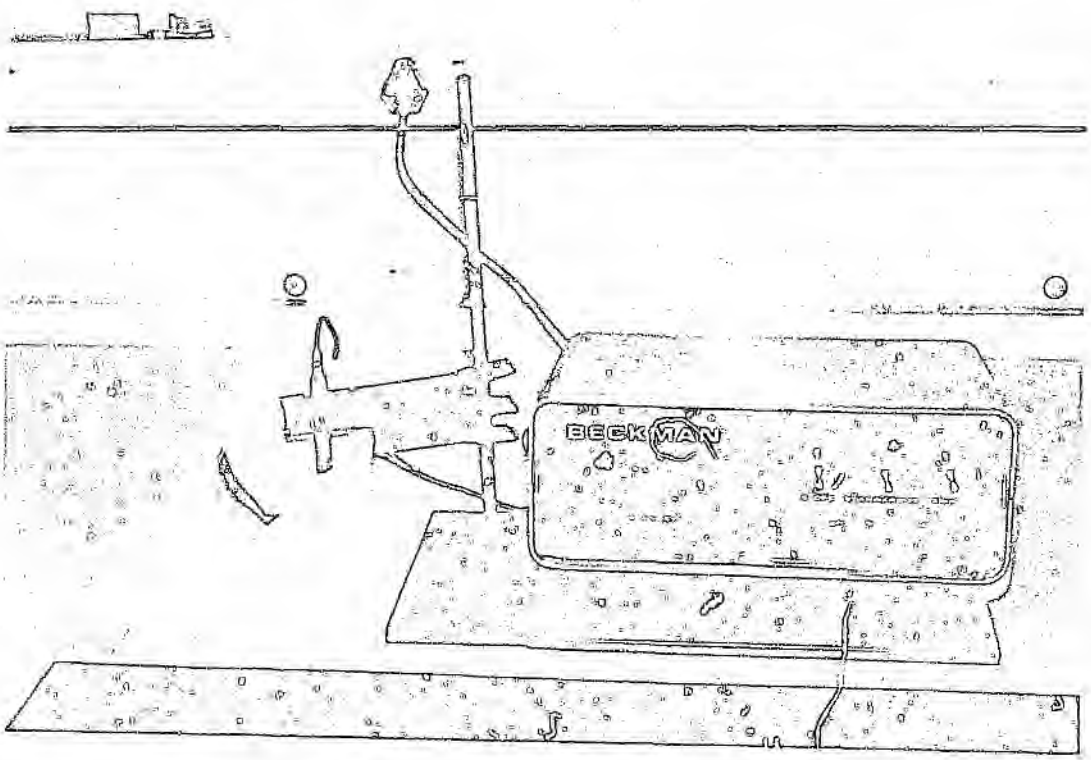


Figure 9

PROBE IN BAG

4.4.2.1. pH OF FAECES OVER A PERIOD OF 3 HOURS

In 4 patients the effect of time on faecal pH was studied. After a sufficient amount of faeces passed through the colostomy the faeces were incubated at 37^0C and the pH value estimated every 20 minutes. 1 g of faeces was mixed with 10 ml Normal Saline, and the pH of this solution was determined by immersing the same electrode into the emulsion (Walker et al., 1979; Samuelson et al., 1985). (Figure 10)

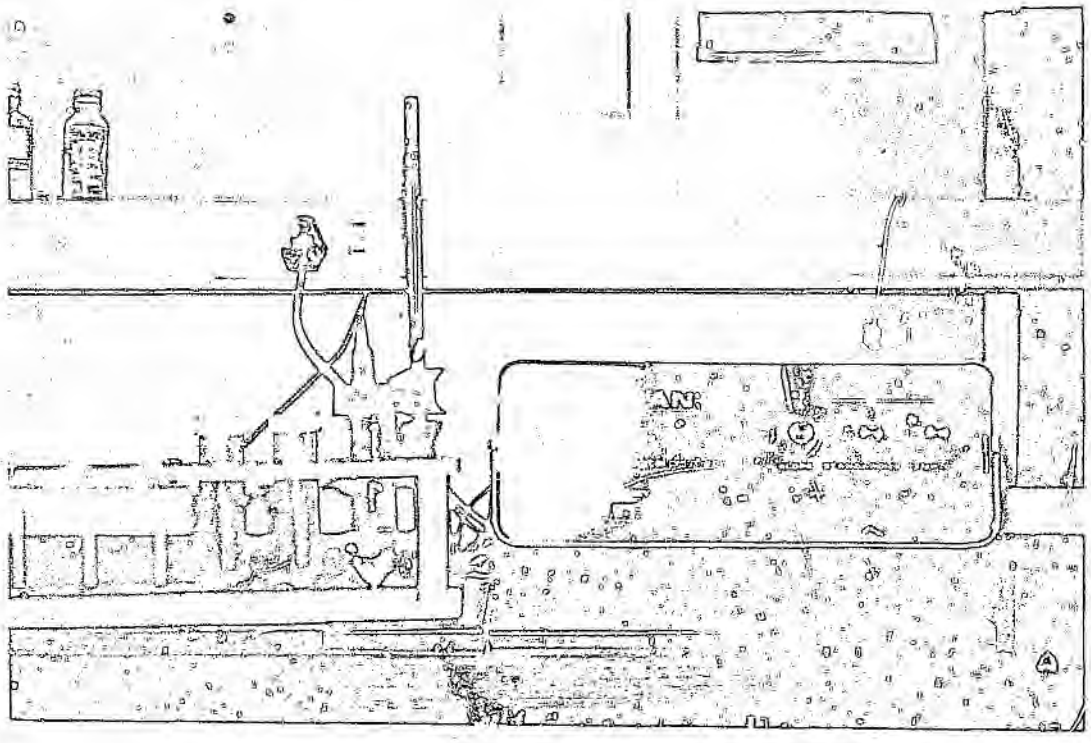


Figure 10

PROBE IN EMULSION OF FAECES

WITH NORMAL SALINE

4.5. BREATH METHANE

4.5.1. COLLECTION OF BREATH METHANE

Collection of breath methane was carried out on the morning of the 5th, and 6th day of each diet (Figure 11).

TIME INTERVAL BREATH METHANE COLLECTION

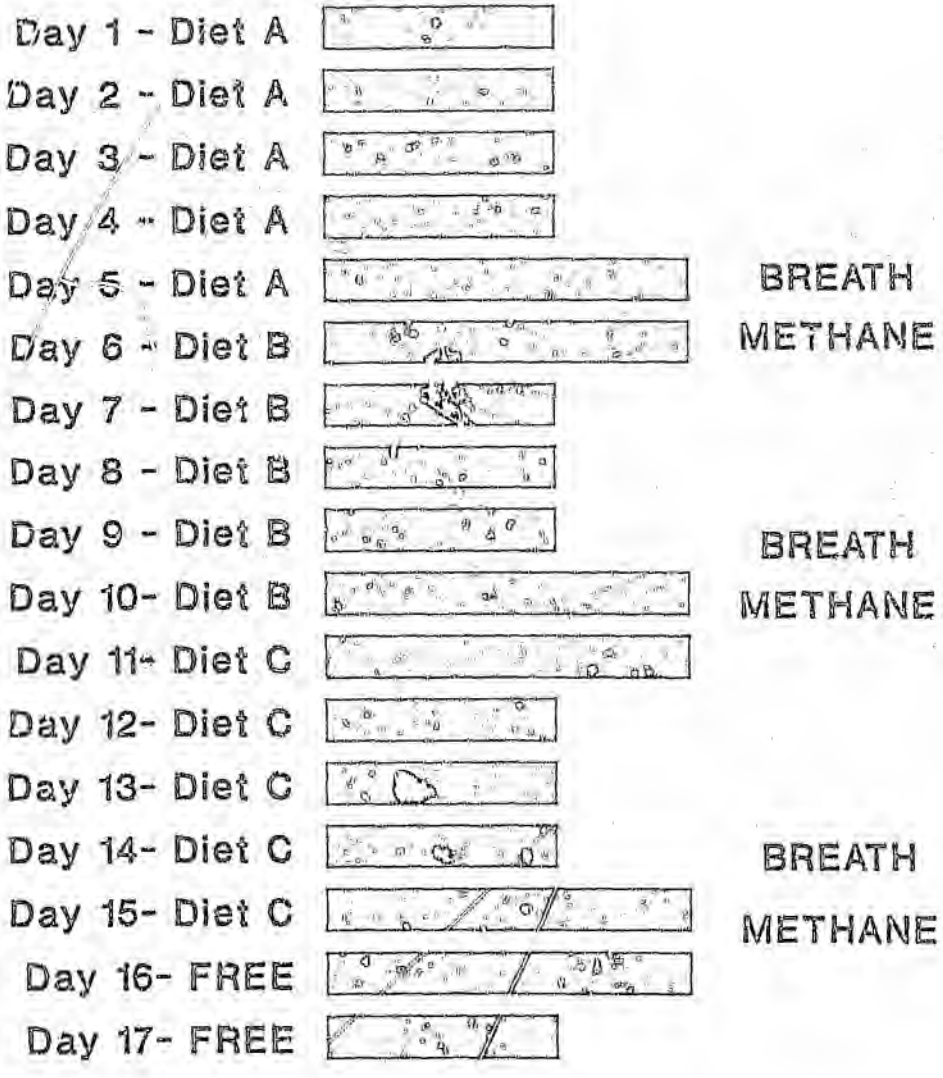


Figure 11

Triplicate end expiratory breath samples were collected via a Wiggins end expiratory breath sampler (Pomare et al., 1985) into 20 ml plastic syringes. (Figure 12)

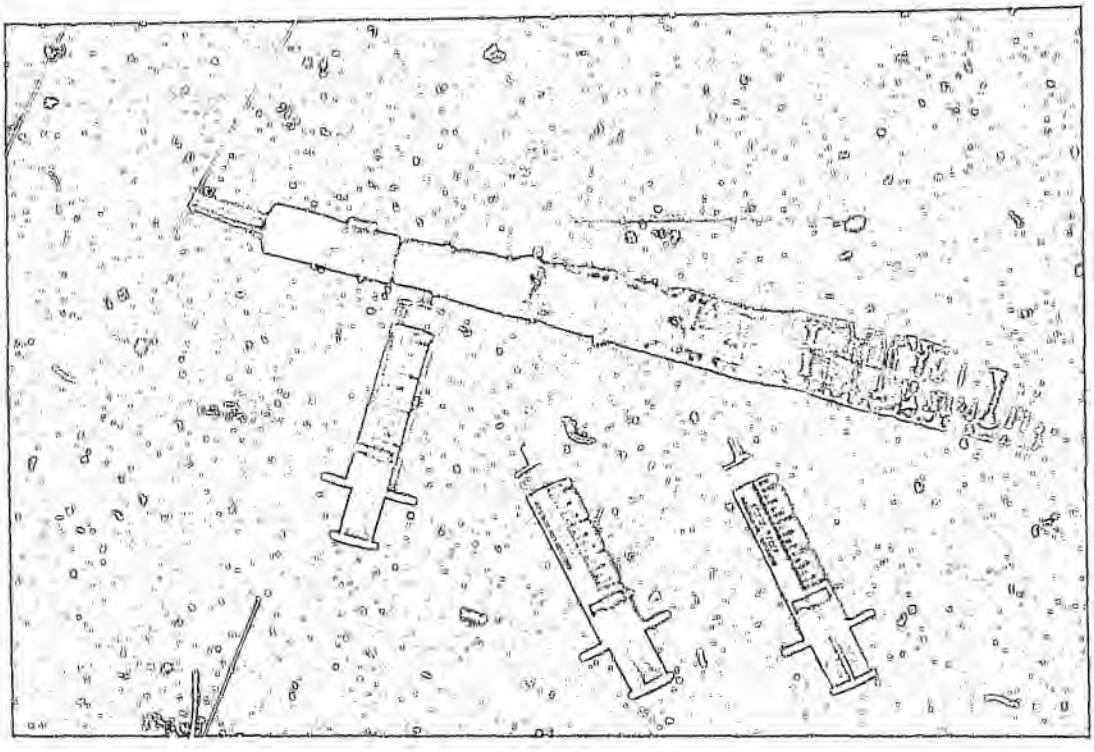


Figure 12

WIGGINS END EXPIRATORY BREATH SAMPLER

4.5.2. DETERMINATION OF METHANE CONCENTRATION

Methane concentration was determined by gas chromatography using a Phillips Pye Unicam PU-4500 equipped with a hydrogen ionisation detector (Figure 13). 1,5 meters by 4mm glass column was packed with molecular sieve 5A 80/100 mesh (supelco cat. No. 2-303). The carrier gas was nitrogen. A sample of room air was taken at the time of each breath sample and the value (usually in the range 1.0-2.0 parts per million (ppm)) was subtracted from the average level of the triplicate breath samples. The peak area of methane was calculated against the peak area of methane standards. Methane producers were defined as those producing more than 1 ppm (parts per million) of methane above the level in the ambient air. (Bond et al., 1971)

