THE DEVELOPMENT AND CLINICAL APPLICATION OF SMALL INTESTINAL PERFUSION STUDIES IN MAN

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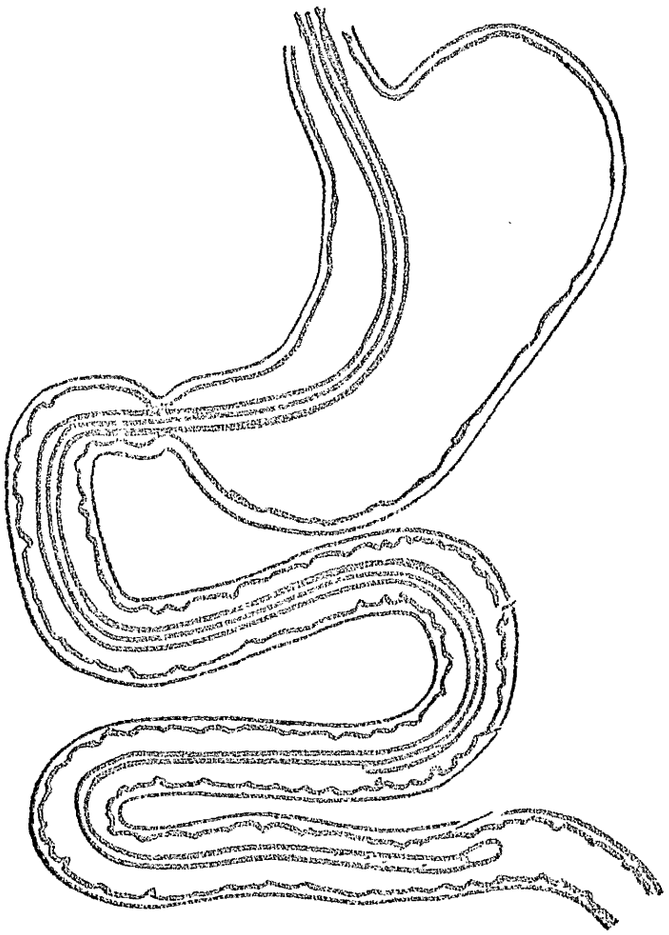

SUMMARY

The subject of this thesis is the technique of small intestinal marker-perfusion applied to the measurement of jejunal absorption in man. The work describes studies performed in developing the technique and applying it to study factors involved in influencing intestinal absorption. Standard methods of investigating problems of maldigestion, malabsorption and various diarrhoeal states are generally indirect, and give little information on the direct absorption capacity of the small intestine. The development of long intestinal tubes opened up the possibility of directly studying the absorptive area of the small intestine. The initial use of non-absorbable, water-soluble markers in conjunction with long intestinal tubes was a major advance in the development of small intestinal intubation techniques. (Borgström, Dalqvist, Lundh et al, 1957). Within a few years of this development, early small intestinal perfusion studies were appearing. Since then, a number of different versions of the technique have appeared. There has, however, been no general agreement as to the best way to perform these studies.

After an introductory section, the background to the development of the technique is described, and the necessary requirements discussed. Section II describes initial studies performed on the development of the perfusion system. The basic principles are defined, and a number of specific problems considered. These include the requirements of an adequate non-absorbable reference substance and the need for new markers, the choice of the perfusion tube system, and problems relating to the influence of flow rate and composition of the perfusate used in individual studies. The need for new and easily measured non-absorbable, water-soluble markers for perfusion studies led to a comparison of $^{51}CrCl_3$ and ^{51}Cr EDTA in the perfusion system by comparing each with phenolsulphonphthalein (PSP). The results show a good correlation for water absorption figures obtained using ^{51}Cr EDTA with those using PSP, and a poor correlation between

water absorption calculated using $^{51}CrCl_3$ compared with those calculated using PSP. ^{51}Cr EDTA thus appears to be a satisfactory non-absorbable water-soluble reference substance for intestinal perfusion studies.

The composition of the perfusate was also investigated with particular reference to the relationship of glucose to sodium and water absorption. Using a triple-lumen tube perfusion system and perfusates consisting of isotonic-saline and glucose-saline with glucose concentrations ranging from 28 mM to 224 mM, it was found that with increasing concentrations of glucose up to 56mM, increasing absorption of water and sodium occurred. With high concentrations of glucose (224 mM) a marked reduction of water and sodium absorption occurred, and net secretion of sodium was found in the jejunum. From these results a glucose concentration of 56 mM in glucose-saline solutions has been taken as being the best basic perfusate for subsequent jejunal perfusion studies.

The effect of different perfusion flow rates on water and electrolyte absorption has also been studied. Infusion rates of 10 ml per minute, 15 ml/minute, 20 ml/minute and 25 ml/minute were investigated and no significant difference was found in the absorption of water and electrolytes at each of these rates. However, a trend towards a reduction of water and electrolyte absorption at 25 ml/minute was noted. From these results an infusion rate of 20 ml/minute was selected as the standard perfusion flow rate for the jejunal perfusion studies subsequently performed.

Finally, in this section, the design of the intestinal tube system itself was studied. A triple-lumen tube perfusion system was designed which incorporated a double-lumen system and a proximal occluding balloon. Absorption measured using the triple-lumen system could therefore be directly compared with that calculated by the double-lumen system in the

same patients at the same time. The effect of balloon inflation on intestinal absorption could also be studied. The results demonstrated that absorption figures using the triple-lumen tube system appeared less variable than those using the double-lumen technique. The double-lumen system gave results in the same patients which were significantly higher than those obtained using the triple-lumen system. Inflation of the proximal occluding balloon was not found to interfere significantly with absorption.

On the basis of these preliminary investigations a standard technique and experimental procedure was devised for use in jejunal perfusion studies in man. In general, a triple-lumen tube system was used, although a double-lumen tube with proximal occluding balloon was considered acceptable. A basic perfusate of glucose-saline containing glucose in a concentration of 56 mM was used, although modifications of this perfusate were made from time to time depending on the investigation being performed. A constant perfusion flow rate of 20 ml/minute was utilised in all the studies. In general, two water-soluble markers were used in each study (usually PSP and ^{51}Cr EDTA), the results being worked out using both markers.

Following the development of this standard technique the system was applied to study the effect of various factors on influencing intestinal absorption from the jejunum. These studies are described in section III. The effect of conjugated and unconjugated bile acids in various concentrations on absorption of water and electrolytes in the proximal jejunum was studied in this way. The trihydroxy bile acid cholic acid and its glycine and taurine conjugates, had no significant effect on water and electrolyte absorption. The dihydroxy bile acid deoxycholic acid, however, significantly inhibited water and electrolyte absorption at low concentration and induced net secretion at higher concentrations. Similar results were found when

taurodeoxycholic and glycodeoxycholic acid were used. The use of chenodeoxycholic acid and its taurine conjugate had a similar effect in inhibiting water and electrolyte absorption and inducing secretion at higher concentration. The results of this study show that dihydroxy bile acids, whether conjugated or unconjugated, inhibit water and electrolyte absorption and may cause net secretion within the proximal jejunum. The results are of importance in the pathogenesis of diarrhoea associated with conditions of bacterial overgrowth (in which deconjugation and dehydroxylation of bile acids may occur) and suggest that bile acids and possibly lipids are of importance in balancing the movement of fluids in the upper small intestine.

This theme is further developed in the following chapter when lipids in the form of an emulsion containing glycerol trioleate (5 mM) and sodium taurocholate (2.5 mM) was perfused and found to inhibit water and electrolyte absorption from the upper jejunum. This effect was considered to be due to the release of oleic acid following lipolysis in the small intestine. Further evidence favouring this was obtained in patients with chronic pancreatitis in which the same lipid emulsion was not found to inhibit water and electrolyte absorption in the jejunum. In such patients lipolysis is inefficient due to pancreatic insufficiency and oleic acid may not be released and thus no damage occurred to the absorption mechanisms in the jejunum. The results of this study further support the concept that the relationship of bile acids and lipids, and the occurrence of fat absorption itself, are important factors in the maintenance of normal fluid balance in the upper small intestine.

The effect of chemical stimulants such as dihydroxy bile acids and lipids on water and electrolyte transport leads to a consideration of the influence of various drugs on jejunal water and electrolyte absorption.

The effect of frusemide administered intravenously on water and electrolyte absorption in the human jejunum was thereby studied using a double-lumen perfusion system with proximal occluding balloon. Frusemide (40 mg) significantly reduced the absorption of water and electrolytes, and caused net secretion in some subjects. No significant change, however, was observed when 20 mg of frusemide was given. These findings may explain the diarrhoea which may be induced by frusemide in some patients, and raises important questions in relation to the absorption of drugs such as digoxin which may be administered at the same time as frusemide.

In Section IV studies are reported in which the intestinal perfusion technique is used to investigate the jejunal absorption abnormalities in patients with coeliac disease and Crohn's disease. In adult coeliac disease it was found that all patients studied had an inhibition of jejunal absorption of water and electrolytes and most exhibited net secretion of water, sodium and chloride. No significant improvement in absorption was observed in patients with adult coeliac disease 3 to 4 months after starting a gluten-free diet, during which time all the patients studied had improved clinically and to some extent histologically. One patient showed an improvement of intestinal absorption one year after gluten withdrawal but one further patient showed no improvement at this time although it was subsequently found that he had not been keeping strictly to his gluten-free diet. This finding accounts for some of the symptomatology of adult coeliac disease, notably abdominal distension, pain and to some extent, the watery diarrhoea which is often present. The lack of response of the absorption abnormality to the exclusion of gluten from the diet within 3-4 months suggests a slow improvement, and it is possible that the absorption abnormality may remain for some time and may only improve in the proximal jejunum, where damage is most marked, much later. The clinical response which often occurs early may be due to improvement in absorption in the

distal jejunum and ileum so that a greater degree of reabsorption of the fluid secreted in the proximal jejunum may occur shortly after starting the gluten-free diet.

In patients with Crohn's disease variable results were obtained but in 4 of the 6 patients studied, an inhibition of water and electrolyte absorption was observed in the jejunum and in two subjects secretion of water, sodium and chloride was found. In only one of these patients was jejunal involvement suggested as being marked on radiological examination and in this patient the diagnosis was confirmed histologically by jejunal biopsy. In all the other patients studied no significant radiological abnormality was noted in the jejunum. The results emphasise the diffuse nature of Crohn's disease and indicate that the intestinal mucosa is involved to a much greater extent than can be judged by radiological appearances alone. It also accounts to some extent for some of the diarrhoea in patients with Crohn's disease although multiple factors are likely to be involved in the pathogenesis of diarrhoea in individual patients with Crohn's disease.

Finally, Section V is given over to considerations on the possible future applications of the small intestinal perfusion technique. The application of the technique to the study of the absorption of folic acid, lipids and drugs is considered, and the technique may be of immense value in these fields. The technique can also be applied to study the activity of membrane digestive enzymes, and then measure pancreatic enzyme activity. The possibility of studying intestinal absorption using a more physiological 'slow marker perfusion' system is considered and the possibility of tailoring the technique to study particular aspects of intestinal physiology and disease processes is discussed.

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SECTION 1

INTRODUCTION

CHAPTER 1

SMALL INTESTINAL FUNCTION AND DYSFUNCTION

FUNCTIONS OF THE SMALL - INTESTINE

The small-intestine, comprising the duodenum, jejunum and ileum has two principal functions - digestion and absorption.

1. DIGESTION

Food leaving the stomach is partially broken down, but little digestion occurs in the stomach, apart from some begun by enzymes produced by the salivary glands. The principal initial phase of digestion occurs within the duodenum and jejunum following the secretion of the pancreatic enzymes amylase, lipase and trypsin. In the case of dietary fat, solubilisation occurs with the aid of bile acids produced in the liver and transported by the bile ducts. Different mechanisms of digestion are adopted for the principal components of ingested food - fat, carbohydrates and protein.

Fat is ingested principally in the form of long-chain triglycerides. (Matson and Volpenheim, 1964). Triglyceride is broken down by pancreatic lipase to eventually form monoglyceride and free fatty acids. These water-insoluble compounds enter the aqueous phase of intraluminal solution by means of the formation of mixed micelles with bile acids (Hofmann and Small, 1967). Carbohydrates are principally ingested as starch, sucrose and lactose. These compounds are broken down by salivary and pancreatic amylases, and intraluminal digestion in the duodenum occurs very rapidly. (Gray, 1970). Maltose, maltotriose, sucrose and lactose are all subsequently hydrolysed by intestinal surface digestion by brush border enzymes (Miller and Crane, 1961). Protein digestion begins in the stomach with the action of pepsin, but most ingested protein is hydrolysed in the duodenum and upper jejunum by pancreatic proteases and peptidases. Oligopeptidases of the intestinal brush border hydrolyse some di, tri and tetrapeptides, although peptides consisting primarily of glycine, proline, hydroxyproline

or dicarboxylic amino acids may be absorbed in peptide form, after which hydrolysis may occur within the cell. (Kania, Santiago and Gray, 1972; Kraft, Geddes, Hyde et al, 1968).

2. ABSORPTION

Following the complex mechanisms of intestinal digestion, absorption occurs in various ways. In the case of lipids, the mixed micelle appears to break up at the intestinal surface membrane, allowing the released monoglyceride and free fatty acids to enter the cell across the lipoprotein membrane. The bile acids remain within the intestinal lumen to form other micelles and themselves are absorbed (principally in the terminal ileum) and re-circulated in an enterohepatic circulation through the liver (Borgström, Lundh and Hofmann, 1963).

The products of carbohydrate digestion are absorbed in two principal ways. Glucose and galactose are actively transported across the intestine by a process requiring energy and sodium ions (Gray, 1970). Active pumping of sodium ions out of the intestinal cells provides energy allowing glucose and galactose to be absorbed against a concentration gradient. (Malawer, Ewton, Fordtran et al, 1965; Olsen and Ingelfinger, 1968; Fisher and Gardner, 1974). Fructose is absorbed by a process of facilitated diffusion.

Amino-acids enter and are transported across the intestinal cell by an active process also involving sodium ions (Gray and Cooper, 1971).

The absorption of water and electrolytes is of special importance to the applications of the small-intestinal perfusion technique described in this work, and is presented in rather more detail.

The intestinal mucosal epithelium may be regarded as a compartment with net movement of substances through the individual cells occurring up or down gradients of concentration, and an electro-chemical potential

existing across the epithelium. If the movement occurs 'up hill', then by definition the movement is 'active' (Parsons, 1971). Sodium is absorbed from the jejunum by an active transport system of this kind which is able to transport sodium from the lumen against gradients of chemical concentration, the negative electrical charge of the intestinal mucosa, and in some circumstances against the bulk flow of water (Schultz and Curran, 1968). The energy for this sodium pumping mechanism appears to be the membrane bound ATPase. (Glynn, 1968). These ATPase systems constitute an integral part of the biochemical architecture of cell membranes. The maintenance of the sodium pump thus requires a continuing metabolism on the part of the cell so as to maintain the supply of ATP. The absorption of sodium is inhibited if the intestine is exposed to metabolically unsuitable conditions. (Parsons, 1968). The active transport of sodium also appears to be related to the absorption of glucose and certain amino-acids (Schultz and Zalusky, 1964; Munck, 1972; Hellier, Thirumalai and Holdsworth, 1973). Factors such as intraluminal pH (Rousseau and Sladen, 1971) and intestinal blood flow (Love, Matthews, and Veall, 1972) also appear to be important in the absorption of sodium from the jejunum. In man, the electro-chemical potentials against which sodium is absorbed increase aborally. Thus sodium is absorbed against greater gradients in the distal small bowel compared to the jejunum (Fordtran, Rector, and Carter, 1968).

The mechanism of water absorption is considered to be passive, secondary to the absorption of solutes (Schultz and Curran, 1968; Parsons, 1968). Transport of solute, such as sodium chloride, creates an osmotic gradient for water movement (Parsons, 1967; Love, Mitchell and Phillips, 1968). Malabsorption of water is also secondary to malabsorption of solutes.

Absorption of potassium appears to occur in the jejunum mainly by the solvent drag, and in the ileum is associated more specifically with

the transport of sodium (Turnberg, 1972). Intestinal absorption of anions appears to be a complex process. Movement of chloride and bicarbonate are closely coupled. Chloride–bicarbonate exchange in the ileum has been related to sodium and hydrogen ion transport (Turnberg, Fordtran, Carter et al, 1970; Turnberg, Bieberdorf, Morawski et al, 1970). It appears that chloride and bicarbonate can be absorbed together in the jejunum but in the ileum and colon, chloride is usually absorbed and bicarbonate secreted. Chloride concentrations decrease, bicarbonate concentrations increase and the pH rises the more distally the fluid passes down the intestine. The secretion of bicarbonate into the lumen of ileum appears to depend upon the presence of chloride ions within the intestinal lumen (Hubel, 1969).

The net absorption of salt is thus essentially a two-way process, net absorption being associated with the occurrence of massive bidirectional fluxes across the cells. Water absorption is passive and secondary to the absorption of solute.

Absorption of iron and calcium occur principally from the duodenum and involve complex control mechanisms (Manis and Schachter, 1962; Taylor and Wasserman, 1969).

Absorption of vitamins depends upon whether they are fat-soluble or water-soluble. The fat-soluble vitamins, A, D, E, and K, depend upon the mechanisms of lipid absorption previously described, and water-soluble vitamins appear to be absorbed by a passive mechanism. Folic acid is absorbed principally in the jejunum, in the form of pteroylglutamic acid. The polyglutamates (the principal form of dietary folate) must be deconjugated prior to absorption. (Rosenberg and Godwin, 1971; Hoffbrand, 1971). Finally, Vitamin B_{12} is absorbed

by an active intrinsic factor-mediated process in the terminal ileum (Corcino, Waxman and Herbert, 1970).

SMALL INTESTINAL DYSFUNCTION

Small-intestinal dysfunction resulting in deficiency of absorption of essential substances into the bloodstream may result from disorders of digestion of ingested food, or of disorders of absorption across the intestinal mucosa. A classification of the principal causes of maldigestion and malabsorption is shown in Table 1.1.

Maldigestion

Maldigestion will occur when ingested foodstuffs are inadequately digested due to the lack of, or inadequate production of, digestive enzymes. This, in turn, leads to a secondary intestinal absorption. Digestion of fat, protein and carbohydrate, requires pancreatic enzymes, and thus diseases of the pancreas may produce a maldigestion state. This will follow chronic pancreatitis and cystic fibrosis, and in some instances, carcinoma of pancreas (Cerda and Brooks, 1967; Rastogi and Brown, 1967). Deficient intraluminal bile acid concentration will lead to fat maldigestion with steatorrhoea, and may follow extrahepatic biliary obstruction (Atkinson, Nordin and Sherlock, 1956) or chronic intrahepatic disease (Marin, Clark and Senior, 1969). Fat maldigestion may also follow breaking of the enterohepatic circulation of bile acids due to severe disease of the terminal ileum as in Crohn's disease, or after ileal resection (Hofmann, 1967). It may also follow jejunal bacterial overgrowth due to intestinal stasis as in the blind loop syndrome, strictures, or multiple jejunal diverticula. (Rosenberg, Hardison and Bull, 1967). A cholecystocholic fistula may also lead to bile acid insufficiency and fat maldigestion. Inadequate mixing following gastric surgery, as with partial gastrectomy, may also lead to a maldigestion state. (Corsini, Gandolfi, Bonechi et al, 1966).

Malabsorption

Disorders of malabsorption broadly refer to conditions leading to damage of the actual absorption process itself. This may follow damage to the small intestinal mucosa giving rise to subtotal or partial villous atrophy, as in coeliac disease (Benson, Kowlessar and Sleisenger, 1964) in which intestinal damage is caused by ingested gluten in the diet. Other causes of mucosal malabsorption include tropical sprue (Klipstein, 1968), and rarer conditions include small intestinal resection or bypass (Kalser, Roth, Trueman et al, 1960; Hardison and Rosenberg, 1967), radiation damage to the small intestine (Tankel, Clark and Lee, 1965), Whipple's disease (Gross, Wollaeger, Sauer et al, 1959), intestinal lymphoma (Eidelman, Parkins and Rubin, 1966) intestinal lymphangiectasia (Waldemann, Steinfield, Dutcher, et al, 1961), abetalipoproteinaemia (Isselbacher, Scheig, Protgin et al, 1964), amyloidosis (Gilat, Revach, Sohar, 1969), hypogammaglobulinaemia (Ament and Rubin, 1972), small intestinal ischaemia (Birchir, Bartholomew, Cain et al, 1966), and parasitic infections, such as giardiasis (Hoskins, Winawar, Broitman et al, 1967) and hookworm (Meronei, Cox and Solar, 1962).

Malabsorption may also accompany endocrine conditions such as diabetes mellitus (Rubel, and Kalsar, 1964) scleroderma (Khan, Jeffries, and Sleisenger, 1966), thyroid (Crane and Evans, 1966) and parathyroid disease (Russell, 1967). Drugs such as Cholestyramine (Zurier, Hashim and Van Itallie, 1965) and Neomycin (Rogers, Vlodman, Blum et al, 1966), may also cause malabsorption states.

Symptomatology of Maldigestion and Malabsorption

A wide range of symptomatology may follow the development of maldigestion and malabsorption states. This can be classified into two main groups (Gray, 1973).

A. Local gastro-intestinal symptoms - Abdominal pain, distension and diarrhoea are common features of maldigestion and malabsorption states. These symptoms may follow malabsorption of fluid and may also in some cases be due to a secretion of fluid from the intestinal mucosa. If steatorrhoea is present, the classical features of large, bulky, greasy, foul-smelling stools which float on water, may be present. In most patients the passage of two or three soft bowel motions per day may be present for some time before symptoms become severe, and in some patients, no gastro-intestinal symptoms may be present.

B. Deficiency states following malabsorption

A state of malnutrition and cachexia may occur late in malabsorption and maldigestion states if early symptomatology is overlooked. Deficiencies of fat-soluble vitamins may cause hyperkeratosis of the skin, (Vitamin A), ecchymosis and haematuria (Vitamin K) and parathesia, tetany and bone pain (Vitamin D). Malabsorption of the water-soluble vitamin B complex may result in glossitis, cheilosis and peripheral neuropathy. Anaemias are common and may be due to iron, folic acid or vitamin B_{12} deficiency. Osteomalacia, (Calcium and Vitamin D), muscle weakness (Potassium), tetany (Calcium, magnesium) or peripheral oedema (albumin) may be present.

As mentioned above, one of the principal symptoms related to small-intestinal dysfunction is diarrhoea. In recent years, technical advances (including information obtained from intestinal perfusion studies) have given us a greater knowledge of the pathogenesis of diarrhoea, and the

complex pathogenesis of this symptom requires further discussion.

The Pathophysiology of Diarrhoea

Patients with malabsorption states may have diarrhoea, but there are many other causes of diarrhoea unassociated with maldigestion or malabsorption. Some of these causes may be due to dysfunction of the small intestine and others due to colonic dysfunction.

Diarrhoea may be classified in three principal ways: (Phillips, 1972).

(a) Osmotic retardation of water absorption.

(b) Abnormal electrolyte and water transport.

(c) Disorders of transit.

(a) Osmotic factors

(i) Overload:

Diarrhoea may follow dietary indiscretions, although individual and racial variations in the digestion and absorption of carbohydrate may be a factor in many such cases (Bayless, Paige and Ferry, 1971). Sometimes diarrhoea may occur when the normal compensatory capacity to digest and absorb is exceeded, which may occur under certain dietary circumstances.

(ii) Malabsorption:

Diarrhoea is a frequent, but not an invariable, symptom of malabsorption of carbohydrate, fat and protein, and may follow a wide variety of gastro-intestinal diseases. The presence of unabsorbed dietary components in the bowel lumen also constitutes an abnormal osmotic load. Malabsorption of carbohydrate may also occur in disacharidase deficiency secondary to coeliac disease, and often other pathological conditions of the small intestine (Welsh, Zschiesche, Anderson et al, 1969).

Disacharidase deficiency may cause diarrhoea by osmotic effects, and may also have an effect of decreasing the pH of the colon (Christopher and Bayless, 1971). Malabsorption of fat leads to diarrhoea by complicated mechanisms in which hydroxy fatty acids may play a part. (Wiggins, Pearson, Russell, et al, 1973). Malabsorption of protein is an infrequent cause of diarrhoea. The mechanism of diarrhoea associated with carbohydrate and lipid malabsorption is clearly complex in origin and requires further study.

(b) Abnormal electrolyte and water transport

As previously described, the net absorption of salt (and thus of water), is essentially a two-way process, the net absorption being associated with the occurrence of bidirectional fluxes across the cells. It is clear that this absorption mechanism is finely balanced and if destroyed, may well lead to a state of reduced absorption or net secretion.

It has been suggested that a number of abnormal factors may lead to stimulation of intestinal secretion, thus altering electrolyte and water transport. Even a modest impairment of reabsorption in one area, if not compensated by increased absorption in another area, can lead to diarrhoea. (Phillips, 1972). Toxic, chemical, humoral and mucosal factors may lead to diarrhoea by altering water and electrolyte absorption in the small intestine, but further information and evaluation of diarrhoeal disease following these causes requires further investigation (Field, 1974). This aspect will be dealt with in greater detail in a later part of this work, as the study of such influences on water and electrolyte absorption is a major application of the technique of small intestinal perfusion.

(c) Disorders of transit

For absorption to proceed normally, chyme must be mixed and digested sufficiently, and exposed to an adequate mucosal surface for a critical minimum time. Mixing and propulsive factors thus play a part in the normal absorption mechanisms. Thus conditions leading to hypomotility and stasis, such as strictures, diverticula, blind loops or muscular disease may be followed by bacterial overgrowth in the small intestine. (Gorbach, 1971). Steatorrhoea may follow and may be due to bacterial deconjugation of bile acids; watery diarrhoea also frequently follows these overgrowth states. The basic cause of the watery diarrhoea of this type is as yet unknown. An association between rapid intestinal transit and malabsorption is not clearly proven, and when they do co-exist the cause and effect in relationships are often poorly defined. (Connell, 1961). A number of factors such as fatty acids, bile acids and hormones may have an effect on intestinal smooth muscle function. (Farrar and Zafass, 1967; Misiewicz, Waller, Kiley et al, 1969).

Much information is thus still to be obtained on the mechanisms of malabsorption states and the pathogenesis of diarrhoea. It seems possible that the technique of small-intestinal perfusion may be helpful in providing some of these answers.

TABLE 1.1

CLASSIFICATION OF THE PRINCIPAL CAUSES OF MALDIGESTION AND MALABSORPTION.

MALDIGESTION

1. PANCREATIC INSUFFICIENCY
   - Chronic pancreatitis
   - Cystic fibrosis
   - Cancer of pancreas
2. INTRALUMINAL BILE ACID DEFICIENCY
   - Biliary tract obstruction
   - Chronic intrahepatic obstruction
   - Interruption of enterohepatic circulation of bile acids
   - Intestinal stasis with bacterial overgrowth. (blind loops, strictures, scleroderma).
   - Cholecystocholic fistulae
3. INADEQUATE MIXING - following gastric surgery

MALABSORPTION

1. MUCOSAL DAMAGE
   - Coeliac disease
   - Tropical sprue
   - Crohn's disease
   - Whipple's disease
   - Amyloidosis
   - Radiation
   - Parasitic infection
2. INTESTINAL RESECTION

TABLE 1.1 (ctd.)

3. VASCULAR
   - Superior mesenteric artery occlusion
   - Atheroma
4. LYMPHATIC OBSTRUCTION
   - Reticulosis
   - Intestinal lymphangiectasia
5. BACTERIAL OVERGROWTH
6. DRUGS
   - Neomycin, Colchicine, Cholestyramine, Diphenylhydantoin
7. MISCELLANEOUS
   - Hyperthyroidism, hypothyroidism, Parathyroid disease, Addison's disease.
   - Diabetes mellitus
   - Hypogammaglobulinaemia
   - Abetalipoproteinaemia

CHAPTER 2

THE INVESTIGATION OF SMALL INTESTINAL FUNCTION

STANDARD CLINICAL TESTS OF DIGESTION AND ABSORPTION

A wide variety of tests have been devised for the measurement of digestion and absorption in the small intestine. Most of these tests are indirect and do not give specific information about the ability of the small intestine to absorb digested food.

Many of the tests available are balance studies, tolerance tests or the measurement of deficiency states by the direct estimation of blood levels. Thus the quantitative estimation of faecal fat has remained the standard test for many years for the presence of steatorrhoea, but provides no information about the actual ability of the small-intestinal mucosa to absorb lipids (Van de Kamer, Huinink, and Weyers, 1949). The pancreatic stage of digestion can be tested by use of the secretin test (Dreiling and Janowitz, 1962) or by the Lundh test meal (Lundh, 1962). Both of these methods are liable to inaccuracies in borderline cases of pancreatic insufficiency. Carbohydrate absorption can be measured indirectly by the use of tolerance tests such as glucose tolerance test and the d-xylose excretion test (Finlay, Hogarth and Wightman, 1964). Blood levels are often used to establish the presence of deficiencies, although no information is obtained from these tests as to the absorption of the specific substances. Blood levels of Vitamin B_{12} iron, folic acid, calcium, magnesium and phosphorus are such examples.

The use of radio-isotopes is of value in various absorption studies. Thus, Vitamin B_{12} absorption tests provide information about the ileal intestinal phase of absorption, and a wide range of isotopes have been studied over the years as being of possible value in the measurement of lipid absorption. (Bonnet, Hightower and Rodardte, 1962).

Assay of small intestine digestive enzymes provides information about surface stage digestion (Dahlqvist, 1968). Small-intestinal radiology, such as small bowel enema, may also provide useful information in some patients, but provides no information about the absorptive capacity of the small intestine (Marshak and Lindner, 1966).

Suction biopsy of the small intestine gives essential information about the histology of the intestinal mucosa, and in recent years a number of successful techniques have been devised to obtain biopsies (Crosby, Kugler, 1957; Rubin and Dobbins, 1965). A study of the histological appearances of the small intestinal mucosa, however, gives no information about the ability of that mucosa to maintain its normal absorption capacity.

All of these methods may be of value in any individual patient in building up a composite picture of deficiencies, histological change in the intestinal mucosa, enzyme activity in the mucosa, pancreatic enzyme activity etc., but none of them specifically offer information about the ability of the intestinal mucosa to absorb water, electrolytes or other substances.

THE DEVELOPMENT OF LONG TUBE SYSTEMS FOR STUDYING SMALL INTESTINAL FUNCTION

Traditional methods for studying the function of the small intestine give only a very rough approximation of intestinal absorption in man. Tolerance and balance studies can give at best only indirect estimates of overall intestinal function. New and more direct methods are required to study the function of the small intestine.

The concept of intubation of the gastro-intestinal tract is an old one, but modern technical development such as image intensification and the introduction of new materials for making intestinal tubes has made

the whole concept more practical, and has allowed the development of the sophisticated intubation systems at present available for small intestinal intubation. Scheltema, (1908) first intubated the human subject successfully and subsequently a "duodenal pump" was introduced by Einhorn (1926). Successful small-intestinal intubation was subsequently accomplished by Einhorn (Einhorn, 1919) and McClendon (McClendon, Bissell, Lowe et al, 1920). The use of a collapsable balloon on the distal end of an intestinal tube was first reported by Jones and Pearce (1931), and a double-lumen tube was first described successfully by Miller and Abbott (1934). The technique of the use of such a tube was improved upon, and soon studies using it for the measurement of intestinal absorption were appearing (Abbott and Miller, 1936; Nicolson and Chornock, 1942; Abbott, Karr and Miller, 1937). With these original methods, gut segments were isolated by balloons which probably affected intestinal motility and blood supply. The solutions were introduced directly to the lumen and unabsorbed material was recovered after a set time interval. The results from these studies were probably unreliable because of leakage past the balloons and difficulty in recovering unabsorbed material rapidly and completely.