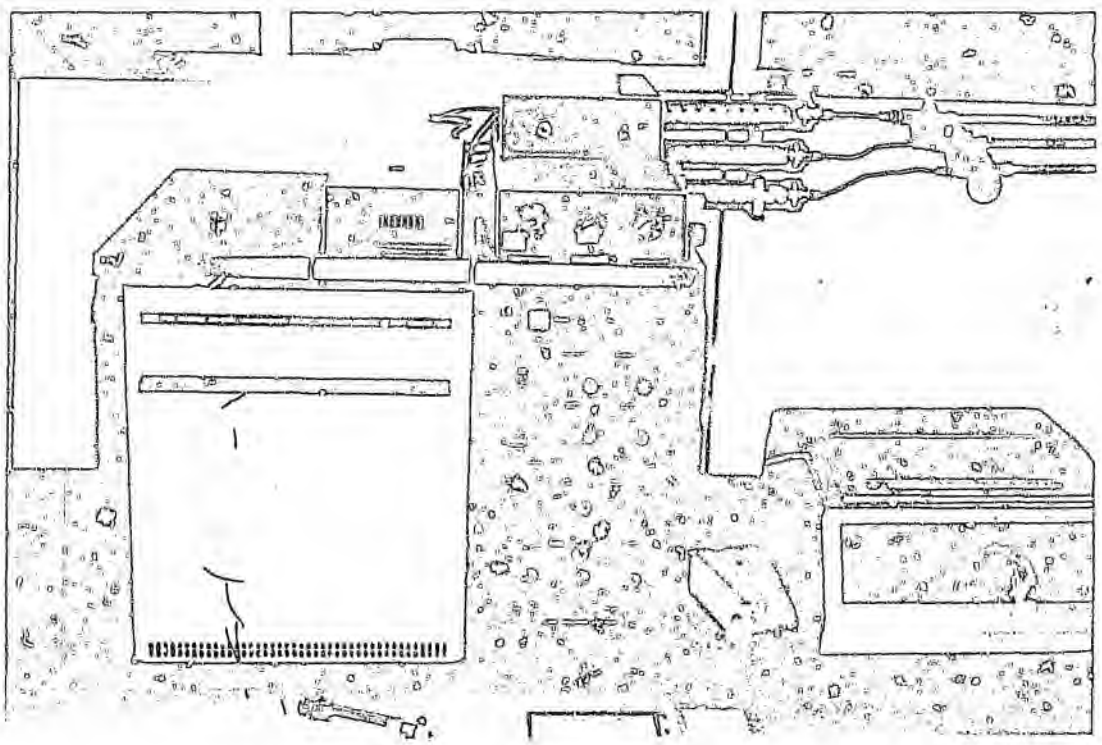


Figure 13

PHILLIPS Pye Unicam PU-4500,

METHANE DETECTOR

- CHAPTER 5-

STATISTICAL ANALYSIS

Analysis of variance with the following factors and interactions in the model were carried out. Type of diet, side of colon*, diet x side, diet x placement**, and also the second order interaction, diet x side x placement. Finally persons were nested within side (right or left side). After the analysis of variance, whenever a factor was significant the Bonferroni adjustment for pairwise comparison between means was carried out. The Bonferroni adjustment implies dividing the significance level (0.05) by the number of comparisons that were done.

Analysis was done with the SAS programme package. (SAS Users Guide, 1985).

* Side of colon = right colon or left colon

** Placement = siting where the pH measurements were carried out. viz luminal pH; colonic mucosal pH; defunctioned loop pH; faecal pH;

Regression lines were fitted for each patient with time as predictor and pH as dependant variable. If the slopes of the patients did not differ significantly the data of all patient were pulled out and a common slope was calculated and tested for significance.

- CHAPTER 6 -

RESULTS

6.1. pH RESULTS

The pH results of each patient are shown below:

According to Diet

Placement

Site

Time

6.1.1. pH RESULTS OF INDIVIDUAL PATIENTS

6.1.1.1. RIGHT COLONIC TRAUMA

6.1.1.1.1. Rt colonic trauma - 1st Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	----	7.62	8.07	6.13
11:00	----	7.76	8.12	5.42
14:00	----	7.60	8.19	6.75
17:00	----	7.12	7.99	7.03
20:00	----	7.52	8.08	6.24
08:00 Day 6	----	7.98	8.04	5.20
08:00 Day 7	----	7.95	8.00	6.14
Mean value:	----	7.65	8.07	6.13
DIET B:				
08:00	----	7.81	8.06	4 99
11:00	----	8.11	8.33	5.44
14:00	----	7.62	8.21	5.24
17:00	----	7.58	7.96	4.61
20:00	----	7.80	8.01	4.62
08:00 Day 6	----	7.93	8.08	5.11
08:00 Day 7	----	7.96	7.98	4.71
Mean value:	----	7.83	8.09	4.96
DIET C:				
08:00	----	8.01	8.29	4.77
11:00	----	8.02	8.22	6.56
14:00	----	7.91	8.02	5.91
17:00	----	7.90	8.00	5.13
20:00	----	8.10	8.03	6.08
08:00 Day 6	----	7.89	8.09	4.63
08:00 Day 7	----	8.15	8.12	4.41
Mean value:	----	7.99	8.11	5.35

6.1.1.1.2. Rt colonic trauma - 2nd Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	----	8.03	----	6.95
11:00	----	8.02	----	6.60
14:00	----	8.12	----	6.83
17:00	----	7.80	----	7.40
20:00	----	7.84	----	7.20
08:00 Day 6	----	8.21	----	6.31
08:00 Day 7	----	8.13	----	6.24
Mean value:	----	8.01	----	6.79
DIET B:				
08:00	----	7.98	----	6.01
11:00	----	7.90	----	6.41
14:00	----	7.95	----	6.35
17:00	----	7.93	----	7.09
20:00	----	7.70	----	5.91
08:00 Day 6	----	7.68	----	6.47
08:00 Day 7	----	7.94	----	5.23
Mean value:	----	7.86	----	6.21
DIET C:				
08:00	----	8.03	----	5.55
11:00	----	7.72	----	7.55
14:00	----	7.73	----	6.71
17:00	----	7.93	----	7.32
20:00	----	7.93	----	6.7
08:00 Day 6	----	7.98	----	6.25
08:00 Day 7	----	7.98	----	5.33
Mean value:	----	7.90	----	6.41

6.1.1.1.3. Rt colonic trauma - 3rd Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	7.91	8.37	----	----
11:00	----	8.03	----	----
14:00	7.43	7.78	----	6.58
17:00	7.48	7.94	----	5.97
20:00	7.62	7.83	----	6.41
08:00 Day 6	6.95	7.52	----	5.09
08:00 Day 7	7.11	7.91	----	5.27
Mean value:	7.41	7.91	----	5.86
DIET B:				
08:00	7.70	8.05	----	6.57
11:00	7.81	8.05	----	7.61
14:00	7.98	8.15	----	7.03
17:00	7.65	7.91	----	6.81
20:00	7.78	8.11	----	7.28
08:00 Day 6	7.59	8.25	----	5.34
08:00 Day 7	7.67	8.11	----	5.42
Mean value:	7.74	8.09	----	6.58
DIET C:				
08:00	7.78	7.89	----	6.18
11:00	7.18	7.75	----	6.46
14:00	8.02	8.19	----	6.73
17:00	7.65	7.90	----	7.12
20:00	7.70	7.93	----	6.16
08:00 Day 6	----	7.59	----	5.39
08:00 Day 7	7.43	7.81	----	5.39
Mean value:	7.63	7.87	----	6.20

6.1.1.1.4. Rt colonic trauma - 4th Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	----	7.89	----	5.53
11:00	7.84	7.62	----	5.87
14:00	----	7.80	----	4.95
17:00	7.89	8.11	----	5.83
20:00	7.77	7.92	----	6.39
08:00 Day 6	7.79	7.99	----	5.41
08:00 Day 7	7.82	8.03	----	4.92
Mean value:	7.82	7.91	----	5.56
DIET B:				
08:00	7.15	7.88	----	5.79
11:00	7.22	7.83	----	5.91
14:00	7.13	7.83	----	6.15
17:00	7.18	7.89	----	----
20:00	----	7.76	----	6.27
08:00 Day 6	7.21	7.71	----	5.23
08:00 Day 7	----	8.11	----	5.29
Mean value:	7.18	7.86	----	5.77
DIET C:				
08:00	7.47	7.99	----	5.21
11:00	7.38	7.95	----	5.53
14:00	7.35	7.99	----	5.08
17:00	7.05	7.68	----	4.99
20:00	7.81	8.12	----	5.28
08:00 Day 6	7.47	7.99	----	5.09
08:00 Day 7	7.65	7.88	----	5.10
Mean value:	7.45	7.94	----	5.18

6.1.1.1.5. Rt colonic trauma - 5th Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	----	8.00	8.08	5.42
11:00	7.62	8.27	8.00	6.35
14:00	----	7.82	8.03	4.99
17:00	7.51	8.01	8.09	5.09
20:00	7.73	----	8.11	5.73
08:00 Day 6	7.58	7.83	8.07	5.31
08:00 Day 7	7.52	8.03	8.13	4.97
Mean value:	7.59	7.99	8.07	5.41
DIET B:				
08:00	7.52	8.01	7.99	5.01
11:00	7.54	7.96	8.03	5.33
14:00	7.66	7.95	8.01	4.96
17:00	7.47	7.88	7.95	4.98
20:00	7.49	8.03	8.11	4.93
08:00 Day 6	7.45	7.99	8.07	5.13
08:00 Day 7	----	8.10	8.03	5.06
Mean value:	7.52	7.99	8.03	5.06
DIET C:				
08:00	7.67	7.99	7.96	4.99
11:00	7.63	8.11	8.01	5.32
14:00	7.70	7.79	7.88	----
17:00	----	7.97	7.99	4.87
20:00	7.77	7.96	8.01	4.93
08:00 Day 6	7.81	7.99	8.03	4.89
08:00 Day 7	7.39	8.00	8.11	4.95
Mean value:	7.66	7.97	8.00	4.99

6.1.1.2. LEFT COLONIC TRAUMA

6.1.1.2.1. Lt colonic trauma - 1st Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	----	8.19	8.31	----
11:00	----	7.89	8.08	----
14:00	----	8.13	8.09	----
17:00	----	8.09	8.11	----
20:00	----	7.98	8.11	----
08:00 Day 6	----	8.03	8.18	7.67
08:00 Day 7	7.07	7.67	8.11	7.32
Mean value:	7.07	7.99	8.14	7.49
DIET B:				
08:00	7.69	8.03	8.29	6.74
11:00	7.97	8.15	8.61	----
14:00	----	8.28	8.44	7.89
17:00	7.51	7.91	8.11	6.48
20:00	7.85	8.10	8.13	5.91
08:00 Day 6	7.75	7.93	8.39	7.29
08:00 Day 7	7.38	7.93	8.32	7.15
Mean value:	7.69	8.05	8.33	6.74
DIET C:				
08:00	7.56	8.19	8.26	6.51
11:00	7.19	----	8.11	6.62
14:00	----	8.19	8.29	----
17:00	7.43	7.85	8.15	6.33
20:00	7.70	8.11	8.02	----
08:00 Day 6	7.44	8.11	8.26	----
08:00 Day 7	7.63	7.89	8.42	6.61
Mean value:	7.49	8.06	8.22	6.52