The use of non-absorbable markers or reference substances in human intubation studies was a major advance in the small-intestinal intubation techniques (Borgström, Dahlqvist, Lundh and Sjövall, 1957). This technique meant that quantitative recovery of unabsorbed material was not essential as the change in concentration of the marker substances allowed the calculation of absorption rates. The essential properties of these reference substances are particularly important for the accuracy of intubation studies and will be dealt with in a

later chapter. Following the introduction of non-absorbable markers, the technique of segmental intestinal perfusion was proposed by Schedl and Clifton (1961) and by Fordtran, Levitan, Bikerman et al (1961) using multi-lumen tube methods. The initial perfusion technique has been shown to function effectively in the measurement of water and electrolyte absorption (Schedl and Clifton, 1963; Cooper, Levitan, Fordtran et al, 1966; Whalen, Harris, Greenan et al, 1966). Important technical improvements and modifications have appeared, such as the use of the triple-lumen tube system with a mixing segment, initially suggested by Ingelfinger (1964), and successfully used by Cooper, Levitan, Fordtran et al (1966), and Whalen, Harris, Greenan et al (1966), and a proximal occluding balloon with a double-lumen tube, introduced by Phillips and Summerskill (1966).

The technique has been successfully used in the measurement of intestinal absorption of substances other than water and electrolytes (Holdsworth and Dawson, 1964; McMichael, Webb, and Dawson, 1967; Adibi and Gray, 1967; Silk, Kumar, Perrett et al, 1974).

The technique is constantly being assessed, developed and applied to new absorption systems, and a number of critical and constructive assessments of the indications and limitations of the technique have appeared (Fordtran 1966; Goulston, Olsen and Harris, 1966; Sladen, 1968; Fordtran, 1969; Soergel, 1971; Modigliani, Rambaud and Bernier, 1973).

CHAPTER 3

AIMS OF THE STUDIES

The development of the small intestinal marker perfusion technique using double-lumen or triple-lumen tubes has opened up a major new investigative area in the direct study of small intestinal function in man. The studies to be reported in this work are concerned with the further development and application of the technique. For the purposes of these investigations the technique has been limited to the study of water and electrolyte transport in the jejunum, although small intestinal perfusion systems have been used to study the absorption of carbohydrate, protein, lipids, folic acid and a number of other substances.

The investigations fall into a number of well-defined categories:

A. Studies of the technique of small intestinal marker perfusion in the jejunum.

In this section investigations will be described into various accuracy factors in perfusion studies, together with a study of the best way to apply the technique, and the type of tube systems used. The importance of perfusion flow rates, the composition of perfusates and the validation of new water-soluble markers for the perfusion technique itself have all been studied.

B. Studies of factors influencing water and electrolyte absorption.

In this section investigations will be described in which the small intestinal perfusion technique is applied to study normal intestinal transport of water and electrolytes in the jejunum, together with factors influencing solute and water movement.

The effect of different types and concentrations of bile acids, and of lipids, on jejunal absorption of water and electrolytes will be described and the importance investigated of a balance between various chemical factors in the small intestine and the effect of imbalance on solute and water transport will be studied.

The effect of the diuretic drug Frusemide on small intestinal absorption of water and electrolytes will also be described.

As a result of these studies more information on the pathogenesis of secretory diarrhoea has been obtained, and a fuller classification of the causes of this type of diarrhoea can then be made.

C. Jejunal absorption abnormalities in coeliac disease and Crohn's disease.

In this section the nature of the jejunal absorption abnormalities of water and electrolytes in disease processes such as Crohn's disease and coeliac disease will be described, together with the effect of therapy such as gluten-free diet on the abnormalities found in coeliac disease.

Finally, these studies are only a part of an ongoing programme of research into intestinal absorption and its abnormalities, and some considerations will be made on possible future applications of the small intestinal perfusion technique.

SECTION II

STUDIES ON THE TECHNIQUE OF SMALL INTESTINAL PERFUSION

CHAPTER 4

PRINCIPLES AND PROBLEMS OF THE TECHNIQUE.

BASIC PRINCIPLES OF THE TECHNIQUE

The basic principle of the small intestinal marker-perfusion technique is straightforward. A test solution containing an inert reference substance is infused into the jejunum at a constant rate, and intestinal contents are sampled continuously distal to this point. The infusion flow rate is known, and the flow rate past the sampling point can be calculated accurately from the infusion rate and the difference in concentration of markers between the test solution and aspirated samples. (Fordtran, 1966; Soergel, 1971). The principle of the use of non-absorbable water-soluble markers is described in Figure 4.1. The absorption of solute or water calculated from the change in marker concentration within the test segment refers to net absorption or secretion, and thus measures flux across the mucosa. The terms absorption or secretion, when applied to the results of a small intestinal perfusion study, thus refer to the direction of net movement from the intestinal lumen towards blood, or from blood towards the intestinal lumen.

The calculation of net absorption or secretion using the basic double-lumen perfusion system is shown in Table 4.1.For accurate results to be obtained from marker-perfusion studies, the following conditions must be present.

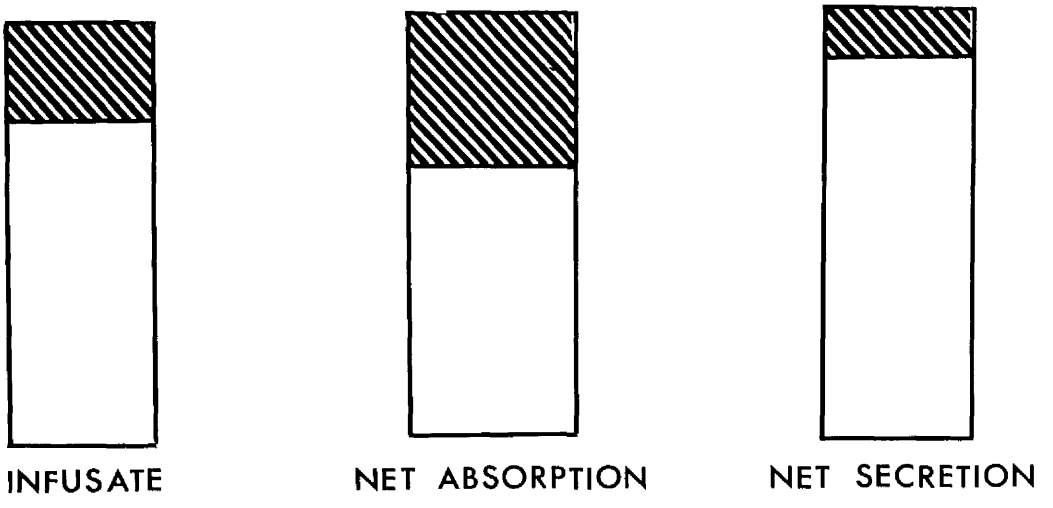
(a) The marker must be uniformly distributed in the luminal contents.

(b) Marker concentration of the sample must be identical to that of the intestinal content.

(c) The rates at which the marker enters and leaves the study segment must be equal.

FIGURE 4.1

PRINCIPLE OF THE USE OF NON-ABSORBABLE MARKERS



Hatched areas represent concentration of marker.

TABLE 4.1

CALCULATION OF ABSORPTION OR SECRETION

USING DOUBLE-LUMEN TUBE

PERFUSION SYSTEM

$$\text{Net water absorption} = I \times \left(1 - \frac{M_1}{M_X}\right)$$

$$\text{Net solute absorption} = I \times \left(S_1 - S_x \frac{M_1}{M_x}\right)$$

I - initial infusion rate

M_1 - marker concentration in perfusate

M_X - marker concentration in collections

S_1 - solute concentration in perfusate

S_X - solute concentration in collections

These conditions may be difficult to obtain in a perfusion study and are difficult to prove to be present in any study. They depend upon two principal factors - the existence of a steady state within the test segment, and the homogeneity of the intestinal contents (Modigliani, Rambaud and Bernier, 1973). The achievement of the steady-state system means that the flow rate and luminal solute concentration at any given point are constant with respect to time, but they change with respect to distance. A steady-state system is not immediately attained and requires a period of preliminary perfusion or equilibration. This period requires to be larger in time when the test segment is longer in length and the rate of perfusion lower. In general, a period of time from 45-60 minutes is required for the achievement of an acceptable steady state. It is unlikely, however, that a perfect steady-state is ever really obtained in the test segment owing to factors such as unavoidable alterations in intrinsic intestinal motility (Groissier and Farrar, 1962), change in the average diameter of the small intestine (Dillard, Eastman and Fordtran, 1965), and mucosal blood flow (Sladen, 1968). Contamination of the test segment with endogenous secretions and reflux of the perfusate proximal to the infusion point will also alter the steady-state. Although marker concentration in fluid sampled distal to the infusion point will measure an average flow rate past this site, the rate of inflow into the study segment is unknown, and therefore net fluid movement cannot be accurately calculated. Various attempts have been made to limit contamination by endogenous secretions. Initially, suction was applied proximal to the infusion site (Fordtran, Levitan, Bikerman et al, 1961), and subsequently by the introduction of a mixing segment. This allows the infused test material and endogenous secretions to mix thoroughly

and samples of the perfusion solution can be collected from two sites distal to the infusion point (Ingelfinger, 1964). Intestinal absorption can then be calculated in this test segment by the change in marker concentration between the two collection sites. This practical development of the triple-lumen tube system was first used by Cooper and his colleagues, 1966, and Whalen et al, 1966. Calculation of absorption or secretion within the test segment using the triple-lumen tube perfusion system is shown in Table 4.2. It has also been suggested that staggering of collections from the proximal and distal tubes may also give a more accurate estimate of the calculated absorption values (Whalen, Harris, Geenan et al, 1966). The use of a proximal occluding balloon with a basic two-lumen tube system has also been suggested as a means of reducing contamination of the test segment (Phillips and Summerskill, 1966). A diagrammatic representation of the double-lumen tube system with and without a proximal occluding balloon, and the triple-lumen tube system are shown in Figures 4.2, 4.3 and 4.4 respectively.

It is necessary to collect multiple samples of fluid from the test segment over 10-15 minutes each and the absorption calculated over 5 or 6 of these collection periods to minimise the effects of variation in segmental absorption rates (Sladen 1968.)

The homogeneity of intestinal content is also important. The principle of the marker-perfusion method supposes that concentrations of solute in the samples collected are identical to those in the whole intestinal contents which pass the sampling point, and that there is no concentration gradient between the centre and the periphery of the fluid column. This assumption may not be necessarily correct but any error due to this may not necessarily be of importance in any given marker-perfusion study. It is also the case that the measured absorption from

TABLE 4.2

CALCULATION OF ABSORPTION OR SECRETION

USING TRIPLE-LUMEN TUBE PERFUSION SYSTEM

Net water absorption = $Q_p - Q_D$

$$\left(Q_p = I \times \frac{M_1}{M_p} - R_p\right.$$

$$\left.Q_D = Q_p \times \frac{M_p}{M_D}\right)$$

Net solute absorption = $Q_p S_p - Q_D S_D$

Q_p - flow rate entering test segment

Q_D - flow rate leaving test segment

I - initial infusion rate

M_1 - marker concentration in perfusate

M_p - marker concentration in fluid collected from proximal collection point

M_D - marker concentration in fluid collected from distal collection point

R_p - rate of sampling at proximal collection point

S_p - Solute concentration at proximal collection point.

S_D - Solute concentration at distal collection point

FIGURE 4.2

DIAGRAMMATIC REPRESENTATION OF DOUBLE-LUMEN TUBE PERFUSION SYSTEM

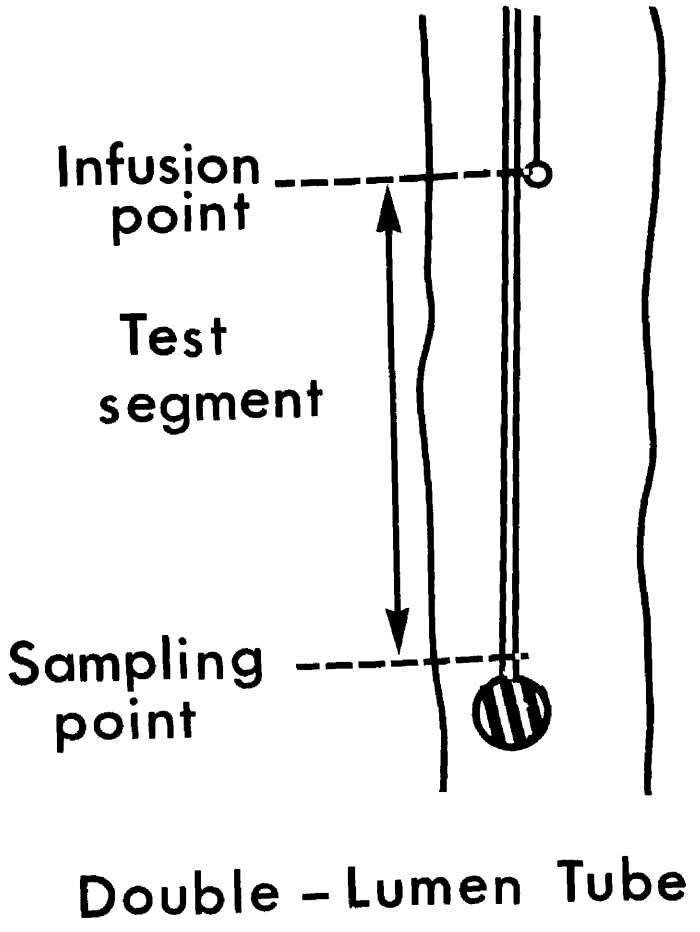
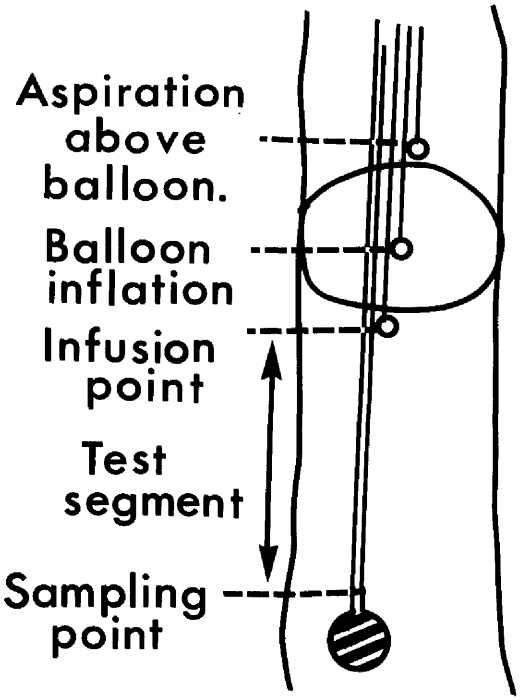


FIGURE 4.3

DIAGRAMMATIC REPRESENTATION OF DOUBLE-LUMEN TUBE

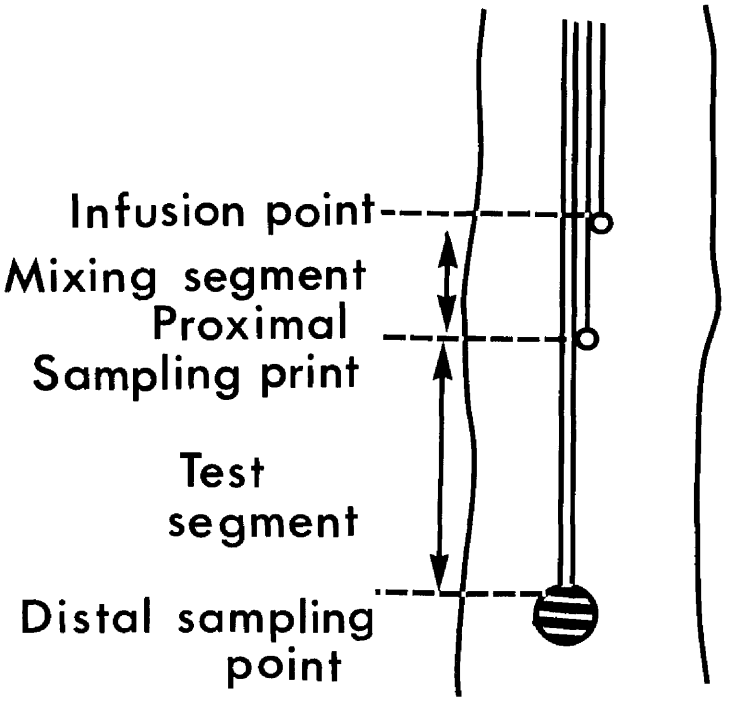
SYSTEM WITH PROXIMAL OCCLUDING BALLOON



Double-Lumen tube with Proximal Occluding Balloon.

FIGURE 4.4

DIAGRAMMATIC REPRESENTATION OF TRIPLE-LUMEN TUBE PERFUSION SYSTEM



a column of fluid in a state of equilibrium within a limited segment of intestine, as in a perfusion study, is probably somewhat different from the absorption of a meal or of a normally ingested solution.

Absorption rates obtained from intestinal perfusion vary widely from individual to individual, but appear to be quite constant during prolonged perfusion of the same subject. Thus the more effective application of small intestinal marker-perfusion studies is by the use of a paired study design, whereby the effect of a particular substance or compound on intestinal absorption is investigated rather than by comparing results from different groups of subjects.

SPECIFIC PROBLEMS OF THE TECHNIQUE

A number of specific problems exist with the small intestinal perfusion technique, and require to be carefully considered. These are as follows:-

1. Provision of an adequate steady-state:

The problem of mucosal surface area variations remains. Telescoping of the intestine around the tube undoubtedly occurs to some extent although variably, and it is known that the mucosal surface area relative to intestinal length varies in different parts of the intestine. The establishment of an adequate steady-state by allowing a long equilibration period is important.

2. Choice of marker

In order to measure differences in flow rate along a perfused segment, non-absorbable reference substances have been incorporated into the infused test solution and the change in concentration of these markers calculated. The requirements of an acceptable marker are discussed in Chapter 5.

Recovery studies under conditions which simulate as nearly as possible the actual experimental conditions of a perfusion should be conducted before using a suggested non-absorbable marker (Bloom, Jacobson and Grossman, 1966). The present chemical and radio-isotopic markers are limited in number, and all have some defect as will be described in the next Chapter. There is a case for studying new markers, which, before use in perfusion studies should be correctly investigated in relation to the criteria for acceptance. It has been shown that adequate water-soluble non-absorbable markers stay evenly mixed during transit through the small intestine (Fordtran, Levitan, Bikerman et al, 1971; Schedl, Miller and White, 1966), and they are not absorbed by mucus (Jacobson, Brody, Broitman et al, 1963). Marker concentrations in small samples of a perfusion solution are almost identical to that of the entire perfusion solution passing the sampling point (Goldstein, Olsen and Harris, 1966). Thus the principle of the marker method appears to be sound for measuring flow rates and volumes of intestinal contents. On the other hand, existing water-soluble reference substances all leave something to be desired in relation to the criteria for accuracy of markers, and there is certainly a place for new and different types or markers. This will be further discussed in the study described in Chapter 5.

3. Choice of perfusion tube system

The two basic tubes available use the double-lumen and the triple-lumen tube systems (Schedl and Clifton, 1961). The use of the basic double-lumen tube for intestinal perfusion studies is complicated by contamination of the test segment with endogenous secretions. The use of a proximal occluding balloon should prevent this problem. (Phillips

and Summerskill, 1966.) The triple-lumen tube aims at avoiding contamination and improving mixing by the use of a mixing segment proximal to the test segment.

It has been considered by some authors that the measurement of intestinal absorption of water and sodium in man may not vary significantly using the double and triple-lumen tube methods (Sladen and Dawson, 1968a), although this claim is refuted by others (Fordtran, 1969). Specific information is required on this point using the same patients perfused on the same day by both methods, so that the relative accuracies of the double and triple-lumen systems can be resolved satisfactorily. The effects of a proximal occluding balloon in possibly altering absorption when inflated also requires to be studied.

4. Choice of flow rate

The choice of the perfusion flow rate is somewhat arbitrary, and variable flow rates have been used in studies reported, ranging from 8-20 ml. per minute. The flow rate of the normal intestinal contents after a meal is not accurately known, but has been indirectly estimated at about 10 ml/minute in the proximal jejunum (Sladen 1968). The effect of the perfusion flow rate on the measurement of intestinal absorption is of practical importance. A compromise must be reached between the provision of a flow rate fast enough to allow adequate collections to be made and the prevention of the development of laminal flow with the loss of the central liquid column. Further experimental information is required on the effect of various flow rates on water and electrolyte absorption using the perfusion system.

5. Problems related to the level and length of the perfused intestine

The positioning of the perfusion tube prior to the start of the perfusion is vitally important. It is essential that radiological

checking of the position should be performed at the beginning and end of each perfusion. As previously mentioned, the intestine may shorten during the perfusion following telescoping of the intestine, the loops may come closer together so that the actual length of the test segment is greater (Blankenhorn, Hirsch and Ahrens, 1955). The actual area of the intestine under study cannot be accurately determined during perfusion. This fact also emphasises the importance of establishing an adequate steady-state prior to starting the perfusion, and also emphasises the ideal application of the perfusion technique in investigating the effect of individual substances on the absorption of water and electrolytes, rather than comparing groups of results from separate patients.

6. Problems of concentration variations throughout the test segment

The intraluminal concentration of perfusate in the intestine varies continually throughout the test segment. To establish an accurate relationship between the rate of transport and intraluminal concentration, it is desirable to know the theoretical concentration which, if uniformly distributed throughout the test segment, would give the rate of transport measured. As this concentration is unknown, individual authors have used various mathematical ways to determine these relationships. The difference between these values is negligible when the concentration varies little within the test segment but this is not the case when variations are considerable. Fisher and Parsons (1953) demonstrated in isolated loops that the concentration of solute in the loop falls progressively with time owing to absorption of a certain quantity of the solute. As concentration influences the rate of absorption, the rate of absorption thus varies with time. A correction factor in relation to the percentage falling concentration

between the initial and final figures can be made, and has been used as a correction co-efficient in intestinal perfusion studies by Sladen and Dawson (1970).

A greater understanding of the many factors affecting results of intestinal perfusion studies in man has come from the realisation and investigation of the many variables to which these studies are liable. More information is still required regarding many of the factors which may affect the accuracy of the results of perfusion studies.

CHAPTER 5

ASSESSMENT OF $^{51}CrCl_3$ AND ^{51}Cr E.D.T.A. AS NON-ABSORBABLE, WATER-SOLUBLE MARKERS FOR SMALL INTESTINAL PERFUSION STUDIES

INTRODUCTION

Criteria for acceptability of markers

The criteria for an ideal reference or inert marker substance have been defined by Fordtran (1966).

(i) It should be strictly non-absorbable - slowly absorbed markers under-estimate absorption rates to some extent.

(ii) It should not be degraded in the intestinal lumen.

(iii) It should not be trapped by mucus or other intestinal content.

(iv) It should not influence intestinal motility.

(v) It should be able to be measured easily and with accuracy.

(vi) It should have the same solubility characteristics as the test substance.

(vii) It must not influence the digestion of the test substance.

(viii) It must not be toxic.

The ideal non-absorbable inert marker substance has not yet been found. All the markers at present in use have some deficiencies but these are generally consistent and predictable enough to allow the marker to be used with care in the measurement of the absorption of water-soluble substances from the small intestine.

Existing markers

The most widely used non-absorbable, water-soluble marker is polyethylene glycol (PEG). The water-soluble properties of this compound were confirmed by Schaffer, Critchfield and Naiv (1950). Polyethylene glycol 4000 is a mixture of molecules of varying sizes with a mean molecular weight between 3,200 and 3,700. PEG 4000 is highly water-soluble and non-absorbable (Jacobson, Bondi, Broitman et al, 1963; Soergel

and Hogan, 1967; Soergel, 1968). After oral administration, 96.9% of the ingested dose of PEG may be recovered in the intestinal contents through an ileostomy, (Soergel and Hogan, 1967) and 98.2% in the stools of normal subjects (Hyden, 1956). There is also evidence that polyethylene glycol does not adhere to proteins and mucus (Jacobson, et al, 1963). One of the problems in the use of polyethylene glycol is the potential inaccuracy of its analysis using the turbidometric method (Hyden, 1956). This problem still remains in spite of some recent improvements in the analysis. (Malawer, and Powell, 1967; Boulter and McMichael, 1970). Recently, a greater accuracy of measurement of polyethylene glycol has been achieved by the use of 14C labelling. The isotope is more easily and accurately measured, and a good comparison has been obtained between the stable and 14C-labelled varieties (Miller and Schedl, 1970; Wingate, Sandberg and Phillips, 1972).

A number of other non-absorbable water-soluble markers have been used over the years. Phenol red (PSP, phenolsulphonphthalein) is absorbed by the gastro-intestinal tract in man at a rate of about 1% per hour of the administered dose (McLeod, French, Good et al, 1968). 91.8% of an ingested dose of PSP is recovered in the intestinal content from an ileostomy (Soergel and Hogan, 1967). It is considered that the stomach is partly responsible for this absorption (Bloom, Jacobson and Grossman, 1967). During segmental intestinal perfusion in man the use of PEG and PSP simultaneously has given identical results (Schedl, 1966; Schedl, Miller and White, 1966; French, Brown, Good et al, 1968). This also applies with PSP and 14C-PEG (Domschke, Demling, Domschke et al, 1973). It thus appears that absorption of PSP is negligible from a limited segment of perfused intestine. PSP binds to proteins such as

casein and albumin (Grollman, 1925), and this may result in the uneven distribution of the marker within the intestinal content. It has also been shown that falsely high readings of absorbance may be obtained with solutions containing PSP in the presence of mucoproteins (McGregor and Meyer, 1974). In spite of these potential disadvantages with the use of the PSP as a non-absorbable water-soluble marker, it does seem to be a satisfactory marker for use in intestinal perfusion studies in man. Phenol red (PSP) has also been used successfully in a number of other studies (Phillips and Summerskill, 1966; Clark and Williams, 1971; Miller and Schedl, 1972).

Bromsulphthalein (BSP) may also be used as a non-absorbable water-soluble marker (Fordtran, Levitan, Bikerman, et al, 1961). Some absorption of the marker does occur however, (Diller, Eastman and Fordtran, 1965), and the estimation may be inaccurate in the presence of bile pigment (McLeod, French, Good et al, 1968).

Indocyanine green is less satisfactory as a marker as there is some evidence that it may adhere to mucus (Maddrey, Serebro, Marcus et al, 1967). Indocyanine green, like BSP, is estimated by a colorimetric method.

The use of radio-active markers has the advantage of easy and rapid estimation. Rose-bengal labelled with 131I has been used as a marker but appears to be absorbed by the small intestine in the rat (Maddrey, Serebro, Marcus et al, 1967). 131I-polyvinyl pyrrolidine (PVP) binds to intestinal mucus (Schedl and Clifton, 1962). 14C-inulin has also been tried, but 14C - PEG may be the most successful radio-active marker yet available. This reference substance was not available for

use at the beginning of the studies currently reported. Radio-active chromium compounds have been suggested as possible inert markers in the past, although intestinal absorption of trace quantities of chromium may occur (Donaldson and Barreras, 1966). With the use of $^{51}CrCl_3$ as an inert water-soluble marker, there may also be loss due to adsorption of chromium, and for this reason an alternative chromium isotope ethylenediamine teta-acetate (EDTA) labelled with ^{51}Cr - has been suggested as a water-soluble reference substance (Lökken and Sögnen, 1967).

Need for new water-soluble non-absorbable markers

There is a requirement for new satisfactory marker substances for perfusion studies. Two markers may be used in perfusion studies so that confirmation of results by obtaining consistent figures worked out using both markers can be obtained. In addition, marker substances may also be used in the study of intestinal flow using bolus propulsion, and if this technique is used in conjunction with absorption studies using a perfusion technique, two or more markers may require to be utilised simultaneously. It is therefore of value to have a selection of inert water-soluble markers of different types which can be used simultaneously in perfusion studies.

In addition, the difficulty of measurement of some of the markers standardly used such as polyethylene glycol, bromsulphthalein and indocyanine green, makes it desirable for new types of markers to be made available. The combination of a chemically and radioactively measured marker may be satisfactory in perfusion studies.

Whereas development and application of small intestinal perfusion studies in studying intestinal absorption in man depends upon the use of satisfactory water-soluble markers, the perfusion technique in itself can be used to assess the efficacy of new markers by comparing

the absorption of water and electrolytes calculated using standard markers with those using the compound under investigation.

AIMS OF STUDY

The aim of this investigation was therefore to assess the effectiveness of two new water-soluble marker substances, ^{51}Cr-chromic chloride ($^{51}CrCl_3$) and ^{51}Cr-ethylenediamine tetra-acetate (^{51}Cr EDTA), as non-absorbable water-soluble reference substances in small intestine perfusion systems in man.

SUBJECTS AND METHODS

Subjects

Each of the chromium markers was compared in a series of volunteer subjects with a known satisfactory marker PSP(Phenolsulphonphthalein) using a jejunal perfusion system. In seven volunteer subjects $^{51}CrCl_3$ was used as a water-soluble marker together with PSP, and in a further 8 volunteers ^{51}Cr EDTA was used together with PSP.

Methods

A triple-lumen tube perfusion system was used (as shown in Figure 4.4). This incorporated a mixing segment of 15 cm and a test segment of 30 cm. The perfusate consisted of an isotonic glucose-saline mixture with a glucose concentration of 56 mM. The infusion rate was 20 ml per minute. An equilibration period of 45-60 minutes was used, following which collections 11-12 ten minute collections were made.

The perfusate contained a small amount of the appropriate chromium marker sufficient to give adequate counts on a scintillation counter (the dose being approximately 100 μc), and PSP in a concentration of 5g/l (Schedl, 1966). The perfusion was started only when accurate positioning of the tube in the proximal jejunum was achieved radiologically, so that the infusion point was sited immediately distal to the duodeno-

jejunal flexure. 150 mg/l of stable chromic chloride was added as a carrier.

On each 10 minute sample collected, the concentration of sodium and chloride was estimated, chromium counts measured by scintillation counting, PSP estimated by spectrophotometry at 580 nm in alkaline solution. In each study, care was taken to eliminate radio-activity from the test tubes used. Standards were taken before and after each perfusion study, and the mean of 4 standards taken. Calculation of absorption of water, sodium and chloride was then made using two sets of data from each subject, one using the chromium figures (either from $^{51}CrCl_3$ or from ^{51}Cr EDTA) and one from the PSP figures. Blood and urine samples were taken from the volunteer subjects one hour after the study, so that an estimate of the absorption of chromium could be made.

Calculation of absorption within the test segment was carried out using the standard formula for a triple-lumen tube system, as shown in Table 4.2.

RESULTS

As the results obtained from the absorption of sodium and chloride are dependent on the figures for water absorption, statistical analysis in this study has been performed on the water absorption figures alone, and the results for absorption of water alone are presented. Similar results relating to the chromium markers were obtained when sodium and chloride absorption was calculated.

The results of mean water absorption in the 7 studies containing $^{51}CrCl3$ and PSP as water-soluble markers are shown in Table 5.1. The mean water absorption is expressed as ml/hr/30 cm. segment, and each

TABLE 5.1

MEAN WATER ABSORPTION CALCULATED FROM STUDIES USING $^{51}CrCl_3$ AND P.S.P.

Subject	Mean water absorption (ml/hour/30 cm. segment)		Correlation (p)
	$^{51}CrCl_3$	P.S.P.	
1	125	232	> 0.05
2	197	234	> 0.05
3	265	159	> 0.05
4	100	158	> 0.05
5	116	224	> 0.05
6	123	195	> 0.05
7	201	228	< 0.05

figure in each column represents the mean water absorption obtained over 11 or 12 ten minute collection periods after the establishment of a steady-state. The correlation between the water absorption figures obtained using $^{51}CrCl_3$ and those obtained using PSP was calculated in each case and expressed for each patient as a p value. In only one patient (patient number 7) was a statistically significant correlation between water absorption results obtained using both of these markers. The results of water absorption from each of the ten minute collection periods were used for the correlation statistics.