6.1.1.2.2. Lt colonic trauma - 2nd Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	7.86	8.05	7.99	6.94
11:00	----	7.74	7.88	6.95
14:00	7.20	----	7.60	6.86
17:00	7.26	7.75	7.81	6.89
20:00	----	7.87	7.83	7.04
08:00 Day 6	----	7.69	7.93	6.90
08:00 Day 7	----	7.84	7.90	6.84
Mean value:	7.44	7.82	7.85	6.92
DIET B:				
08:00	----	7.96	7.89	6.76
11:00	----	7.67	7.66	6.96
14:00	7.41	7.90	7.93	6.66
17:00	6.72	7.96	8.02	6.72
20:00	7.32	7.94	7.86	6.66
08:00 Day 6	----	7.82	7.83	6.89
08:00 Day 7	----	7.79	7.85	6.79
Mean value:	7.15	7.86	7.86	6.78
DIET C:				
08:00	----	7.70	7.93	6.81
11:00	7.32	7.09	7.93	6.79
14:00	7.79	7.77	7.29	3.86
17:00	7.89	7.18	8.14	7.18
20:00	7.04	7.93	8.15	6.46
08:00 Day 6	----	8.03	8.13	6.49
08:00 Day 7	7.40	8.07	8.06	6.96
Mean value:	7.49	7.68	7.95	6.79

6.1.1.2.3. Lt colonic trauma - 3rd Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	7.23	----	8.32	5.29
11:00	7.64	----	8.02	5.50
14:00	6.89	----	7.89	5.90
17:00	7.24	7.80	7.89	4.69
20:00	8.08	----	8.09	5.65
08:00 Day 6	7.29	7.62	8.12	5.33
08:00 Day 7	7.53	7.87	8.05	4.79
Mean value:	7.41	7.76	8.05	5.31
DIET B:				
08:00	----	8.04	8.05	5.79
11:00	7.49	8.16	8.09	5.66
14:00	7.86	8.04	8.02	5.79
17:00	7.63	8.12	8.09	6.16
20:00	7.54	7.92	8.04	5.91
08:00 Day 6	7.69	7.92	7.99	5.18
08:00 Day 7	7.62	8.01	8.03	5.69
Mean value:	7.64	8.03	8.04	5.74
DIET C:				
08:00	7.83	----	7.85	5.01
11:00	7.31	7.96	7.92	4.91
14:00	7.91	8.22	7.07	4.73
17:00	7.98	7.34	7.66	5.51
20:00	----	8.01	8.03	4.69
08:00 Day 6	8.02	8.23	8.13	5.07
08:00 Day 7	7.83	8.29	8.17	5.23
Mean value:	7.81	8.00	7.83	5.02

6.1.1.2.4. Lt colonic trauma - 4th Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	7.54	8.26	----	6.73
11:00	7.79	8.34	----	----
14:00	7.27	7.99	----	6.41
17:00	----	8.28	----	6.37
20:00	7.78	8.27	----	5.87
08:00 Day 6	7.19	7.99	----	6.49
08:00 Day 7	7.57	8.02	----	6.95
Mean value:	7.52	8.16	----	6.44
DIET B:				
08:00	7.90	8.05	----	5.73
11:00	----	8.05	----	6.57
14:00	----	8.27	----	----
17:00	7.83	8.11	----	5.24
20:00	7.95	7.69	----	----
08:00 Day 6	8.15	8.20	----	6.59
08:00 Day 7	7.59	----	----	6.54
Mean value:	7.88	8.06	----	6.14
DIET C:				
08:00	----	8.37	----	6.33
11:00	8.02	8.11	----	----
14:00	----	8.23	----	----
17:00	----	8.11	----	----
20:00	7.96	8.09	----	6.65
08:00 Day 6	7.51	8.16	----	6.83
08:00 Day 7	7.63	8.15	----	6.49
Mean value:	7.78	8.18	----	6.58

6.1.1.2.5. Lt colonic trauma - 5th Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	----	8.	8.29	6.71
11:00	----	8.	8.33	----
14:00	----	8.1	8.16	5.98
17:00	7.59	8.15	8.16	----
20:00	7.23	8.33	8.30	5.99
08:00 Day 6	7.88	8.28	8.34	6.38
08:00 Day 7	----	8.28	8.25	6.45
Mean value:	7.57	8.25	8.26	6.30
DIET B:				
08:00	7.87	8.27	8.10	6.15
11:00	----	8.08	8.09	5.71
14:00	----	7.59	8.19	4.55
17:00	----	8.05	8.07	4.81
20:00	----	7.93	8.28	----
08:00 Day 6	7.63	8.02	8.04	6.55
08:00 Day 7	8.08	8.20	8.09	5.49
Mean value:	7.86	8.02	8.12	5.54
DIET C:				
08:00	8.21	8.28	8.05	6.22
11:00	----	8.12	8.25	6.90
14:00	7.76	7.90	8.13	6.11
17:00	----	8.15	8.13	----
20:00	7.89	8.18	8.35	4.93
08:00 Day 6	7.65	8.10	8.35	5.64
08:00 Day 7	7.66	7.99	7.93	6.17
Mean value:	7.83	8.10	8.17	6.00

6.1.1.3. COLONIC CANCER PATIENTS

6.1.1.3.1. Lt colonic cancer - 1st Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	----	8.07	----	5.83
11:00	7.81	8.51	----	----
14:00	----	8.03	----	6.42
17:00	7.68	7.95	----	4.90
20:00	----	8.03	----	----
08:00 Day 6	7.61	7.98	----	5.85
08:00 Day 7	7.66	8.09	----	6.03
Mean value:	7.69	8.09	----	5.81
DIET B:				
08:00	----	7.84	----	6.57
11:00	----	8.15	----	7.04
14:00	----	8.00	----	7.18
17:00	----	7.90	----	6.33
20:00	----	7.88	----	----
08:00 Day 6	7.55	7.91	----	5.99
08:00 Day 7	----	7.99	----	6.43
Mean value:	7.55	7.95	----	6.59
DIET C:				
08:00	----	7.97	----	6.88
11:00	7.55	7.76	----	7.36
14:00	7.94	8.09	----	6.22
17:00	----	7.98	----	----
20:00	7.59	8.01	----	6.39
08:00 Day 6	7.66	8.04	----	7.40
08:00 Day 7	7.79	7.98	----	7.21
Mean value:	7.71	7.98	----	6.91

6.1.1.3.2. Lt colonic cancer - 2nd Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	7.92	8.10	----	6.69
11:00	----	8.28	----	7.94
14:00	7.57	8.04	----	6.11
17:00	7.39	7.99	----	5.53
20:00	7.01	7.89	----	6.55
08:00 Day 6	7.56	8.03	----	----
08:00 Day 7	7.98	8.45	----	7.41
Mean value:	7.57	8.11	----	6.71
DIET B:				
08:00	7.35	----	----	7.17
11:00	7.54	8.16	----	7.22
14:00	----	8.22	----	6.69
17:00	7.54	8.14	----	----
20:00	7.49	7.91	----	7.63
08:00 Day 6	7.29	7.89	----	6.98
08:00 Day 7	----	7.92	----	7.46
Mean value:	7.44	8.04	----	7.19
DIET C:				
08:00	7.54	8.05	----	7.01
11:00	7.65	7.89	----	----
14:00	7.78	8.27	----	6.88
17:00	----	7.98	----	----
20:00	7.63	----	----	----
08:00 Day 6	7.51	8.19	----	7.17
08:00 Day 7	7.23	8.03	----	7.63
Mean value:	7.56	8.07	----	7.17

6.1.1.3.3. Lt colonic cancer - 3rd Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	7.45	7.99	----	7.01
11:00	7.47	8.05	----	7.20
14:00	7.47	----	----	7.00
17:00	7.51	7.95	----	7.09
20:00	7.55	7.98	----	6.94
08:00 Day 6	7.45	----	----	6.82
08:00 Day 7	7.37	----	----	6.90
Mean value:	7.47	7.99	----	6.99
DIET B:				
08:00	----	7.99	----	5.86
11:00	7.20	7.89	----	6.54
14:00	----	8.09	----	----
17:00	7.47	7.81	----	5.15
20:00	7.38	8.03	----	5.24
08:00 Day 6	7.26	7.98	----	6.81
08:00 Day 7	7.25	8.07	----	6.01
Mean value:	7.31	7.98	----	5.94
DIET C:				
08:00	7.75	----	----	6.98
11:00	----	7.87	----	7.12
14:00	7.62	----	----	7.08
17:00	7.24	7.71	----	7.04
20:00	7.41	7.93	----	6.99
08:00 Day 6	7.47	7.99	----	6.98
08:00 Day 7	7.39	7.87	----	6.73
Mean value:	7.48	7.87	----	6.99

6.1.1.3.4. Lt colonic cancer - 4th Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	7.51	7.79	----	6.61
11:00	7.55	8.03	----	----
14:00	7.48	----	----	6.82
17:00	----	7.72	----	7.06
20:00	7.25	7.89	----	6.15
08:00 Day 6	7.24	8.03	----	6.36
08:00 Day 7	8.08	8.28	----	6.78
Mean value:	7.52	7.96	----	6.63
DIET B:				
08:00	7.64	----	----	6.43
11:00	7.49	8.01	----	----
14:00	7.45	7.99	----	6.11
17:00	7.56	8.13	----	6.39
20:00	----	8.09	----	6.67
08:00 Day 6	8.15	8.49	----	7.07
08:00 Day 7	----	7.95	----	6.05
Mean value:	7.66	8.11	----	6.45
DIET C:				
08:00	7.66	8.14	----	5.89
11:00	7.54	7.91	----	5.95
14:00	7.29	8.03	----	5.59
17:00	8.04	8.23	----	5.26
20:00	----	8.21	----	5.69
08:00 Day 6	----	8.25	----	5.31
08:00 Day 7	----	8.07	----	5.24
Mean value:	7.63	8.13	----	5.56

6.1.1.3.5. Lt colonic cancer - 5th Patient

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
08:00	7.87	7.90	----	6.68
11:00	7.60	7.81	----	----
14:00	7.86	7.92	----	7.29
17:00	----	----	----	----
20:00	7.72	8.03	----	7.13
08:00 Day 6	7.28	7.88	----	6.78
08:00 Day 7	----	7.98	----	5.60
Mean value:	7.67	7.92	----	6.70
DIET B:				
08:00	----	8.07	----	6.64
11:00	7.60	7.99	----	6.53
14:00	7.46	8.03	----	6.83
17:00	7.27	----	----	6.86
20:00	7.41	8.17	----	6.73
08:00 Day 6	7.64	----	----	7.15
08:00 Day 7	----	8.03	----	5.66
Mean value:	7.48	8.06	----	6.63
DIET C:				
08:00	7.65	8.21	----	6.70
11:00	7.77	8.41	----	6.73
14:00	7.70	8.21	----	----
17:00	7.93	----	----	6.56
20:00	7.80	8.05	----	6.61
08:00 Day 6	7.39	----	----	6.83
08:00 Day 7	7.27	8.09	----	6.79
Mean value:	7.64	8.19	----	6.70

6.1.2. MEAN pH RESULTS OF THE DIFFERENT GROUPS

The summary of the results of each patient are shown below:

6.1.2.1. MEAN pH OF RIGHT TRAUMA PATIENTS

(Diet x Side x Placement)