The results obtained using ^{51}Cr EDTA and PSP as water soluble-markers in 8 volunteer subjects are shown in Table 5.2. Again the water absorption was expressed by ml/hour/30 cm segment, and each figure in each column refers to the mean water absorption obtained over 11 to 12 collection periods in each volunteer subject. In each case a good correlation was found between the water absorption results obtained using ^{51}Cr EDTA as a water-soluble marker compared with PSP.

The tests of radio-activity in blood and urine showed no significant absorption of ^{51}Cr from either the two markers tested. From these results it was found that the mean radio-activity absorbed from the small intestine during the perfusion (expressed as a percentage of activity in the infusate) was 1.5% from the studies using $^{51}CrCl_3$ and 2.2% from studies using ^{51}Cr EDTA.

DISCUSSION

The results of this study indicate that ^{51}Cr EDTA correlates well with PSP as a water-soluble marker in the measurement of water absorption from the proximal jejunum in volunteer subjects. A poor correlation was found between $^{51}CrCl_3$ and PSP. It would appear from this study that ^{51}Cr EDTA is satisfactory as a water-soluble non-absorbable reference

TABLE 5.2

MEAN WATER ABSORPTION CALCULATED FROM STUDIES USING

^{51}Cr-E.D.T.A. AND P.S.P.

Subject	Mean Water Absorption (ml/hour/30 cm.segment)		Correlation
	^{51}Cr-E.D.T.A.	P.S.P.	
1	346	361	$p < 0.005$
2	131	142	$p < 0.05$
3	169	172	$p < 0.001$
4	238	224	$p < 0.05$
5	143	151	$p < 0.005$
6	250	243	$p < 0.001$
7	174	163	$p < 0.005$
8	408	432	$p < 0.001$

substance for perfusion studies in man.

No significant absorption of 51Cr occurred from either compound during the studies.

The use of 51Cr EDTA represents the incorporation of the analytical advantages of the 51Cr isotope in a water-soluble, highly stable chelated compound. 51Cr EDTA was first used as a chromium marker for absorption studies in sheep. In this investigation 51Cr EDTA was compared as a reference substance with polyethylene glycol (PEG) (Downes and MacDonald, 1964). 85% to 91% of the dose of 51Cr EDTA was recovered in the faeces in the first 10 days, after which time small amounts of 51Cr were still being excreted. That study concluded that 51Cr EDTA is satisfactory as a reference substance, and that it appeared 'move' together with PEG. Lökken and Sögnen, (1967) found that 5% of the activity of 51Cr was absorbed in rats during infusion of the small intestine 'in situ', and the behaviour of 51Cr EDTA was comparable to that of PSP. These workers concluded that in spite of a small but consistent absorption, 51Cr EDTA appeared to be preferable to PSP as a marker because of its "analytical advantages and pharmacodynamic inertness." The results of the present study confirm the advantages of 51Cr EDTA as a non-absorbable water-soluble reference substance in perfusion studies in man.

51Cr EDTA has been widely used in the measurement of glomerular filtration rate (Stacey, and Thorburn, 1966; Garnett, Parsons and Veall, 1967; Heath, Knapp and Walker, 1968). The compound has been shown to have no toxic effects and is chemically inert. It is unlikely that exchange reactions occur with other metal ions (Johnson and Seven, 1960). It has no chelating action. The dose of chromium in 51Cr EDTA is slightly more than that given when $^{51}CrCl_3$ is used as a water-soluble marker, but with the latter isotope an additional amount of stable

chromic chloride is required as a carrier. It would appear from the results that a significant loss of chromium from the $^{51}CrCl_3$ occurs in the jejunum. As absorption of chromium does not appear to be an important factor, even from the $^{51}CrCl_3$, adsorption to mucus or to the small intestinal mucosa may be occurring with this isotope. The degree of loss of chromium from $^{51}CrCl_3$ appears to be significantly large, and occurs with such regularity that this compound cannot be used as a satisfactory water-soluble marker. On the other hand ^{51}Cr EDTA appears to be a reliable marker, giving results which correlate significantly well with absorption figures calculated from PSP. ^{51}Cr EDTA thus appears to be a safe, stable and chemically inert marker substance suitable as a non-absorbable water-soluble compound in measuring water absorption in the intestinal studies in man.

SUMMARY

A comparison of $^{51}CrCl_3$ and ^{51}Cr EDTA as non-absorbable water-soluble gastro-intestinal markers has been made by comparing each with PSP as a marker for water absorption in jejunal perfusion studies using a triple-lumen tube system.

The results show that figures for water absorption obtained using ^{51}CrEDTA correlate well with those using PSP, and a poor correlation was found between the water absorption calculated from $^{51}CrCl_3$ compared with that calculated using PSP.

^{51}Cr EDTA thus appears to be a satisfactory non-absorbable water-soluble reference substance for intestinal perfusion studies in man.

CHAPTER 6

COMPOSITION OF THE PERFUSATE : THE RELATIONSHIP OF GLUCOSE TO SODIUM AND WATER ABSORPTION

INTRODUCTION

The composition of the perfusate in small intestinal perfusion studies is of great importance. Different perfusates may be used to investigate particular functions of intestinal absorption in different absorption sites such as jejunum and ileum. It is important in each small intestinal perfusion investigation that the composition of the perfusate is selected to allow optimal conditions to exist such as to provide an adequate answer to the questions being posed. It is also important that the perfusate be iso-osmolar. Hypertonic or hypotonic solutions markedly alter the balance of intestinal absorption and secretion in the jejunum, and prevent accurate data from being obtained. During absorption or secretion when iso-osmolar solutions are used, the intraluminal fluid remains at the same osmotic pressure as plasma, thus suggesting that the fluid absorbed or secreted has the same osmotic pressure (Sladen and Dawson, 1969a). The osmolality of the perfusing solution is thus very important.

The relationship of glucose to the absorption of water, sodium and chloride is also an important factor. The presence of sodium is important for the active absorption of glucose (Riklis and Quastel, 1958; Crane, 1965; Olsen and Ingelfinger, 1968). Conversely, it has also been shown that the presence of glucose within the jejunal lumen stimulates the absorption of sodium. This has been demonstrated by in vitro studies (Schultz and Zaluski, 1964; Barry, Smyth and Wright, 1965), animal studies (Fullerton and Parsons, 1966; Livinson and Schedl, 1966; Summers and Schedl, 1968; Schultz and Curran, 1970), and in man (Malawer, Ewton, Fordtran et al, 1965; Fordtran, Rector and Carter, 1964; Sladen and Dawson, 1969; Modigliani and Bernier, 1971). The absorption of water is, like that of sodium, stimulated by glucose (Sladen and Dawson, 1969).

It would appear from these studies that glucose is necessary in the jejunal lumen for active transport of sodium to occur, and following this, absorption of water. The optimal concentration of glucose in the perfusate suitable for jejunal studies required to be studied prior to the full applications of the technique.

AIMS OF STUDY

The aim of the present study was thus to investigate the necessity for glucose in the perfusate for jejunal perfusion studies, and the optimal concentration which leads to adequate absorption of sodium and water from the jejunum.

SUBJECTS AND METHODS

Subjects

Jejunal perfusion of the solutions studied was performed in 10 normal volunteer subjects. There was no evidence of any gastro-intestinal disease in any of these volunteers.

Methods

A triple-lumen tube perfusion system was used (Figure 4.4) The tube incorporated a mixing segment of 15 cm and a test segment of 30 cm.

Composition of perfusing solutions

Five different perfusing solutions were used in this investigation. These were as follows:

A. Isotonic saline.

B. Glucose saline with a glucose concentration of 28 mM

C. Glucose saline with a glucose concentration of 56 mM

D. Glucose saline with a glucose concentration of 112 mM

E. Glucose saline with a glucose concentration of 224 mM

All the solutions used were iso-osmotic (300 milliosmols/l).

Phenolsulphonphthalein (PSP) in a concentration of 5 g/l and 51Cr EDTA were used as water-soluble, non-absorbable markers in each of the studies.

Experimental Procedure and analytical methods

The subjects fasted overnight and the perfusion tube was passed by mouth on the following morning. The tube position was checked radiologically and perfusion started when the infusion point was sited distal to the duodeno-jejunal junction. The perfusate was passed through a water bath at a constant temperature of 37°C, and introduced into the jejunum at a constant flow rate of 20 ml per minute. Infusion and collection was performed using two Watson-Marlow constant flow-inducer pumps, one of which was fitted with a 10 channel Delta pump attachment.

After the start of infusion, an equilibration period of 45-60 minutes was allowed in each study so that a steady-state could be established. In each patient several of the solutions described above were introduced in succession, time being allowed for equilibration after each solution was changed. After the establishment of a steady-state 4-6 ten minute collections were made in each subject.

Water absorption was determined by the change in concentration of both markers in the test segment. PSP was analysed by spectrophotometry, and 51Cr EDTA by an automatic gamma counting system. The concentration of sodium was measured in each collection.

The method used to calculate absorption was that described in the formula for the triple-lumen perfusion technique. (Table 4.2).

RESULTS

Ten studies were performed using isotonic saline alone, eight with glucose-saline with a glucose concentration of 28 mM, ten at 56 mM, six at 112 mM and six studies at a glucose concentration of 224 mM.

The absorption of sodium is expressed as m.mol/hour/30 cm segment and the absorption of water as ml/hour/30 cm segment.

The mean sodium absorption figures (± SEM) from the various solutions studied is shown in Table 6.1. Very little absorption of sodium was found to occur from isotonic saline alone. (5.6 ± 2.7 m.mol/hour). The absorption of sodium from glucose-saline 28mM (14.6 ± 3.0 m.mol/hour) and from glucose saline 56 mM (23.4 ± 3.2 m.mol/hour) was statistically significantly greater than the absorption of sodium from isotonic saline solution alone ($p < 0.05$ respectively). The absorption of sodium from glucose saline 112 mM was 4.4 ± 2.5 m.mol/hour. This was statistically significantly less than from the solutions containing 28 mM and 56 mM ($p < 0.05$ respectively). When glucose in a concentration of 224 mM was perfused into the jejunum a net sodium secretion was found to occur in the subjects studied (-16.3 ± 4.2 m.mol/hour).

The mean water absorption from isotonic saline and the glucose-saline solutions is shown in 6.2. Very little water was absorbed from isotonic saline solution alone (27 ± 16 ml/hour). From glucose-saline solutions containing 28 mM a mean water absorptionof 131 ± 25 ml/hour was obtained, statistically significantly greater than from isotonic saline alone ($p < 0.05$). When glucose-saline in a concentration of 56 mM was perfused, however, a further large increase in water absorption was observed, the mean level being 263 ± 40 ml/hour. This was statistically significantly greater than the water absorption from isotonic saline alone ($p < 0.001$) and from glucose-saline with a glucose concentration of 28 mM ($p < 0.05$). A falling-off of water absorption was observed when glucose-saline was perfused with a glucose concentration 224 mM, the mean level in the group being 83 ± 49 ml/hour. This figure was significantly

TABLE 6.1.

MEAN SODIUM ABSORPTION (± S.E.M.) FROM ISOTONIC - SALINE AND GLUCOSE - SALINE SOLUTIONS CONTAINING DIFFERENT CONCENTRATIONS OF GLUCOSE

Perfusing Solutions	No. of Studies	Mean Sodium Absorption (m.mol/hr/30 cm. segment)
A. Isotonic saline	10	5.6 ± 2.7
B. Glucose - saline 28mM	8	14.6 ± 3.0
C. Glucose - saline 56mM	10	23.4 ± 3.2
D. Glucose - saline 112mM	6	4.4 ± 2.5
E. Glucose - saline 224mM	6	-16.3 ± 4.2

TABLE 6.2

MEAN WATER ABSORPTION ($\pm$ S.E.M.) FROM ISOTONIC - SALINE

AND GLUCOSE - SALINE SOLUTIONS CONTAINING DIFFERENT CONCENTRATIONS OF GLUCOSE

Perfusing Solution	No. of Studies	Mean Water Absorption (ml/hr/30 sm. segment).
A. Isotonic saline	10	27 ± 16
B. Glucose - saline 28mM	8	131 ± 25
C. Glucose - saline 56mM	10	263 ± 40
D. Glucose - saline 112mM	6	202 ± 38
E. Glucose - saline 224mM	6	83 ± 49

less than that found with glucose–saline solutions with concentrations of 28 mM, 56 mM, 112 mM, but not significantly different from the water absorption which occurred from the isotonic saline solution alone.

DISCUSSION

The results of this study confirm the importance of glucose in the absorption of sodium and water from the upper jejunum in man. The selection of concentrations of glucose in the various glucose–saline solutions was to some extent arbitrary in this study, but the results show a rapid rise in sodium absorption when glucose is introduced into the perfusate. No information is available from this study as to the minimal concentration of glucose necessary to provide adequate absorption of sodium. Some sodium absorption does appear to occur from isotonic saline but with concentrations of 28 and 56 mM statistically significant increases in the absorption of sodium were observed. The results confirm the figures obtained by Sladen and Dawson (1969a), but using a triple-lumen tube system. A more graded response of sodium absorption (than by Sladen and Dawson) to increasing concentrations of glucose was found in this study and by Modigliani and Bernier (1971) using a perfusion technique with proximal occluding balloon. A graded response to the introduction of glucose was also observed with the water absorption figures.

A fall-off of absorption of both water and sodium was observed with high concentrations of glucose and in the case of sodium, net secretion was observed when glucose–saline with a glucose concentration of 224 mM was introduced into the jejunum.

The results of this study show that glucose does stimulate absorption of sodium and water. Active transport of glucose is probably important in this stimulation. Reduction in the absorption of water and sodium

with high concentrations of glucose confirms the previous observation of Holdsworth and Dawson, (1964) although the mechanisms of this is not yet fully understood. The close association between water and solute absorption has been noted for many years, initially with in vivo animal preparations (Curran and Solomon, 1957) and is probably the general rule in absorption from isotonic solutions (Fordtran and Dietschy, 1966). Glucose may stimulate the absorption of sodium initially, and water absorption probably follows passively with the development of local osmotic gradients.

Other factors other than glucose may stimulate the absorption of sodium in the jejunum. This may occur with amino acids (Adibi, 1970) and bicarbonate ions in the perfusate also stimulate the absorption of sodium (Sladen and Dawson, 1968b; Klotz and Schloerb, 1971).

SUMMARY

The importance of glucose in the absorption of sodium and water from the jejunum has been studied using a triple-lumen tube perfusion technique in man. Little or no absorption of water or sodium was observed with isotonic saline. With increasing concentrations of glucose in glucose-saline solutions up to 56 mM, increasing absorption of water and sodium was found. With high concentrations of glucose (224 mM) a marked reduction of water and sodium absorption occurred and net secretion of sodium occurred in the jejunum.

From these results a concentration of glucose of 56 mM in glucose-saline solutions has been taken as being the best basic perfusate for the jejunal perfusion studies subsequently to be described.

CHAPTER 7

PERFUSION FLOW RATE : THE EFFECT OF DIFFERENT FLOW RATES ON WATER AND ELECTROLYTE ABSORPTION

INTRODUCTION

The rate of introduction of the perfusate into the small intestine is particularly important in the technique of small intestinal perfusion. A wide range of infusion rates has been used in the past (8–30 ml/min.) and the selection of the perfusion flow rate has been largely arbitrary.