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
1.	----	7.65	8.07	6.13
2.	----	8.01	----	6.79
3.	7.41	7.91	----	5.86
4.	7.82	7.91	----	5.56
5.	7.59	7.99	8.07	5.
Mean value:	7.61 (0.11)	7.89 (0.064)	8.07 (0)	5.98 (0.27)
DIET B:				
1.	----	7.83	8.09	4.96
2.	----	7.86	----	6.21
3.	7.74	8.09	----	6.58
4.	7.18	7.86	----	5.77
5.	7.52	7.99	8.03	5.06
Mean value:	7.48 (0.162)	7.92 (0.049)	8.06 (0.03)	5.72 (0.32)
DIET C:				
1.	----	7.99	8.11	5.35
2.	----	7.90	----	6.41
3	7.63	7.87	----	6.20
4.	7.45	7.94	----	5.18
5.	7.66	7.97	8.00	4.99
Mean value:	7.58 (0.066)	7.93 (0.022)	8.06 (0.055)	5.63 (0.28)

6.1.2.2. MEAN pH OF LEFT TRAUMA PATIENTS

(Diet x Side x Placement)

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
DIET A:				
1.	7.07	7.99	8.14	7.49
2.	7.44	7.82	7.85	6.92
3.	7.41	7.76	8.05	5.31
4.	7.52	8.16	----	6.44
5.	7.57	8.25	8.26	6.30
Mean value:	7.40 (0.088)	7.99 (0.094)	8.08 (0.086)	6.49 (0.361)
DIET B:				
1.	7.69	8.05	8.33	6.74
2.	7.15	7.86	7.86	6.78
3.	7.64	8.03	8.04	5.74
4.	7.88	8.06	----	6.14
5.	7.86	8.02	8.12	5.54
Mean value:	7.64 (0.132)	8.00 (0.037)	8.09 (0.097)	6.18 (0.252)
DIET C:				
1.	7.49	8.06	8.22	6.52
2.	7.49	7.68	7.95	6.79
3.	7.81	8.00	7.83	5.02
4.	7.78	8.18	----	6.58
5.	7.83	8.10	8.17	6.00
Mean value:	7.68 (0.078)	8.00 (0.086)	8.04 (0.092)	6.18 (0.318)

6.1.2.3. MEAN pH OF LEFT CANCER PATIENTS

(Diet x Side x Placement)

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faeca pH
DIET A:				
1.	7.69	8.09	----	5.81
2.	7.57	8.11	----	6.71
3.	7.47	7.99	----	6.99
4.	7.52	7.96	----	6.63
5.	7.67	7.92	----	6.70
Mean value:	7.58 (0.042)	8.01 (0.037)	----	6.57 (0.199)
DIET B:				
1.	7.55	7.95	----	6.59
2	7.44	8.04	----	7.19
3.	7.31	7.98	----	5.94
4.	7.66	8.11	----	6.45
5.	7.48	8.06	----	6.63
Mean value:	7.49 (0.058)	8.03 (0.028)	----	6.56 (0.199)
DIET C:				
1.	7.71	7.98	----	6.91
2.	7.56	8.07	----	7.17
3.	7.48	7.87	----	6.99
4.	7.63	8.13	----	5.56
5.	7.64	8.19	----	6.70
Mean value:	7.60 (0.039)	8.05 (0.056)	----	6.67 (0.286)

6.1.3. MEAN pH VALUES INCLUDING (S.E.M.)

The mean pH values including the standard error means are shown below: (Diet x Side x Placement)

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
		RIGHT COLON (TRAUMA PATIENTS)		
DIET A:	7.6 (0.111)	7.9 (0.064)	8.1 (0.00)	6.0 (0.027)
DIET B:	7.5 (0.162)	7.9 (0.049)	8.1 (0.03)	5.7 (0.32)
DIET C:	7.6 (0.066)	7.9 (0.022)	8.1 (0.055)	5.6 (0.28)
Mean value:	7.6 (0.119)	7.9 (0.048)	8.1 (0.036)	5.8 (0.245)
		LEFT COLON (TRAUMA PATIENTS)		
DIET A:	7.4 (0.088)	8.0 (0.094)	8.1 (0.086)	6.5 (0.361)
DIET B:	7.6 (0.132)	8.0 (0.037)	8.1 (0.097)	6.2 (0.252)
DIET C:	7.7 (0.078)	8.0 (0.086)	8.0 (0.092)	6.2 (0.318)
Mean value:	7.6 (0.102)	8.0 (0.077)	8.1 (0.092)	6.3 (0.314)
		COLORECTAL CANCER PATIENTS		
DIET A:	7.6 (0.042)	8.0 (0.037)	----	6.6 (0.199)
DIET B:	7.5 (0.058)	8.0 (0.028)	----	6.6 (0.199)
DIET C:	7.6 (0.039)	8.1 (0.056)	----	6.7 (0.286)
Mean value:	7.6 (0.047)	8.0 (0.042)	----	6.6 (0.232)

6.1.4. SUMMARY OF pH RESULTS

The summary of the results of each group of patients including the standard error means are shown below, as Side of colon x Placement

	Luminal pH	Mucosal pH proximal loop	Mucosal pH defunct. loop	Faecal pH
RIGHT TRAUMA	7.6 (0.119)	7.9 (0.048)	8.1 (0.036)	5.8 (0.245)
LEFT TRAUMA	7.6 (0.102)	8.0 (0.077)	8.1 (0.092)	6.3 (0.314)
CANCER	7.6 (0.047)	8.0 (0.042)	----	6.6 (0.232)

6.1.5. pH RESULTS OF FAECES OVER A PERIOD OF 3 HOURS

The pH results of the 4 faecal specimens which were incubated at 37^0C for 3 hours, as discussed in 4.2.1. are shown below:

	Patient A	Patient B	Patient C	Patient
00 min.	7.30	8.09	7.34	8.08
20 min.	7.00	7.30	7.30	7.81
40 min.	6.95	7.00	6.99	7.05
60 min.	6.60	7.00	7.08	6.87
80 min.	6.7	7.00	7.08	6.95
100 min.	6.37	7.3	6.35	7.19
120 min.	6.16	7.10	6.22	7.21
140 min.	6.70	7.00	6.17	7.18
160 min.	6.1	6.7	6.17	7.15
180 min.	5.9	6.68	6.14	7.13

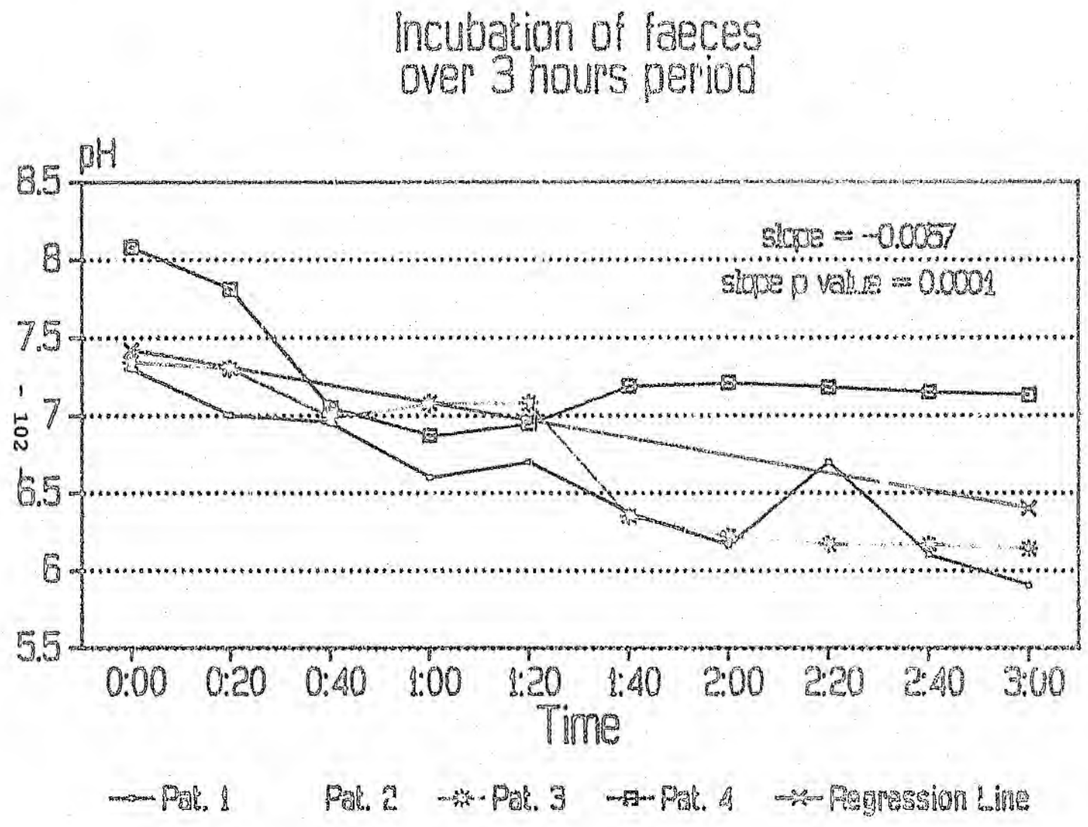

Incubation of faeces
over 3 hours period
pH
8.5
8
7.5
7
6.5
6
5.5
slope = -0.0057
slope p value = 0.0001
0:00
0:20
0:40
1:00
1:20
1:40
2:00
2:20
2:40
3:00
Time
Pat. 1
Pat. 2
Pat. 3
Pat. 4
Regression Line

Figure 14

6.2. BREATH METHANE RESULTS

Breath Methane measurements were done on twelve out of the fifteen patients.

The results for each patient were as follows:

6.2.1. RIGHT COLONIC TRAUMA PATIENTS

	Day 5	Day 6	Mean:
1st patient:			
Diet A:	1.9 ppm	1.56ppm	1.7 ppm
Diet B:	2.9 ppm	2.2 ppm	2.6 ppm
Diet C:	1.0 ppm	0.54ppm	0.8 ppm
2nd patient:			
Diet A:	8.4 ppm	18.77ppm	13.6 ppm
Diet B:	13.8 ppm	21.3 ppm	22.2 ppm
Diet C:	3.19ppm	1.5 ppm	2.35ppm
3rd patient:			
Diet A:	15.48ppm	14.2 ppm	14.84 ppm
Diet B:	12.63ppm	13.58ppm	13.1 ppm
Diet C:	0.99ppm	3.8 ppm	2.4 ppm

6.2.2. LEFT COLONIC TRAUMA PATIENTS

	Day 5	Day 6	Mean:
1st patient:			
Diet A:	32.6 ppm	46.2 ppm	39.4 ppm
Diet B:	45.1 ppm	45.1 ppm	38.5 ppm
Diet C:	45.7 ppm	45.1 ppm	45.4 ppm
2nd patient:			
Diet A:	5.86ppm	23.7 ppm	14.8 ppm
Diet B:	15.62ppm	10.99ppm	13.3 ppm
Diet C:	2.7 ppm	2.3 ppm	2.5 ppm
3rd patient:			
Diet A:	1.5 ppm	1.9 ppm	1.7 ppm
Diet B:	2.07ppm	1.0 ppm	1.5 ppm
Diet C:	3.6 ppm	6.07ppm	4.8 ppm
4th patient:			
Diet A:	13.9 ppm	15.6 ppm	14.8 ppm
Diet B:	9.1 ppm	1.4 ppm	5.3 ppm
Diet C:	13.28ppm	9.8 ppm	11.5 ppm

6.2.3. LEFT COLONIC CANCER PATIENTS

	Day 5	Day 6	Mean:
1st patient:			
Diet A:	4.5 ppm	1.5 ppm	3.0 ppm
Diet B:	---	---	
Diet C:	32.06ppm	47.9 ppm	40.0 ppm
2nd patient:			
Diet A:	21.6 ppm	17.3 ppm	19.5 ppm
Diet B:	---	---	
Diet C:	5.8 ppm	15.2 ppm	10.5 ppm
3rd patient			
Diet A:	11.04ppm	22.14ppm	16.6 ppm
Diet B:	19.0 ppm	5.51ppm	12.3 ppm
Diet C:	10.79ppm	12.61ppm	11.7 ppm

	Day 5	Day 6	Mean:
4th patient:			
Diet A:	3.5 ppm	2.6 ppm	3.1 ppm
Diet B:	2.1 ppm	0.11ppm	1.1 ppm
Diet C:	0.54ppm	0.54ppm	0.5 ppm
5th Patient			
Diet A:	23.5 ppm	31.3ppm	27.4 ppm
Diet B:	15.0 ppm	21.0ppm	18.0 ppm
Diet C:	52.8 ppm	30.0ppm	41.4 ppm

6.2.4. MEAN BREATH METHANE FOR THE DIFFERENT DIETS

Mean of diet A: 13.9 ppm

Mean of diet B: 12.8 ppm

Mean of diet C: 12.9 ppm

6.2.5. MEAN BREATH METHANE FOR THE DIFFERENT SIDES OF COLOSTOMIES

Mean of Rt side: 7.7 ppm

Mean of Lt side: 16.7 ppm

Mean of Cancer Lt: 15.3 ppm

* ppm = part per million

6.3. COMMENTS ON RESULTS

6.3.1. SITUATIONS IN WHICH SIGNIFICANT pH DIFFERENCES OBSERVED ($p = < 0.05$)

1. Luminal pH was lower than mucosal pH in:
   a) left colostomy patients ($p = 0.0009$) and
   b) cancer patients ($p = 0.003$)

   Within the right colon the difference just failed to reach significance ($p = 0.052$)

2. Faecal pH was lower than luminal and mucosal pH (significant differences at all sites) ($p = < 0.0001$)

3. Faecal pH of right colon was lower than left colon ($p = < 0.0003$)

4. Faecal pH of left (healthy) colostomies was lower than colon cancer colostomies ($p = 0.0003$)

6.3.2. SITUATIONS WHERE SIGNIFICANT pH DIFFERENCES NOT OBSERVED

1. No significant differences between dietary effects were observed. ($p = 0.6707$)

2. No significant difference between luminal pH (7.6) and mucosal pH (8.0) ($p = 0.3724$)

3. Diet x Side interaction was not significant ($p = 0.9096$)

6.3.3. SITUATIONS WHERE SIGNIFICANT METHANE DIFFERENCES OBSERVED

1. Methane production in Right side lower than Left 'healthy' and Cancer colostomates. ($p = 0.0009$ and 0.005 respectively)

NOTE: Diet evoked no significant difference in methane production in all groups.

6.3.4. MUCOSAL pH

The results show that the mear mucosal pH was 7.9 in right colostomies, 8.0 in left colostomies, 8.1 in the defunctioned loop and 8.0 in colon cancer patients (Figure 15). This suggests that the mucosal pH of the colon was alkaline, and was similar on both sides of the colon (including the defunctioned loop) of healthy colostomates.

It was also shown that the mucosal pH was independent of dietary changes (Figure 16).

6.3.5. LUMINAL pH

Luminal pH was similar on the two sides of the colon in the trauma patients and was similar in the left colon of trauma and cancer patients (7.6). (Figure 17). This suggests that the faeces in the colon have a stable pH. Diets did not make any differences to mean pH values. (Figure 18)

The luminal pH in left sided colostomies and cancer patients was significantly lower than mucosal pH ($p=0.0009$ and 0.0003 respectively), but just failed to reach significance on the right side ($p=0.052$) (Figure 19). This indicates that the presence of faeces slightly lowers the pH of the colon.

6.3.6. FAECAL pH

The lowest pH's recorded were faecal pH's. Mean faecal pH in right colostomies was 5.8, left colostomies 6.3, and in cancer patients 6.6.

Faecal pH of the left colon was significantly higher than that of the right colon. ($p = <0.0003$) and faecal pH in cancer patients was significantly higher than in left-sided colostomies ($p = <0.0003$) (Figure 20).

Faecal pH, in all situations, was significantly lower ($p = <0.0001$) than mucosal and luminal pH (Figure 21).

6.3.7. DIETS & pH

Different diets did not show any significant difference of pH in any placement ($p = 0.067$). However subjects consuming a high fibre diet and a high starch diet tended to have a lower faecal pH than subjects consuming a western diet.