Although the faster infusion rates have been criticised as being unphysiological, segmental perfusion at rates greater than 7ml/minute is very unlikely to allow the establishment of physiological conditions in the intestine (Soergel, 1971). The intestine is distended at fast flow rates and wide variations in flow velocity are likely to follow. Increased flow rate through the lumen increases stirring at the mucosal surface and decreases the thickness of unstirred water layers on the mucosa (Lewis and Fordtran, 1975). The flow rate of normal intestinal contents after a meal is not precisely known. Estimates range from 5 ml/minute to 10 ml/minute in the proximal jejunum (Sladen 1968). The liquid flow rate entering the jejunum after the ingestion of 50 g of glucose in 250 ml of water has been estimated at 7 ml/minute (Bernier and Lebart 1971). After a solid meal, 750 ml of fluid may enter the proximal jejunum during the two hours following ingestion of the meal (Fordtran and Locklear, 1966). However, Soergel and Herbert (1970) found only a slight increase in flow rate over fasting values in the mid-part of the jejunum following a meal. The influence of flow rate in the jejunum on intestinal absorption raises a number of other important factors which will be discussed later. It may thus be considered that physiological intestinal flow rate is about 5 ml/minute, and with perfusion rates faster than this, the intestinal lumen may be distended and that fluctuations in flow rate may occur without any change in flow velocity. Many of the theoretical objections to fast infusion rates in small

intestinal studies are valid if one is attempting to use the technique to accurately measure intestinal absorption values. However, the technique is limited in this respect in any case, as a true steady-state system is unlikely ever to be achieved, and certainly not in a truly physiological way using the small intestinal perfusion technique. Knowing the limitations of the technique, however, and using it within its limitations, the system is acceptable and the perfusion flow rate, although in many instances unphysiological, may be acceptable in most studies.

AIM OF STUDY

A relatively fast flow rate, certainly greater than 5 ml/minute, is necessary to provide adequate collections during studies. The aim of this investigation was to determine the effect of variation of the perfusion flow rate on the absorption of water and electrolytes using the small intestinal perfusion technique, with the object of determining if fast perfusion flow rates are likely to give grossly inaccurate results.

SUBJECTS AND METHODS.

Subjects

15 normal volunteer subjects underwent the investigation, none of whom had any significant gastro-intestinal symptomatology.

Composition of perfusing solutions

The perfusing solutions all consisted of solutions of glucose and saline, each containing a glucose concentration of 56 mM. Care was taken that each of the solutions used was iso-osmotic (300 m.osmol/l), Phenolsulphonphthalein (PSP) in a concentration of 5 g/litre, and 51Cr EDTA were used as water soluble, non-absorbable markers.

Technique, experimental procedure and analytical methods

A triple lumen tube perfusion system was used (as shown in Figure 4.4), each tube being constructed from polyvinyl single lumen tubing, of which one was radio-opaque.

The subjects were fasted overnight and the perfusion tube passed the following morning by mouth. The tube position was checked radiologically and perfusion started when the infusion point was sited immediately distal to the duodeno-jejunal junction. The perfusate was passed through a water bath at a temperature of 37°C. Four infusion rates were used in the study, 10 ml/minute, 15 ml/minute, 20 ml/minute and 25 ml/minute. In each subject, two or three of these different perfusion flow rates were utilized in rotation. Infusion and collection was performed using two Watson Marlow constant flow-inducer pumps, one of which was fitted with a ten-channel Delta pump attachment.

An equilibration period of 45-60 minutes was allowed in each study after infusion was started, so that a steady-state could be established. After establishment of the steady-state at each individual flow rate used, two or three ten-minute collections of fluid were made. A further equilibration period and a different flow rate was then established and a new series of collections made. In each subject, two or three different flow rates were studied in this way.

The measurement of PSP, ^{51}Cr EDTA, and electrolytes were as previously described and absorption of water and electrolytes calculated using the formula for the triple-lumen tube.

RESULTS

The results of the various flow rates on mean water absorption are shown in Table 7.1. At 10 ml/minute the mean water absorption ($\pm$ SEM) was 216 $\pm$ 37 ml/minute; at 15 ml/minute; water absorption was

TABLE 7.1

MEAN (± S.E.M.) WATER ABSORPTION AT DIFFERENT PERFUSION FLOW RATES

Flow Rate (ml/min)	No. of Studies	Mean Water Absorption (ml/hr/30 cm. segment).
10	10	216 ± 37
15	7	240 ± 51
20	10	221 ± 18
25	8	180 ± 27

240 ± 51 ml/minute; at 20 ml/minute, the mean water absorption was 221 ± 18 ml/minute, and at 25 ml/minute, mean water absorption was 180 ± 27 ml/minute. There was no statistically significant difference between the means of water absorption at any of the flow rates studied using Student's t-test.

The effect of the flow rates studied on mean sodium and chloride absorption are shown in Table 7.2. Again, there was no significant alteration in sodium and chloride absorption between the different flow rates studied. For water, sodium and chloride absorption however, there was possibly a trend towards some reduction in absorption of water and the electrolytes at the fastest flow rates studied (25 ml/minute). However, this trend did not reach statistical significance.

DISCUSSION

The result of this investigation indicates that altering perfusion flow rate from 10 ml/minute up to 25 ml/minute in the triple-lumen tube perfusion system, resulted in no significant alteration of absorption of water, sodium and chloride. There was possibly the beginning of a trend towards reduction in absorption of water and electrolytes at the fastest flow rates studied, but no statistical significance was obtained.

The results of this study tend to confirm those of Modigliani and Bernier (1971), who found a trend towards less water and sodium being absorbed when the infusion rate was increased. These authors found statistically significant reduction in sodium absorption between 10 and 15 ml/minute and between 10 and 20 ml/minute. Sladen and Dawson (1969b) failed to show any significant effect of the flow rate (varying from 11 ml/minute to 28 ml/minute) on absorption of water and sodium in man.

The effect of flow rate on the absorption of glucose has also

TABLE 7.2

MEAN (± S.E.M.) SODIUM AND CHLORIDE ABSORPTION AT DIFFERENT PERFUSION FLOW RATES

Flow Rate (ml/min)	No. of Studies	Mean Sodium Absorption (m.mol/hr/30cm. segment)	Mean Chloride Absorption (m.mol/hr/30cm. segment)
10	10	22.9 ± 2.4	19.6 ± 2.3
15	7	19.4 ± 2.4	21.4 ± 1.7
20	10	21.3 ± 1.9	20.2 ± 1.4
25	8	17.4 ± 2.2	18.4 ± 1.9

been studied (Sladen and Dawson, 1969b; Modigliani and Bernier, 1971). These authors found that increasing flow rates increased the absorption rate of glucose.

The influence of flow rate on intestinal absorption is an important problem raising a number of theoretical factors. It has been demonstrated that at flow rates between 3 and 7 ml/minute, intestinal volume increases proportionately and the average transit time varies little (Dillard, Eastman and Fordtran, 1965). At faster flow rates the increased volume in the intestine is dealt with by a proportional decrease in transit time; thus the contact time of the perfused solution with the intestinal mucosa may be reduced with large flows. It seems probable that high rates of perfusion may induce the development of laminar flows in the intestine (Ardaillou, Sreer and Richet, 1970). This may allow the central liquid column to escape the influence of variations in peripheral concentrations. The effects of intestinal motor function on the mucosal contact time in intestinal perfusion are replaced by the flow of velocity artificially created by the rate of the perfusion pump (Soergel, 1971). Thus the rate of perfusion and the concentration of the perfused solutions appear to determine the load offered to the intestinal segment under study. The effect of very fast perfusion rates on intestinal absorption has been studied recently by Lewis and Fordtran (1975). These authors found that the effective mucosal surface area through which absorption takes place is increased by about 40% as perfusion rate is increased from 1 ml/min. to 100 ml/min. Increasing perfusion rate also reduces the thickness of the unstirred water layer on the surface of the mucosal cells, thus leading to an increase of solute absorption. Fast perfusion rates do not however, increase intraluminal pressure to the level at which mucosal

blood flow is reduced.

The results of the present investigation indicate that the actual rate of perfusion may not matter too much when the intestinal perfusion technique is used within its limitations, and preferably when an individual patient is used as control in a perfusion study. The infusion rate should be within the 10 - 25 ml/minute range and preferably 15 - 20 ml/minute. The criteria for the selection of the perfusion rate depends also upon the length of gut perfused and the concentration of the perfusate. It is vitally important that the infusion rate remain constant throughout any individual perfusion. For the purposes of the further studies to be described, a constant infusion rate of 20 ml/minute has been selected on the basis of the results of the study. This infusion rate gives satisfactory absorption figures for water, sodium and chloride absorption and allows adequate fluid collection during the ten minute collection periods.

SUMMARY

The effect of different infusion rates on the absorption of water, sodium and chloride has been studied. Infusion rates of 10 ml/minute, 15 ml/minute, 20 ml/minute, 25 ml/minute were used and no significant difference was found in the absorption of water, sodium and chloride at each of these perfusion flow rates. There was a trend towards a reduction of absorption of water and electrolytes at 25 ml/minute, although these figures were not statistically significantly different from the absorption figures obtained at lower flow rates.

On the basis of these results an infusion rate of 20 ml/minute has been selected as a standard perfusion flow rate for the small intestinal perfusion studies to be subsequently described. Although to some extent unphysiological, flow rates above 5 ml/minute are acceptable within the context of the limitations of the small intestinal perfusion technique.

CHAPTER 8

COMPARISON OF DOUBLE - LUMEN AND TRIPLE - LUMEN PERFUSION TUBE SYSTEMS AND THE EFFECT OF A PROXIMAL OCCLUDING BALLOON ON WATER AND ELECTROLYTE ABSORPTION

INTRODUCTION

Early small intestinal perfusion techniques made use of the simple double-lumen tube (Schedl and Clifton, 1961). A theoretical disadvantage of this technique is that it makes no allowance for endogenous gut secretions which may contaminate the study segment. Attempts were initially made to eliminate these secretions by applying suction proximal to the infusion site (Fordtran, Levitan, Bikerman et al, 1961), although this method proved to be unsuccessful. Reflux of the perfused solution proximal to the infusion point may also lead to inaccuracy in the double-lumen tube system. Aspirations performed up to 15 cm proximal to the point of perfusion in one study were found to contain significant quantities of the test solution (Fordtran, 1969). The existence of such reflux suggests that an overestimation of absorption may occur in relation to length as a segment of intestine greater than the length under study will in fact be participating in the absorption. This additional segmental length probably varies from time to time within the same subject, and from one subject to another (Modigliani, Rambaud and Bernier, 1973).

The inherent theoretical disadvantages of the double-lumen tube system may be overcome in two ways. Firstly, a triple-lumen tube system may be used. This system incorporates a mixing segment proximal to the test segment. This segment thus lies between the infusion point and the proximal collection point. This upper segment allows mixing of the infused test material with endogenous secretions. If any reflux exists, it will occur proximal to the mixing segment, and thus will not interfere with the results obtained. These are calculated from the differences in marker concentrations between the proximal and distal collection points. This system has now been used successfully by a number of authors (Cooper et al, 1966; Whalen, et al, 1966). A second method of avoiding the

theoretical problems raised by the simple double-lumen tube system is the use of a proximal occluding balloon situated immediately above the point of infusion (Phillips and Summerskill, 1966). A potential disadvantage of this system may be an alteration of segmental absorption rates by interference with intestinal motility or mucosal blood flow by the balloon when inflated, although there is little evidence for this objection.

There has been much discussion and debate as to the accuracy of these different techniques and some studies have been performed in an attempt to compare these methods (Sladen and Dawson, 1968a; Sladen and Dawson, 1970; Modigliani and Bernier, 1971). Some of these studies, however, show conflicting results, and there is still no general agreement as to the most reliable technique which should be used.

AIMS OF STUDY

The aim of this investigation has been to compare the absorption of water from the jejunum in man using a triple-lumen tube perfusion system with that obtained using a double-lumen tube system in the same patient, and also to study the effect of a proximal occluding balloon on water absorption.

SUBJECTS AND METHODS

Subjects

Fifteen normal volunteer subjects participated in the investigation.

Composition of perfusion solutions

The perfusion solutions consisted of glucose-saline with a glucose concentration of 56 mM. The osmolality of this solution was checked in each case, and care taken that the solution was iso-osmotic (300 m.osmol/l). Phenolsulphonphthalein (PSP) in a concentration of 5 g/l and ^{51}Cr EDTA were used as water-soluble, non-absorbable markers.

Technique, experimental procedure and analytical methods

A triple-lumen tube perfusion system was used in this study. The tube incorporated a mixing segment 15 cm long and the test segment 30 cm long, and an occluding balloon situated immediately proximal to the infusion point. This could be easily and rapidly inflated and deflated. A sump tube was sited proximal to the balloon and secretions collecting there were continuously removed.

The subjects fasted overnight and the perfusion tube passed by mouth on the following morning. The tube position was checked radiologically and perfusion started when the infusion point was positioned distal to the duodeno-jejunal flexure. The perfusate was passed through a water bath at a constant temperature of 37°C and introduced into the jejunum at a constant flow rate of 20 ml per minute. Samples were collected at a rate of 2 ml/minute. Infusion and collection was performed using two Watson Marlow constant flow-inducer pumps, one of which was fitted with a ten channel Delta pump attachment. After the start of the perfusion an equilibration period of 45-60 minutes was allowed in each study so that a steady-state could be established. After the establishment of the steady-state 10 minute collections were made for four or five periods. The balloon was then inflated with air and after re-equilibration, three or four further collections were made.

The absorption of water and sodium was calculated by the change in concentration of each of the two markers. The results were worked out using both PSP and 51Cr EDTA as water-soluble non-absorbable markers.

For each subject the water and sodium absorption was calculated using the formula for a triple-lumen tube system (Table 4.2). For each subject, also, water absorption was calculated in the same patient using the formula for the double-lumen tube system based on the difference in

marker concentration between the infusion point and the proximal collection point (15 cm) and furthermore, between the marker concentration difference between the infusion point and the distal collection point, (45 cm). The results were also calculated in each patient before and after balloon inflation.

RESULTS

The results of the study are shown in Table 8.1. The mean water absorption (± S.E.M.) calculated by the triple-lumen tube system was 232 ± 25 ml/hour. The water absorption calculated using the double-lumen system was 405 ± 92 ml/hour (15 cm segment) and 322 ± 68 ml/hour (45 cm segment). The mean water absorption calculated by the triple-lumen tube system was statistically significantly lower than that calculated from the double-lumen system at both segment lengths ($p < 0.05$). There is no statistically significant difference between the water absorption calculated in the double-lumen system using the 15 cm. segment from that of the 45 cm segment, although wide variation of results was obtained (particularly with the 15 cm segment).

Similar results were obtained when sodium absorption was studied. The mean sodium absorption using the triple-lumen system was 21.6 ± 1.4 m.mol/hr and for the double-lumen tube system was 32.0 ± 5.8 m.mol/hr (15 cm segment) and 27.3 ± 3.1 m.mol/hr (45 cm segment). Again the figure for mean sodium absorption using the triple-lumen tube system was statistically significantly lower than with either result calculated using the short or long segment of the double-lumen tube system ($p < 0.05$). No significant difference in absorption was found between periods of balloon inflation and deflation.

TABLE 8.1

MEAN WATER AND SODIUM ABSORPTION (± S.E.M.) IN 15 SUBJECTS CALCULATED USING TRIPLE - LUMEN AND DOUBLE - LUMEN TUBE SYSTEMS.

	Triple - lumen (A)	Double - lumen	
		15 cm Segment (B)	45 cm Segment (C)
Mean Water absorption (ml/hr/segment)	232 ± 25	405 ± 92	322 ± 68
Mean Sodium absorption (m.mol/hr/segment)	21.6 ± 1.4	32.0 ± 5.8	27.3 ± 3.1

DISCUSSION

The results of this study indicate that the mean absorption of water and sodium calculated by the triple-lumen system is statistically significantly lower than that calculated in the same patient using a double-lumen calculation over 15 cm and 45 cm segments. No difference in absorption was found to occur with inflation of the proximal occluding balloon. There is no way of knowing in a study of this nature which is the most accurate figure. However, the large standard error found in the calculations using both double-lumen segments (15 cm and 45 cm) indicates a wide variation in the absorption figures obtained in these two groups. This variation suggests a greater likelihood of error using the double-lumen system. The figures which were obtained using the triple-lumen tube system are of the same order as absorption figures are reported in equivalent studies using a similar system. This suggests that the triple-lumen tube technique may be more reliable in giving consistent results than the double-lumen tube system.