MUCOSAL pH

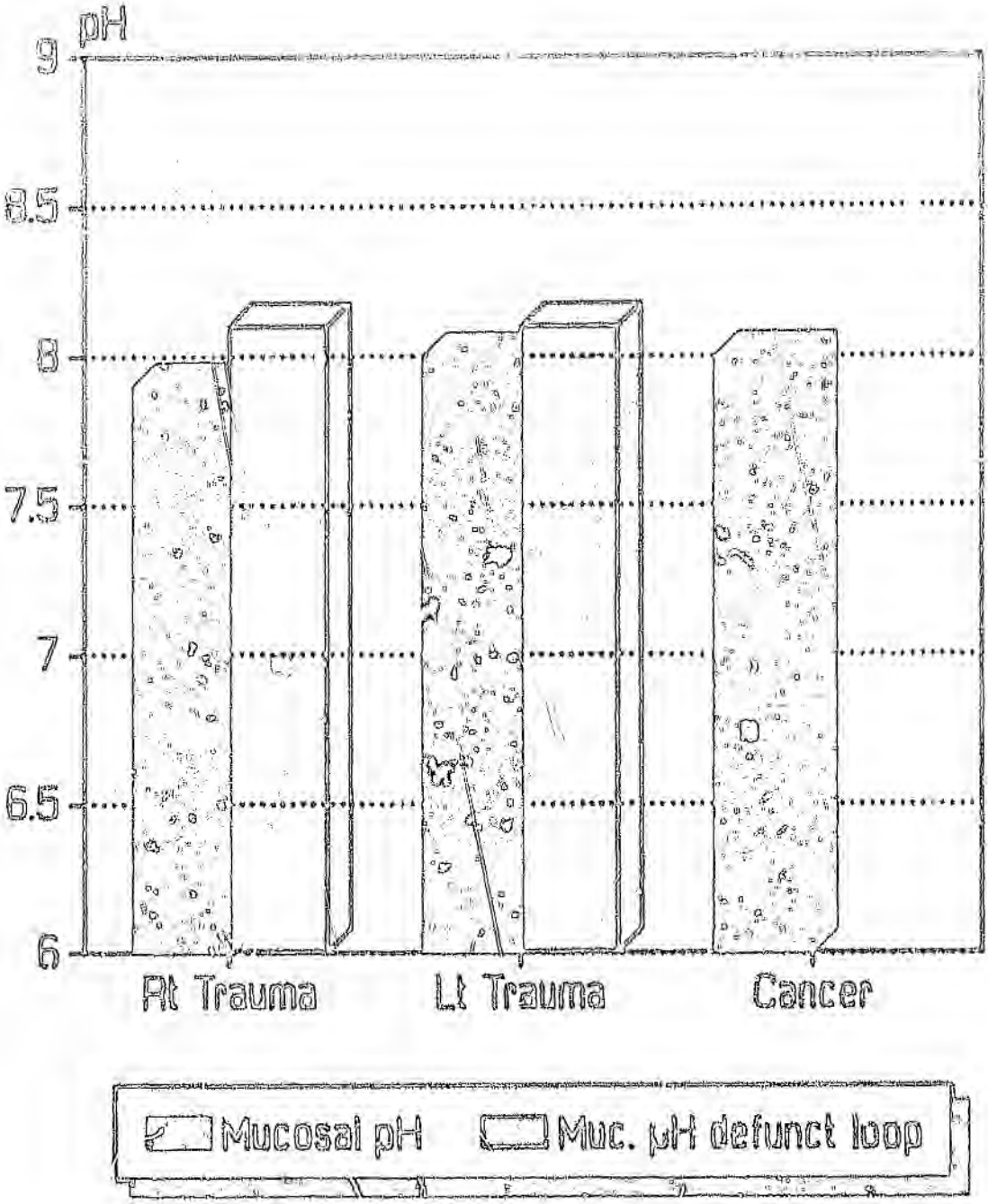


Figure 15

Mucosal pH & Diet

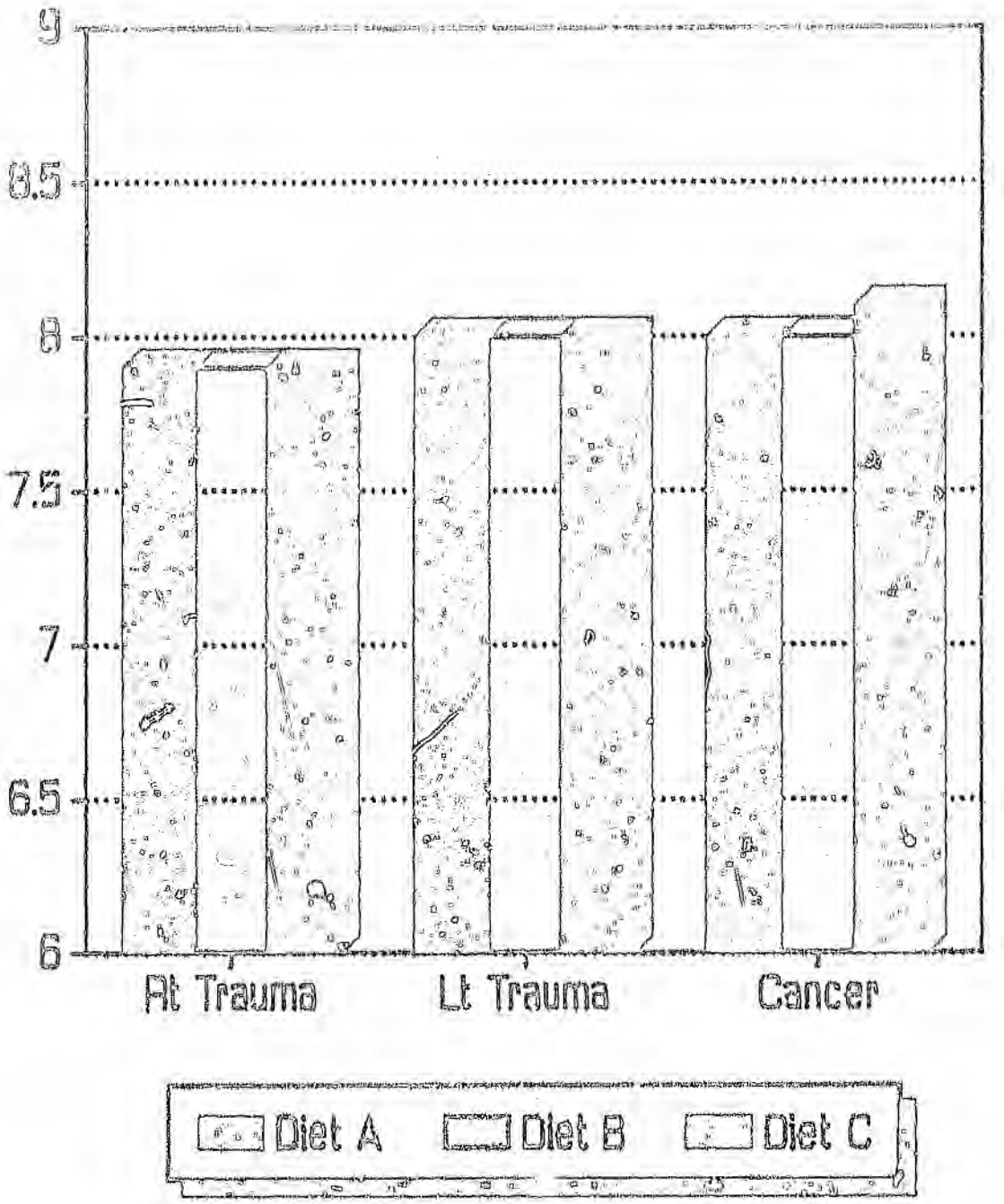


Figure 16

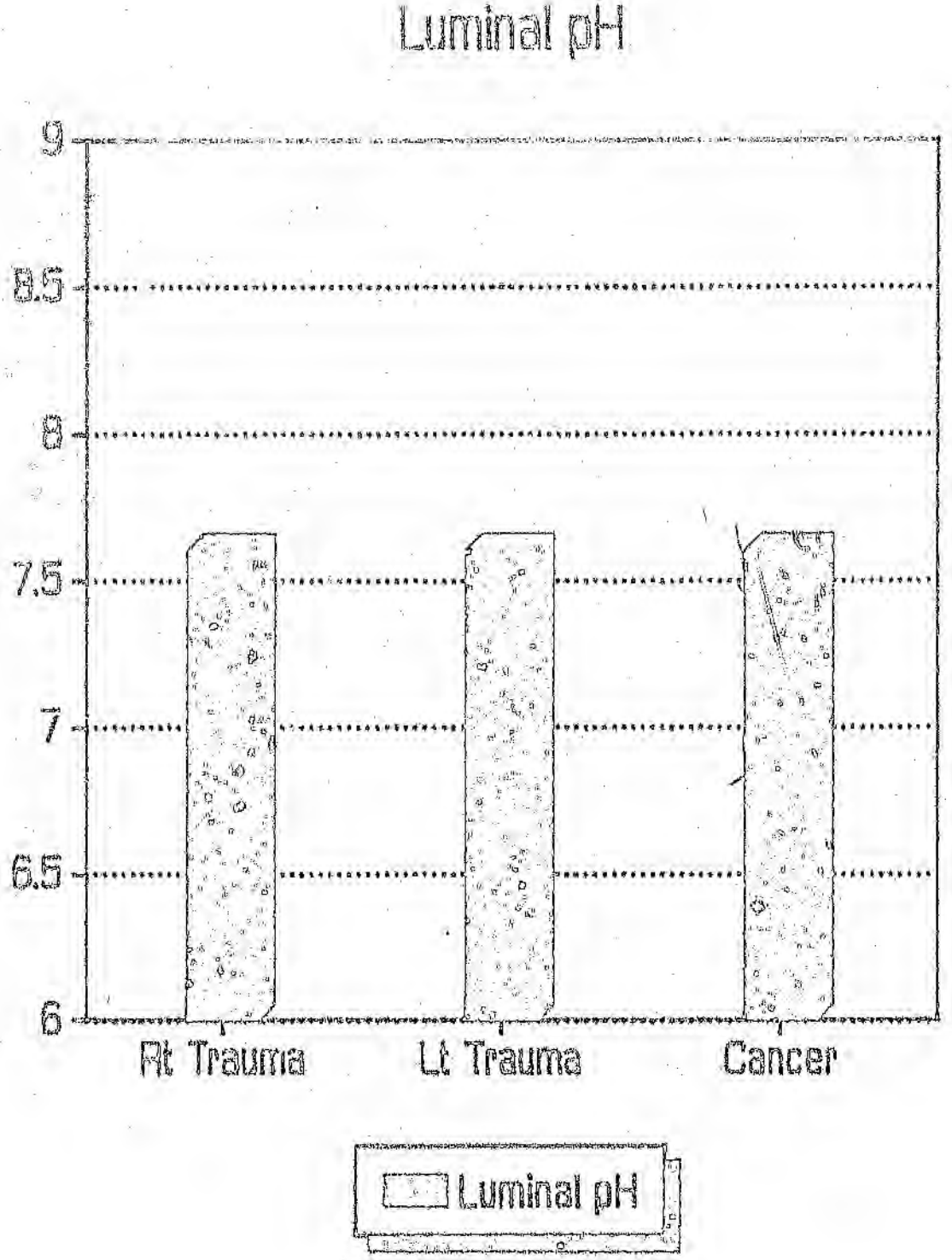

Luminal pH
9
8.5
8
7.5
7
6.5
6
Rt Trauma
Lt Trauma
Cancer
Luminal pH

Figure 17

Luminal pH & Diet

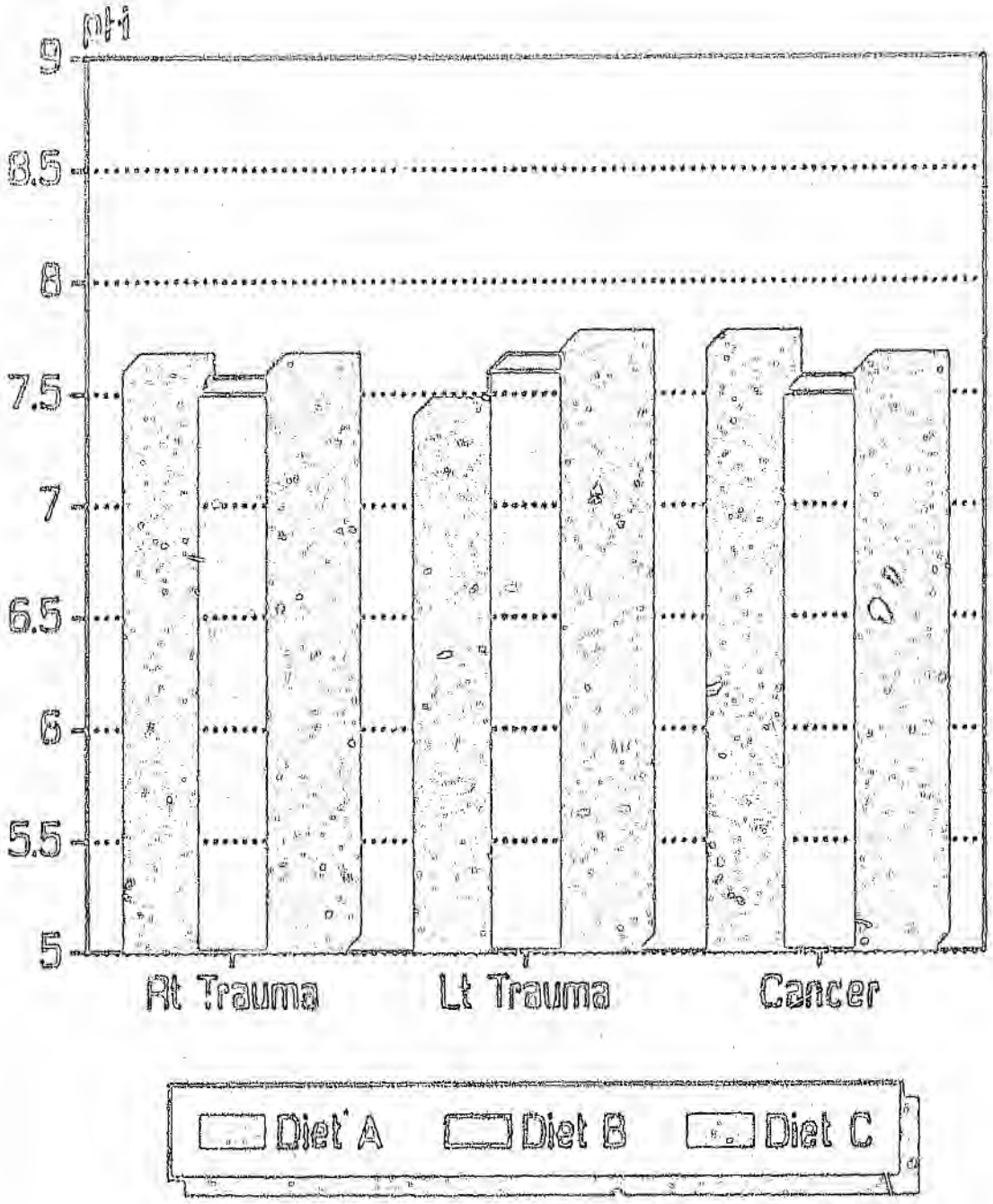


Figure 18

Luminal vs Mucosal pH

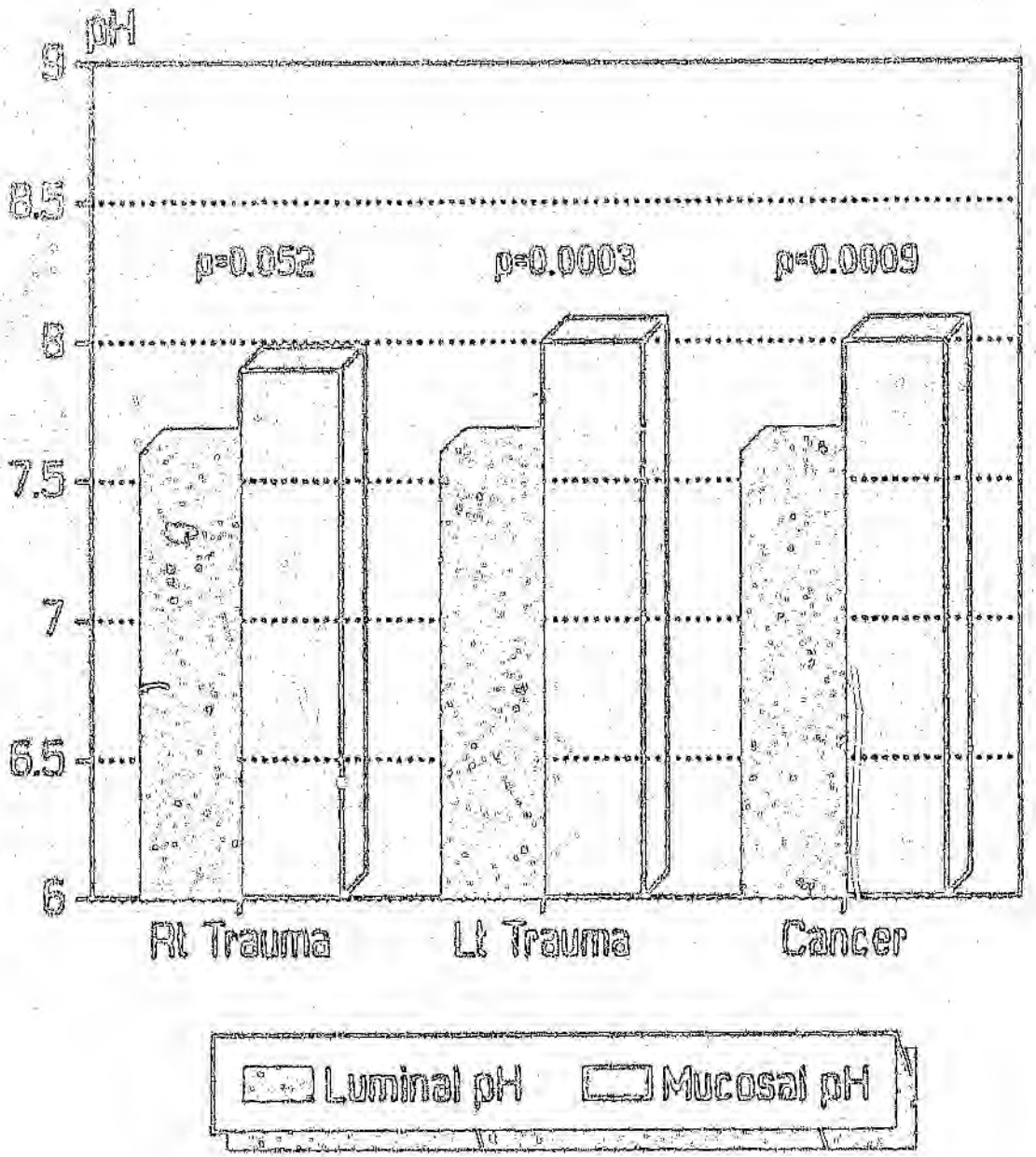


Figure 19

Faecal pH

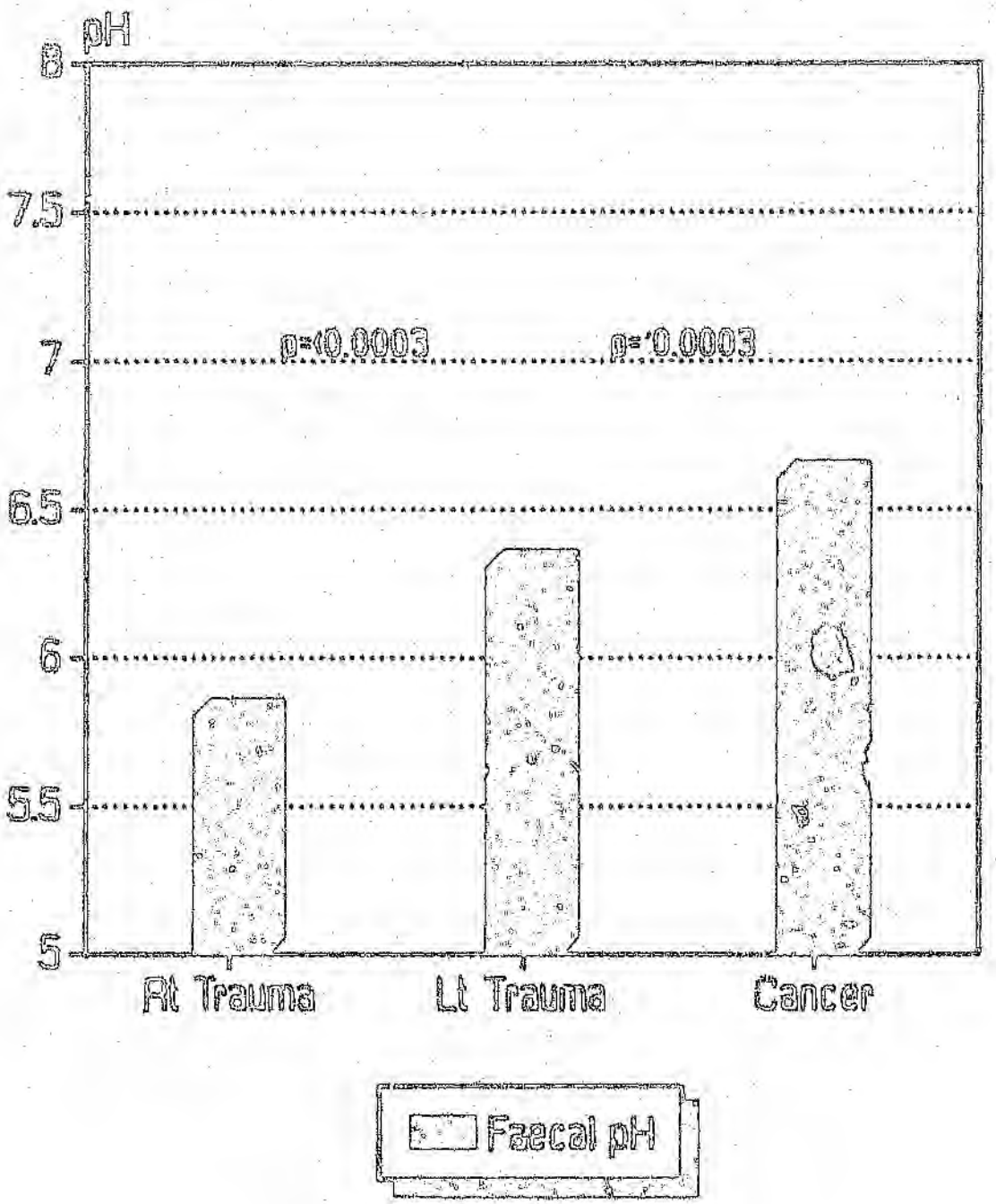


Figure 20

Comparison of Faecal, Luminal, Mucosal pH

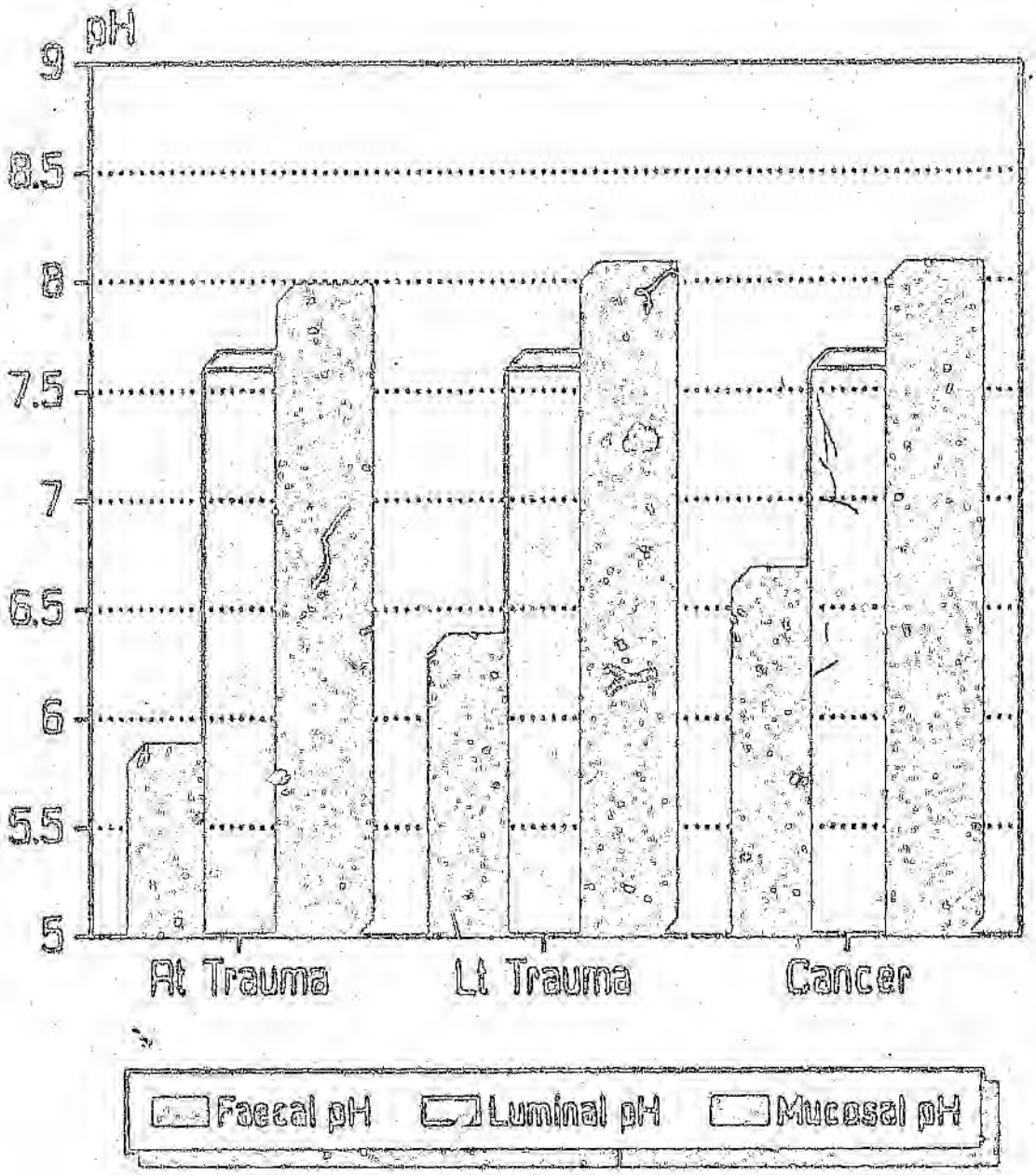


Figure 21

6.3.8. DIETS & BREATH METHANE

It was clear that the different diets did not affect significantly the breath methane (Figure 22).

6.3.9. SIDE & BREATH METHANE

It is evident that the breath methane drops significantly [illegible] part of the colon is excluded; The breath methane in the right sided colostomies was approximately half of the breath methane of the left sided colostomies for trauma and for cancer (Figure 23). This could be explained by the fact that since part of the colon is excluded there is decreased fermentation which will result in decrease in the production of methane.

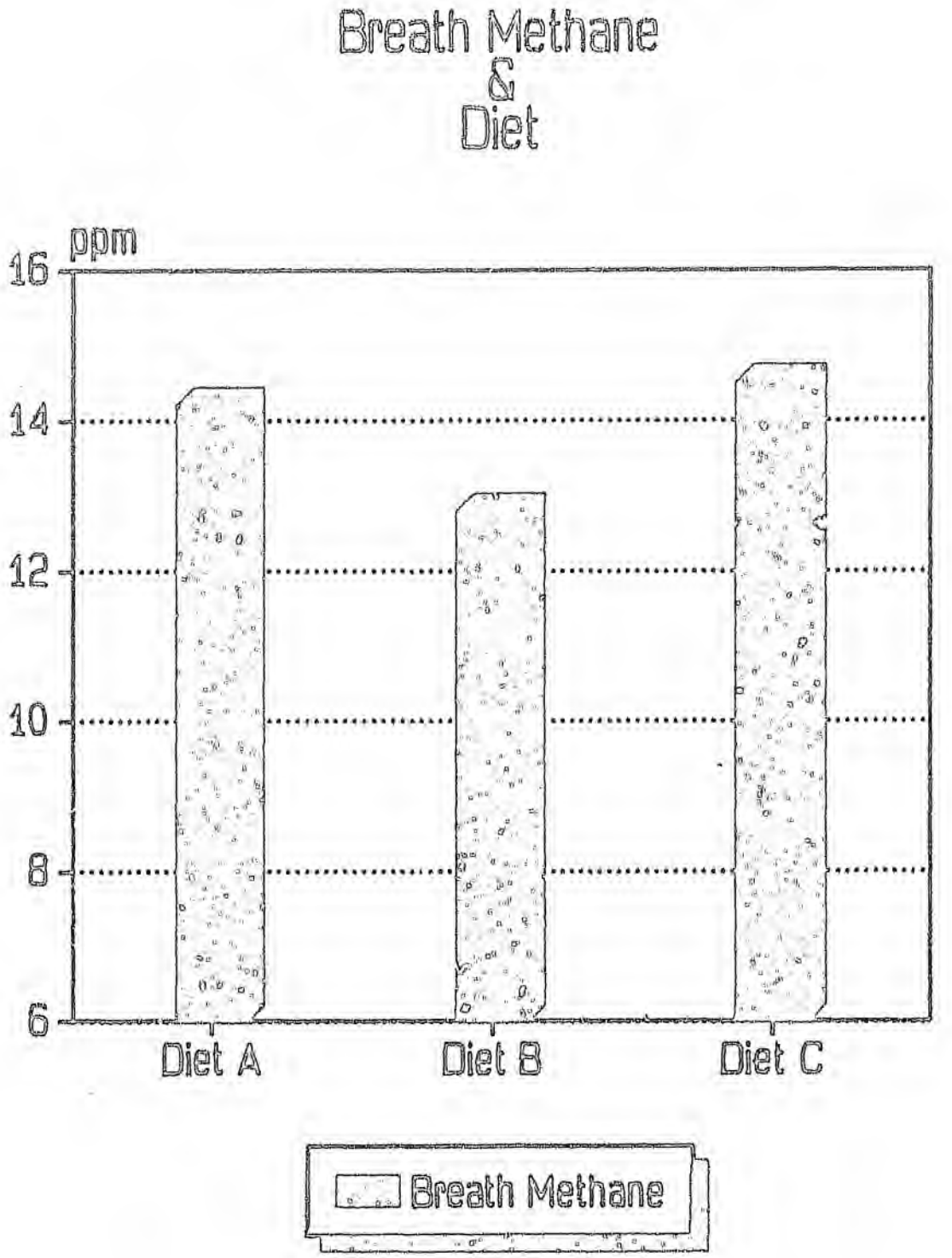

Breath Methane
&
Diet
ppm
16
14
12
10
8
6
Diet A
Diet B
Diet C
Breath Methane

Figure 22

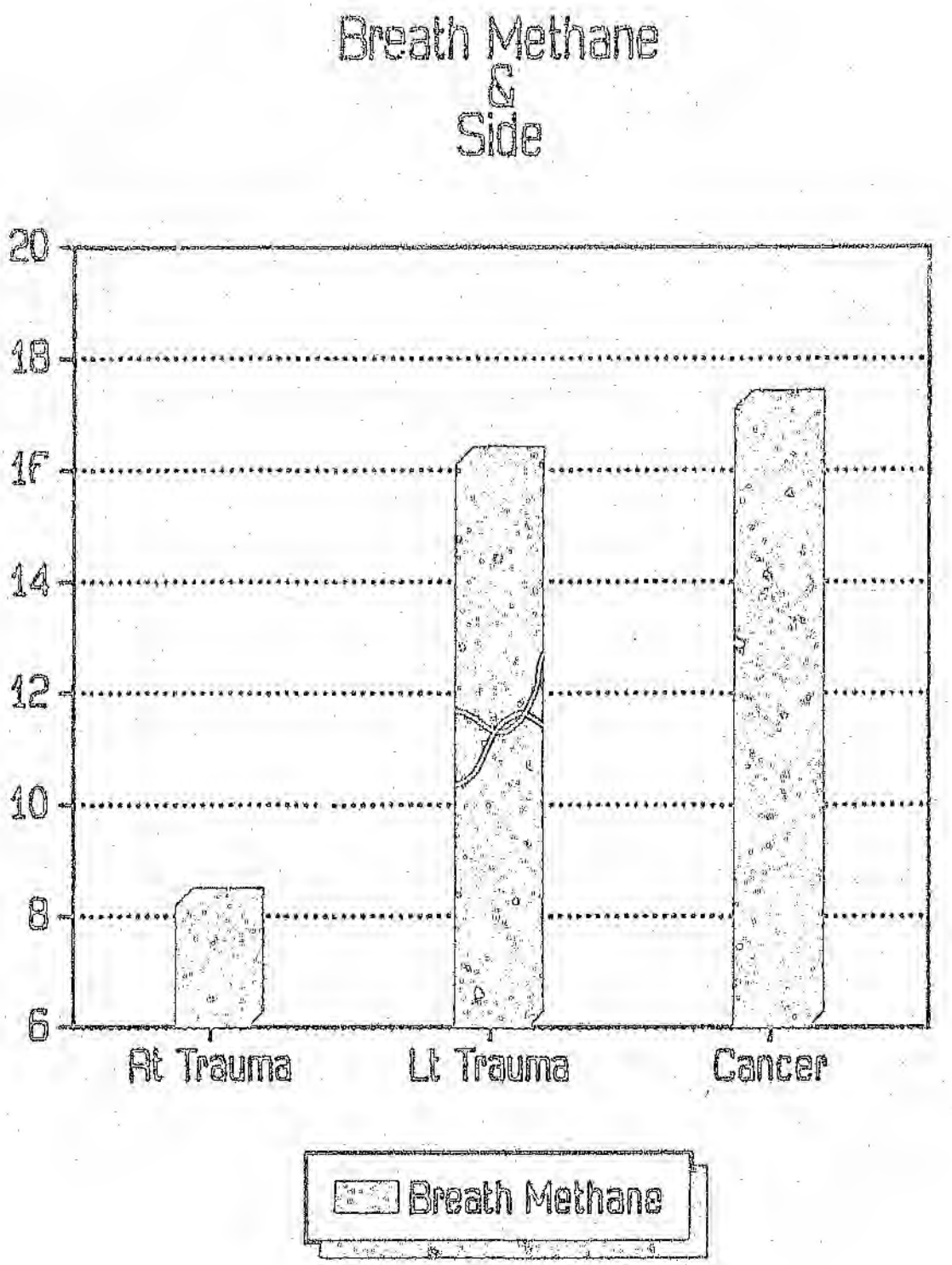

Breath Methane
&
Side
20
18
16
14
12
10
8
6
At Trauma
Lt Trauma
Cancer
Breath Methane

Figure 23

- CHAPTER 7 -

DISCUSSION

The spectrum of luminal pH values in this study was higher than that of Evans (Evans et al., 1988). The reason for this is not known but it is possible that a colostomy alters the normal physiology of the colon. Faecal pH values in my study were identical to those of luminal pH reported by Cummings (Cummings et al., 1990) in which luminal pH was measured at different regions of the colon in autopsy victims within 4 hours of death. The pH ranged from 5.8 in caecum to 6.5 in sigmoid-rectum. In my study faecal pH was 5.8 in right colostomies and 6.5 in left colostomies. The pH values in the above two studies occurred when the faeces were independent of colonic control. -in my study pH of faeces in the colostomy bags were measured, and in Cummings study colonic controlling factors were not viable in the autopsy victims. Presumably fermentation with its acid lowering effect could proceed autonomously.

Mucosal and luminal pH appear to be finely balanced and controlled. However once the faecal contents leave the colonic environment marked changes occur. The faecal pH drops significantly and differences between right and left colostomies and cancer patients are elicited.

Colonic luminal pH is determined by relative concentrations of acids and bases. Ammonia and bicarbonate ion are the main alkaline components and the short chain fatty acids (SCFA) are chiefly responsible for the acidification of colonic contents and faeces (Newmark and Lupton, 1990). Faecal ammonia is derived from bacterial degradation in the large bowel of endogenous urea from tissue fluid and also of proteins and amino acids that escape digestion and absorption in the small intestine (Geigy Scientific Tables, 1981; Deminge and Remsey, 1979). Bicarbonate ion is secreted into the colonic lumen in exchange with SCFA uptake (Umesaki et al., 1980). The SCFA (acetic, butyric and propionic acid) neutralize ammonia and bicarbonate. They are derived from colon microbial fermentation on undigested dietary carbohydrates.

Equimolecular quantities of ammonia and SCFA produce a pH of approximately 7.0 (Newmark and Lupton 1990). Appreciable excesses of ammonia or SCFA are required to shift the pH to the extremes of the 'normal' pH range of 6-8 (outer limits 5.0-9.0) (Newmark and Lupton 1990). Ammonia and SCFA are not present in equimolecular amounts in faeces. Thus the lowered

faecal pH compared to mucosal and luminal pH may be due to an excess of SCFA. This is possibly due to continued (extracolonic) faecal fermentation with SCFA production, and a lessened production of colonic alkaline factors - bicarbonate ion secretion and endogenous urea production.

A high colonic pH has been linked to colon cancer. Thornton (Thornton 1 ^1) proposed that a high colonic pH promotes co-carcinogen formation from either bacterially degraded bile acids or cholesterol, and that acidification of the colon may prevent this process. However my study has shown that there are no differences in mucosal and luminal pH between healthy and colon cancer patients. Pye (Pye et al., 1990) also found no difference in luminal pH among normal subjects and those with colorectal neoplasia in a study measuring pH in normal subjects, patients with colorectal adenomas and patients with colorectal cancer. Tumour site, size of adenoma and stage of carcinoma did not exert any influence.

A perplexing fact, particularly in connection with Thornton's hypothesis is that faecal pH was significantly higher in colon cancer patients than in the healthy colostomates. This may reflect differences in faecal microflora between the groups in accord with Hill's hypothesis (Hill et al., 1971)

The cancer patients (mean age 54 years) were clearly older than the healthy subjects (mean age 30 years). I do not know whether colonic pH changes with age. In this study, however the colonic pH's were similar in both groups.

No significant effect of diet on pH was found, although a high fibre, and a high starch/low fibre diet tended to result in a lower pH than a western diet. There may be several reasons for the lack of a significant effect of the dietary manipulations. These include:- i) The five day regime for each diet may be too short a period ii) Wider differences in the components of the diet may be required to evoke significant pH differences iii) The fibre estimates in our study were obtained from different sources and may not accurately reflect true fibre values. iv) There may have been inadequate

compliance by the patients and v) Newmark and Lupton (1990) proposed that it is not fibre alone but the ratio of the protein to fibre in the diet which markedly affects colon lumen and faecal pH. This change in pH, and the effects it produces on SCFA's, long chain fatty acids, faecal bile acids, and ammonia in the colon has profound effects on the colon epithelium by affecting cancer promotion mechanisms.

In conclusion it is emphasised that the process of establishing colonic pH is complex and dynamic. The rate of production and removal of SCFA's and ammonia at any site in the colon or faeces is an intricate system that varies rapidly depending on the local availability of substrates, effects of other transient food components, and the local microflora. All are constantly changing and vary from subject to subject (Drasar and Hill, 1974)

The results of the methane analysis indicated that the right side of the colon is not the major site of methane production. The implications are not known and further research will have to be done to establish the significance of this finding.

- CHAPTER 8 -

CONCLUSIONS

1. The pH of the colon in absence of faeces is alkaline, and is approximately 8.0.

2. In the presence of faeces the pH decreased but not significantly (pH 7.6).

3. In 'healthy' individuals the faecal pH of the left colon is higher than the faecal pH of the right colon.

4. The faecal pH of patients with colorectal cancer is higher than that the faecal pH of the left colon of 'healthy' patients.

5. The faecal pH is significantly lower than the luminal and mucosal pH.

6. The different diets did not have any significant effect on the faecal pH or the mucosal pH. However a high fibre, and a high starch/low fibre diet tended to result in a lower pH than a western diet.

7. Methane production was significantly lower when the left colon was excluded. The implications for this finding are not known.

SUGGESTIONS
FOR
FURTHER RESEARCH

1. A colostomy is an excellent model to study the physiology of the colon. Projects that could be carried out include infusion studies using short chain fatty acids (SCFA), to observe the effects of the various SCFA on colon physiology. Various drugs could also be tested to assess their effects.

2. The diets should be planned so as to give greater extremes of values in order to evoke more definitive responses; e.g. high fibre diet should consist of 60g dietary fibre and the starch diet should comprise 400g.

3. Cell proliferation studies. The effects of the various diets on cell proliferation. They may answer the questions as to why blacks have such a low incidence of large bowel disease.

4. Secondary bile acids are believed to be important in the aetiology of large bowel cancer. It would be interesting to assess the effects of a high fat

and low fat diet on bile acid production. The faecal contents could be analyzed for primary and secondary bile acids.

5. Breath methane: Bacteriological assays should be done to determine the site of methanogenesis.

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APPENDIX I

DOCUMENT 79/55

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
OFFICE OF THE DEPUTY REGISTRAR (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS

CLEARANCE CERTIFICATE PROTOCOL NO: 45/2/89

PROJECT : PH STUDIES OF COLON IN COLOSTOMY PATIENTS CONSUMING DIFFERENT DIETS

INVESTIGATOR/S : DR D N CHARALAMBIDES

DEPARTMENT : SURGERY, BARAGWANATH HOSPITAL

DATE CONSIDERED 24/2/89

RECOMMENDATION OF COMMITTEE :

NOT APPROVED [ ]

APPROVED [X] subject to the following conditions:

Date : 27/2/89 CHAIRMAN : .....
Professor P E Cleaton-Jones

"INFORMED CONSENT" forms attached - where applicable.
FURTHER "I/C" FORMS AVAILABLE AT FACULTY OFFICE

DECLARATION BY INVESTIGATOR/S

To be completed in duplicate and ONE copy returned to the OFFICE OF THE DEPUTY REGISTRAR (Research), ROOM 10002, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorised to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions.

Should any departure be contemplated from the research procedure as approved I/we undertake to resubmit the Protocol to the Committee.

DATE : ..... SIGNED : 5/3/89

APPENDIX II

LIST OF ABBREVIATIONS

L.C.F.A.	Long Chain Fatty Acids
p.p.m	parts per million
S.C.F.A.	Short Chain Fatty Acid
S.E.M.	Standard Error Mean
V.F.A.	Volatile Fatty Acids

Author: Charalambides, D.C.
Name of thesis: ph Studies in colostomy patients consuming defferent diets

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