As previously mentioned the double-lumen tube system has two principal draw-backs. These are reflux of the perfused solution proximal to the infusion point, and contamination by proximal endogenous secretions. Fordtran (1969) using a triple-lumen tube and a calculation using triple-lumen and double-lumen methods, compared the movements of tritiated water and 14C urea and found that absorption was considerably over-estimated by the double-lumen method. The test segment is increased by a variable unknown length by this reflux. Contamination of the perfused solution by proximal endogenous secretions has also been demonstrated. Whalen and his colleagues (1966) estimated that the flow-rate of fasting intestinal content into the test segment was 2.16 $\pm$ 132 ml/minute in the jejunum and

similar results have been obtained in other studies (Soergel and Herbert, 1970). The likely result of such contamination is underestimation of water and electrolyte absorption. Thus, using the double-lumen tube system, reflux may overestimate intestinal absorption and, contamination by endogenous secretion may result in underestimation of absorption. These two potential errors may cancel themselves out in some instances, but their combined effects must be very variable. The variability of results which were obtained in this study using the double-lumen tube system tends to support this. On the other hand, Sladen and Dawson (1968a) were unable to determine any significant difference between water and electrolyte absorption calculated using both double and triple-lumen tube systems.

Theoretically a double-lumen tube with proximal inflatable balloon (Phillips and Summerskill, 1966) may prevent reflux of infused solution and limit contamination of the test segment by endogenous secretions. This tube has been used successfully now in a number of studies but it has some potential disadvantages. In some patients adequate luminal occlusion may not be possible, perhaps due to flaccidity of the intestinal wall (Sladen, 1970). The balloon, when inflated, may alter segmental absorption rates by affecting intestinal motility or mucosal blood flow. This may not cause significant difference in intestinal absorption in practice, and no significant change was observed in this study, although Modigliani and Bernier (1971) found water absorption to be significantly higher with the balloon deflated than inflated; this may be because of reflux of infused solution proximal to the point of infusion and may be unrelated in practice to the balloon itself. A third potential disadvantage of this system is the possibility of damage to the jejunum by the inflated

balloon. This may possibly occur in a diseased jejunum (Schmid, Phillips and Summerskill, 1968), although it is unlikely to occur in the healthy small intestine.

There is no great difficulty intubating patients with a triple-lumen tube than with a double-lumen tube. There are, however, some potential disadvantages of the triple-lumen tube system. The equilibration period must be of long duration to improve the accuracy of the method. Proximal sampling should be carried out at a constant rate so that the rate of entry remains stable at the proximal end of the test segment. The major potential disadvantage of the triple-lumen tube system, however, is that solute and water may be absorbed by the mixing segment so that the flow rate and composition of fluid entering the study segment may be variable and unreliable. Perfusion flow rates of the solution entering the test segment may be very different from the initial perfusion flow rate at infusion and requires accurate and steady collections at both proximal and distal collection points to ensure reliable figures to calculate absorption rates.

From this and other studies it would appear that the triple-lumen tube system is preferable to the double-lumen system in small intestinal perfusion studies. The double-lumen technique will probably only be used in conjunction with a proximal occluding balloon. The balloon itself does not significantly alter intestinal absorption.

SUMMARY

A comparison of the intestinal absorption of water and sodium has been performed in the same patients on the same occasion, using a triple-lumen tube in which the absorption rates of water and sodium were calculated using both triple-lumen and double-lumen information. A proximal

occluding balloon was also incorporated into the system. The double-lumen results were obtained over 15 cm and 45 cm segments. The results demonstrate that the absorption figures using the triple-lumen tube system appear to be less variable and more akin to figures obtained in other studies. The absorption figures using the double-lumen tube system are very variable and are statistically significantly higher than those obtained using the triple-lumen system. The balloon did not significantly affect intestinal absorption.

It would appear from this investigation and from the study of the literature and other investigations along the same line that intestinal perfusion studies should basically use a triple-lumen tube system for greater accuracy. A double-lumen tube system should only be used in conjunction with a proximal occluding balloon.

CHAPTER 9

CONCLUSIONS

CONCLUSIONS

The principles of the technique of small intestinal perfusion are described and specific problems of the technique are considered. A number of these problems have been studied in detail with the aim of defining standard conditions for the use and subsequent application of the technique.

The problems considered include an assessment of new water-soluble, non-absorbable reference substances, a consideration of the composition of the perfusate with the specific purpose of studying the relationship of glucose to sodium and water absorption, an investigation into the effect of different perfusion flow rates on water and electrolyte absorption with the aim of defining a satisfactory flow rate for routine use, and the study of the tube techniques used themselves, with particular reference to the use of double-lumen and triple-lumen tubes, and the use of tubes with proximal occluding balloons.

The search for an easily measured radio-isotopic non-absorbable water soluble reference substance led to a study assessing $^{51}CrCl_3$ and ^{51}Cr EDTA as markers suitable for small intestinal perfusion studies. The jejunal perfusion system itself was used to assess these markers and comparison made with PSP in each case. The results indicate that figures for water absorption obtained using ^{51}Cr EDTA correlate well with those using PSP, and a poor correlation was found between water absorption calculated from $^{51}CrCl_3$ compared with that calculated using PSP. ^{51}Cr EDTA thus appears to be a suitable non-absorbable, water-soluble marker for use in jejunal perfusion studies. This isotopic marker has been used in most of the studies reported subsequently but usually in conjunction with a chemical marker, usually PSP.

The composition of the perfusate for small intestinal perfusion studies is of immense importance. Many different types of perfusates have been used in the past, and in certain circumstances there may be specific merit in using particular compositions of perfusates. The relationship of glucose to sodium and water absorption was studied in the perfusion system with the aim of determining the importance of glucose in the absorption of sodium and water, and determining the concentration of glucose which allows optimal absorption of sodium and water. Little or no absorption of sodium or water was observed with isotonic saline and an increasing absorption found up to an optimal, which occurred at a glucose concentration of 56 mM. This glucose concentration has thus been taken as the basic glucose concentration suitable for the perfusate in subsequent studies. In most of the studies reported in this work, the basic perfusate has consisted of glucose-saline containing glucose in concentration of 56mM, although modifications and additions have been made if required. There is some merit in using a perfusate which has as simple a composition as is necessary for the purposes of the study being performed. In all the studies reported, however, care was taken to ensure that the perfusing solution was iso-osmotic (300 m.osmol/l). Other problems studied have been the selection of the perfusion flow rate. The purpose of the study was to investigate the effect of different infusion rates on the absorption of water and electrolytes from the jejunum. The infusion rates studied were 10 ml/minute, 15 ml/minute, 20 ml/minute and 25 ml/minute. No significant difference of absorption of water and electrolytes was noted from the jejunum at each of these flow rates, although there was a trend towards reduction of absorption at the highest flow rate studied, 25 ml/minute. On the basis of these results an infusion

rate of 20 ml/minute was selected as a standard perfusion flow rate for the small intestinal perfusion studies subsequently described.

Finally, the design of the intestinal perfusion tube system itself was investigated. A triple-lumen tube perfusion system was designed, in which the absorption of water and electrolytes from the jejunum could be calculated using the triple-lumen system, and also from a double-lumen system within the same tube. The effect of a proximal occluding balloon on intestinal absorption was also studied. The results demonstrated that the absorption figures using the triple-lumen tube system appeared less variable than those using the double-lumen technique. The double-lumen system gave results which were significantly higher than those obtained using the triple-lumen system. The variability of the double-lumen tube figures suggested that more reliable results were likely to be obtained using a triple lumen tube perfusion system. There was also found to be no statistically significant difference in water and electrolyte absorption from the upper small intestine when the balloon was inflated compared to when it was deflated. Thus, balloon inflation does not by itself interfere with absorption measured by the perfusion technique.

On the basis of these preliminary investigations, the following technique and experimental procedure was adopted for use in small intestinal segmental perfusion studies. In most instances a triple-lumen tube system was used, although a double-lumen tube with proximal occluding balloon is acceptable and was used in the study of the effect on frusemide on small intestinal absorption. Balloons were also necessary in the bile acid study to prevent contamination of the test segment with endogenous bile acids. The perfusion tubes were constructed from lengths of polyvinyl single-lumen tubing, of which one was radio-opaque. Each tube had a

diameter of 1.0 mm, and was supplied by Portex Ltd., Hythe, Kent (R/Z SH85 and NT3 SH80). A radio-opaque marker system was devised and incorporated, so that the infusion point could be accurately localised radiologically by an image intensifier. The subjects and patients being perfused fasted overnight and in the morning the perfusion tube was passed by mouth. The tube position was checked radiologically and perfusion started when the perfusion point was sited immediately distal to the duodeno-jejunal flexure. The perfusate was passed in all cases through a water bath at a constant temperature of 37°C, and introduced into the jejunum at a constant flow rate of 20 ml/minute. Samples were collected at a rate of approximately 2 ml/minute. Infusion and collection was performed using two Watson Marlow constant flow-inducer pumps, one of which was fitted with a ten-channel Delta pump attachment. As the establishment of the steady-state is vital to accuracy in perfusion studies, an equilibration period of 45-60 minutes was allowed in each study after infusion was started. When PSP was used as marker the samples were analysed by spectrophotometry at 580 nm in alkaline solution, and when isotopic markers were used, ^{51}Cr radio-activity was counted with an automatic gamma counting system using the pulse-height analyser set to cover the energy range 300-360keV. Sodium concentration was measured in the perfusates and in collections by emmission flame photometry using the IL 143 digital flame photometer. Chloride concentration was estimated by the colorimetric method of Technicon, modified by Skeggs (Zall, Fisher, and Garner, 1956).

Basically the same perfusion technique was used in all the studies described, although minor modifications were made from time to time in particular studies and these will be described in the relevant sections.

SECTION III

FACTORS INFLUENCING WATER AND ELECTROLYTE ABSORPTION IN THE SMALL INTESTINE

INTRODUCTION

Having established a satisfactory practical perfusion technique for the measurement of water and electrolyte absorption from the jejunum in man, areas were considered whereby the particular assets of this technique could be applied to advantage. As previously mentioned, the small intestinal perfusion system is at its most effective when applied to the study of the influence of some factor on the intestinal absorption of water and electrolytes. The subject being perfused thereby acts as his own control. More accurate information is thus obtained in this way rather than by comparing intestinal absorption measurements in groups of patients, perhaps with different disease states. The limitations of the technique and the wide variations in intestinal absorption within patients in health and disease, makes this the most satisfactory application of the system.

As described in Chapter 1, it is now generally recognised that the small intestine, in addition to its absorptive function, is able to secrete large amounts of water and electrolytes. In certain pathological condition, secretion of electrolytes and water is highly relevant to the production of diarrhoea (Hendrix and Bayless, 1970; Field, 1974).

The clinical importance of intestinal secretion becomes apparent when investigators working on cholera started to determine some of the fundamental aspects of the pathophysiology of that condition. The Vibrio cholerae organism produces a protein exotoxin which causes the small intestinal epithelium to secrete water and electrolytes (Greenough, Carpenter, Bayless, 1970; Finkelstein, 1973). This secretion occurs without either light or electron-microscopic evidence of epithelial cell damage (Elliot, Carpenter, Sack et al, 1970) and the disease is

accompanied by a reduction in the bi-directional sodium movement across the mucosa (Love, Phillips, Rohde et al, 1972). The secretory changes are considered to be due to changes in adenyl cyclase activity. (Kimberg, Field, Johnson et al, 1971; Guerrant, Chen and Sharp, 1972). Cholera toxin appears to increase the synthesis of cyclic AMP in the intestinal mucosa, and the cyclic AMP then stimulates secretion or unmasks pre-existing secretion by inhibiting an active absorptive process.

These findings are of clinical importance as the oral administration of glucose and glycine may reduce the rate of water secretion (Nalin, Cash, Rahman, et al, 1970). Other bacterial toxins may have a similar effect on water and sodium movement in the small intestine. This has been demonstrated with Staphylococcus aureus (Sullivan and Asano, 1971), Clostridium perfringems (Strong, Duncan, Perna, 1971) and Escherichia coli (Gorbach, Banwell, Chatterjee, et al, 1971). It is possible that other organisms may produce toxins with similar activities.

A greater understanding of absorption and secretion mechanisms in the upper small intestine has led to the search for other factors which may interfere with the water and electrolyte absorption process, thus leading to the production of diarrhoea and other symptomatology. A greater understanding of the pathophysiology of the various types of diarrhoea can only be of benefit to the patient, as new and more satisfactory methods of treating diarrhoea may follow studies of their pathophysiology. Chemical stimulants, abnormal physical conditions such as mechanical bowel obstruction (Shields, 1964), low pH (Hochman, Kottmeir, Adamson et al, 1970) and after irradiation (Sullivan, 1968), and various humoral factors have been investigated.

The studies reported in this section describe the use of the small intestinal perfusion technique to investigate the effect of factors such as bile acids, lipids and the diuretic drug Frusemide on the absorption and secretion of water and electrolytes in the upper jejunum, in the hope of shedding more light on the pathophysiology of certain diarrhoeal states in man.

CHAPTER 10

THE EFFECT OF CONJUGATED AND UNCONJUGATED BILE ACIDS ON WATER AND ELECTROLYTE ABSORPTION IN THE JEJUNUM

INTRODUCTION

It has been known for some time that the presence of excessive amounts of bile acids in the colon will cause watery diarrhoea. This may follow breaking of the enterohepatic circulation of bile acids (Hofmann, 1967). It has been demonstrated in dogs and in man that conjugated and unconjugated dihydroxy bile acids inhibit water and electrolyte absorption in the colon when present in high concentration, and in man induce secretion of water and electrolytes (Mekhjian and Phillips, 1970; Mekhjian, Phillips and Hofmann, 1971). Forth, Rummell and Glasner (1966) found that unconjugated bile acids inhibit the absorption of water in the rat jejunum. Permeability changes in the isolated rat intestine induced by bile acids have also been reported by Feldman and Gibaldi (1969). Dihydroxy bile acids inhibit water absorption and may cause secretion into the jejunum of hamsters (Teem and Phillips, 1972).

The human jejunum is normally exposed to high concentrations of conjugated bile acids, and in conditions in which bacterial overgrowth occurs, unconjugated bile acids may predominate, (Tabaqchali, Hatzioannou and Booth, 1968).

AIM OF THE STUDY

The aim of this investigation was to study the effect of conjugated and unconjugated bile acids on the absorption of water and electrolytes in the normal human jejunum.

SUBJECTS AND METHODS

Subjects

Jejunal perfusion using solutions containing bile acids was performed on 35 normal healthy subjects. All subjects volunteered for the study after full explanation of its nature.

Composition of perfusing solutions

The perfusing solutions consisted of solutions of glucose and saline or glucose, saline and bile acids, all of which were iso-osmotic (300 mosmol/l). Glucose was present in a concentration of 56 mM. The basic solution in each study consisted of an iso-osmotic glucose-saline mixture, and adjustments were made to produce test solutions containing bile acids. The osmolality of all bile acid-containing solutions was checked by the method of freezing-point depression. Solutions were discarded if they were found not to be iso-osmotic.

The bile acids used in the perfusing solutions consisted of the conjugated and unconjugated forms of the trihydroxy bile acid cholic acid, and the dihydroxy bile acids deoxycholic and chenodeoxycholic acid. The bile acids were of high purity (confirmed by chromatography) and were obtained from Steraloids Ltd, London and Sigma Chemical Co. Ltd., London. The concentration of bile acids present in the perfusing solutions were as follows: 3 mM, 5 mM, 7 mM and 10 mM. The pH of the solutions was approximately 8, and did not change significantly during the perfusion as judged by the pH values of the fluid collected in the studies.

Phenolsulphonphthalein (PSP) in a concentration of 5 g/l and ethylendiamine tetra-acetate (EDTA) labelled with ^{51}Cr were used as water-soluble non-absorbable markers.

Technique, experimental procedure and analytical methods

A five-lumen tube, incorporating a triple-lumen segmental perfusion system, as described in Chapter 4, was used in the study. An occluding balloon was attached to the tube immediately proximal to the infusion point to prevent endogenous secretions and bile from contaminating the test segment. The experimental procedure and analytical methods used

were as previously described. (Chapter 9). The results were calculated using the formula for a triple-lumen tube perfusion system.

Statistical methods

The mean absorption of water and electrolytes from glucose-saline and bile acid solutions were compared in each case by means of Student's paired t-test, each subject in each perfusion study acting as his own control.

RESULTS

The effect of cholic acid in concentrations of 3mM, 5mM, 7 mM and 10 mM on the absorption of water is shown in Table 10.1. From these results it is seen that unconjugated cholic acid, even at a concentration of 10 mM had no apparent effect on net water movement in the small intestine ($p > 0.05$). Glycocholic acid and taurocholic acid, also at high concentrations, did not significantly influence net water movement in the small intestine ($p > 0.05$). The effect of cholic acid (5 mM and 10 mM) on water absorption is shown in more detail in figure 10.1.

Unconjugated deoxycholic acid in all the concentrations tested caused statistically significant inhibition of water absorption (Table 10.2). Net secretion of water and electrolytes occurred with deoxycholic acid in concentrations of 5 mM, 7 mM and 10 mM. Higher concentrations of deoxycholic acid appeared to exert the greatest effect. The effect of deoxycholic acid (5 mM and 10 mM) on water absorption is shown in more detail in Figure 10.2. Taurodeoxycholic acid had a similar effect on water absorption from the jejunum. Net secretion occurred in one patient infused with 3 mM taurodeoxycholic acid, and a statistically significant decrease in water absorption occurred in all patients in this group. Net secretion occurred in all patients perfused with taurodeoxycholic acid in concentrations of 5 mM, 7 mM and 10 mM. Similar results were

TABLE 10.1

MEAN WATER ABSORPTION (± S.E.M.) FROM CONTROL SOLUTIONS AND SOLUTIONS CONTAINING TRIHYDROXY BILE ACIDS

Bile acid	Concentration (mM)	No. of Studies	Water absorption (ml/h per 30 cm segment)	
			Glucose–saline	Bile acid
Cholic acid	3	8	209 ± 22	194 ± 19
	5	8	182 ± 32	156 ± 33
	7	6	174 ± 14	163 ± 15
	10	4	146 ± 18	152 ± 26
Glycocholic acid	3	-	-	-
	5	4	193 ± 15	183 ± 9
	7	4	224 ± 67	235 ± 53
	10	4	148 ± 21	137 ± 11
Taurocholic acid	3	8	190 ± 20	192 ± 18
	5	7	209 ± 34	207 ± 34
	7	4	230 ± 23	200 ± 20
	10	4	223 ± 54	203 ± 49

FIGURE 10.1

EFFECT OF DEOXYCHOLIC ACID (5 mM AND 10 mM)

ON WATER ABSORPTION

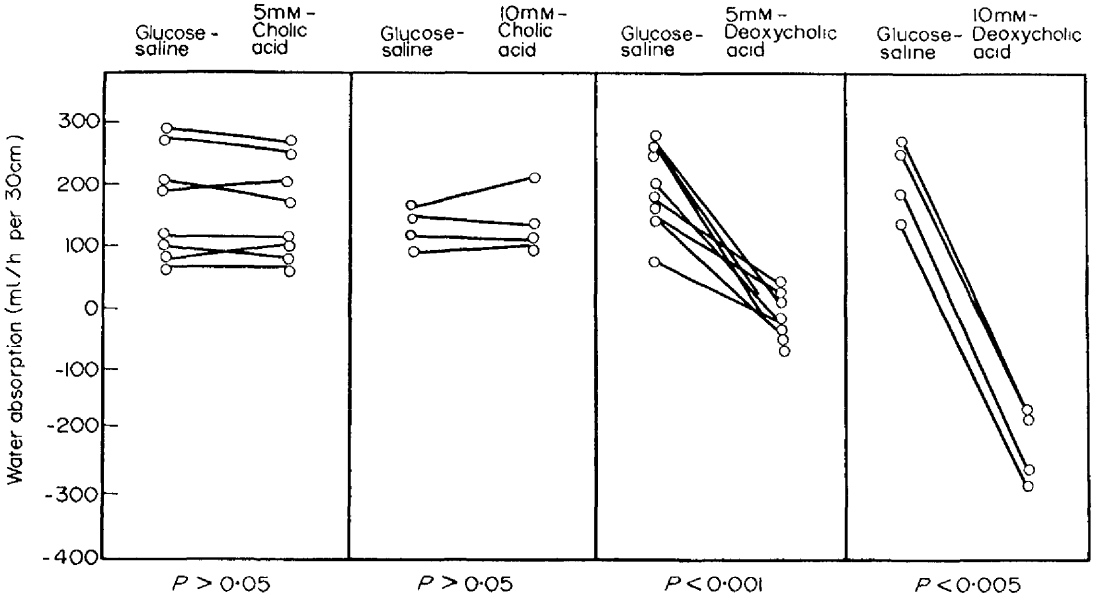


TABLE 10.2

MEAN WATER ABSORPTION (± S.E.M.) FROM CONTROL SOLUTIONS AND SOLUTIONS CONTAINING DEOXYCHOLIC ACID OR ITS GLYCINE AND TAURINE CONJUGATES

Bile acid	Concentration (mM)	No. of Studies	Water absorption (ml/h per 30 cm segment)		Significance of difference
			Glucose–saline	Bile acid	
Deoxycholic acid	3	4	151 ± 20	28 ± 12	$p < 0.02$
	5	8	207 ± 24	−0.8 ± 10	$p < 0.001$
	7	8	221 ± 24	− 99 ± 23	$p < 0.001$
	10	4	227 ± 32	−220 ± 31	$p < 0.005$
Glycodeoxycholic acid	3	4	162 ± 18	34 ± 10	$p < 0.02$
	5	6	176 ± 27	2 ± 12	$p < 0.001$
	7	4	189 ± 30	− 62 ± 18	$p < 0.001$
	10	4	201 ± 28	−129 ± 39	$p < 0.005$
Taurodeoxycholic acid	3	4	231 ± 25	32 ± 20	$p < 0.02$
	5	4	223 ± 17	− 96 ± 19	$p < 0.005$
	7	4	283 ± 46	−158 ± 28	$p < 0.001$
	10	4	259 ± 57	−148 ± 62	$p < 0.025$

obtained on perfusing glycodeoxycholic acid in concentrations of 5 mM and 7 mM in four subjects.

Statistically significant inhibition of absorption of water, and net secretion in all concentrations was found on perfusing unconjugated chenodeoxycholic acid into the jejunum (Table 10.3). Inhibition of absorption occurred with 3 mM solutions of taurochenodeoxycholic acid and net secretion with 10 mM solution.

The effect of the conjugated and unconjugated trihydroxy bile acid, cholic acid on net electrolyte movement in shown in Table 10.4. No statistically significant inhibition of sodium and chloride absorption was found at any of the concentrations tested. The conjugated and unconjugated forms of the dihydroxy bile acids deoxycholic acid and chenodeoxycholic acid did cause a statistically significant inhibition of sodium and chloride absorption at all concentrations, and net secretion was also seen (Table 10.5).

In all the studies in which glucose-saline was again perfused at a later stage, the absorption of water and electrolytes returned to normal very rapidly. The results obtained in one such study using 7 mM deoxycholic acid are shown in Figure 10.2.

DISCUSSION

The results of the present study indicate that the dihydroxy bile acids deoxycholic acid and chenodeoxycholic acid inhibit water, sodium and chloride absorption in the proximal jejunum. They also cause net secretion of water and electrolytes, especially when present in high concentration. These changes occur whether or not the dihydroxy bile acids are present in the free or conjugated forms. In the concentrations studied, the trihydroxy bile acid cholic acid, whether free or conjugated, seems to have no effect on absorption from the jejunum. The effect of

TABLE 10.3

MEAN WATER ABSORPTION (± S.E.M.) FROM CONTROL SOLUTIONS AND SOLUTIONS CONTAINING CHENODEOXYCHOLIC ACID AND ITS TAURINE CONJUGATE

Bile acid	Concentration (mM)	No. of Studies	Water absorption (ml/h per 30 cm segment)		Significance of difference
			Glucose - saline	Bile acid	
Chenodeoxycholic acid	3	4	243 ± 40	-18 ± 22	$p < 0.002$
	5	8	237 ± 22	-165 ± 19	$p < 0.005$
	7	4	239 ± 43	-172 ± 47	$p < 0.005$
	10	4	248 ± 39	-231 ± 40	$p < 0.001$
Taurocheno-deoxycholic acid	3	3	264 ± 40	58 ± 9	$p < 0.02$
	10	3	332 ± 73	-44 ± 8	$p < 0.002$

TABLE 10.4

MEAN SODIUM AND CHLORIDE ABSORPTION (± S.E.M.)

FROM CONTROL SOLUTIONS AND SOLUTIONS

CONTAINING TRIHYDROXY BILE ACIDS.

Bile acid	Concentration (mM)	No. of Studies	Sodium (mmol/h per 20 cm segment)		Chloride (mmol/h per 30 cm segment)	
			Glucose-saline	Bile acid	Glucose-saline	Bile acid
Cholic acid	3	8	23.8 ± 1.1	23.2 ± 1.2	23.1 ± 1.3	22.1 ± 1.2
	5	8	23.6 ± 1.3	23.4 ± 1.4	23.2 ± 1.5	22.6 ± 1.5
	7	6	22.2 ± 1.6	21.1 ± 2.0	21.7 ± 1.9	21.1 ± 1.9
	10	4	22.9 ± 2.2	22.8 ± 3.0	21.5 ± 1.9	21.4 ± 2.6
Glycocholic acid	3	-	-	-	-	-
	5	4	21.7 ± 1.1	22.3 ± 0.9	21.6 ± 1.0	21.5 - 1.4
	7	4	24.3 ± 2.1	24.2 ± 1.8	24.0 ± 1.8	23.0 ± 2.1
	10	4	25.5 ± 2.5	23.6 ± 2.3	25.4 ± 2.1	24.4 ± 2.1
Taurocholic acid	3	8	21.6 ± 1.0	21.5 ± 1.3	22.8 ± 0.8	22.0 ± 1.1
	5	7	23.1 ± 1.7	21.6 ± 1.9	22.9 ± 1.7	21.4 ± 1.6
	7	4	21.3 ± 1.2	20.2 ± 1.2	20.8 ± 0.4	19.8 ± 0.7
	10	4	21.2 ± 1.5	20.2 ± 1.4	21.9 ± 1.2	21.5 ± 1.5

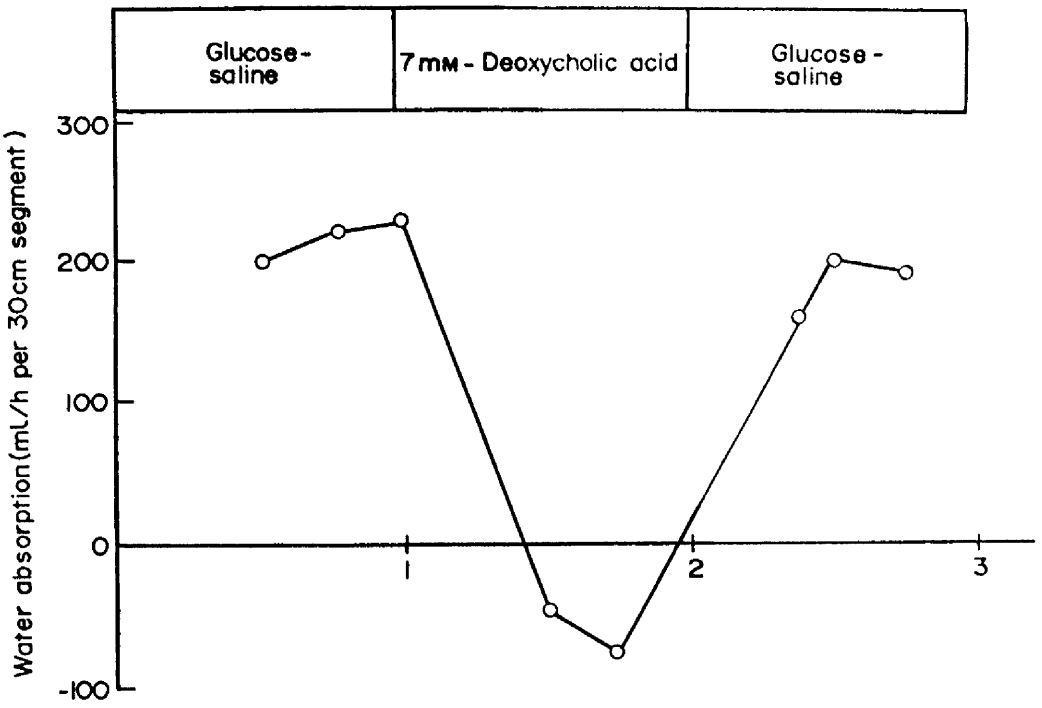
TABLE 10.5

MEAN SODIUM AND CHLORIDE ABSORPTION (± S.E.M.) FROM CONTROL SOLUTIONS AND SOLUTIONS CONTAINING DIHYDROXY BILE ACIDS

Bile acid	Concentration (mM)	No. of Studies	Sodium (mmol/h per 30 cm segment)		Significance of difference	Chloride (mmol/h per 30 cm segment)		Significance of difference
			Glucose-saline	Bile acid		Glucose-saline	Bile acid	
Deoxycholic acid	3	4	22.4 ± 1.0	2.2 ± 0.5	$p<0.02$	21.4 ± 0.4	1.9 ± 0.7	$p<0.02$
	5	8	23.4 ± 1.1	-0.2 ± 1.1	$p<0.001$	21.9 ± 1.3	- 0.3 ± 0.9	$p<0.001$
	7	8	22.2 ± 1.2	-3.2 ± 0.6	$p<0.001$	21.2 ± 1.3	- 2.8 ± 0.4	$p<0.001$
	10	4	26.5 ± 2.3	-11.2 ± 1.4	$p<0.005$	26.4 ± 2.9	-10.9 ± 2.4	$p<0.005$
Taurodeoxycholic acid	3	4	23.3 ± 2.8	2.2 ± 1.4	$p<0.001$	21.8 ± 2.3	1.5 ± 1.1	$p<0.001$
	5	4	25.9 ± 3.0	-6.2 ± 1.1	$p<0.001$	25.3 ± 3.2	- 6.1 ± 1.1	$p<0.001$
	7	4	22.9 ± 0.4	-15.7 ± 2.4	$p<0.005$	22.7 ± 0.5	-13.4 ± 2.2	$p<0.005$
	10	4	25.9 ± 0.9	-13.7 ± 4.1	$p<0.005$	24.6 ± 1.3	-12.2 ± 3.1	$p<0.005$
Chenodeoxycholic acid	3	4	22.3 ± 1.7	- 1.6 ± 0.9	$p<0.001$	21.7 ± 1.8	- 1.4 ± 0.8	$p<0.001$
	5	8	23.5 ± 1.3	-11.9 ± 2.4	$p<0.005$	23.0 ± 1.6	- 8.6 ± 1.8	$p<0.005$
	7	4	23.0 ± 1.0	-15.1 ± 4.1	$p<0.005$	22.5 ± 1.7	-14.4 ± 2.9	$p<0.005$
	10	4	22.3 ± 1.2	-18.9 ± 2.3	$p<0.005$	21.6 ± 1.7	-15.2 ± 2.6	$p<0.005$
Taurochenodeoxycholic acid	3	3	23.5 ± 2.4	4.3 ± 1.3	$p<0.02$	22.0 ± 2.5	3.1 ± 0.8	$p<0.02$
	10	3	23.6 ± 2.3	- 4.9 ± 2.3	$p<0.001$	22.5 ± 2.4	- 5.0 ± 1.8	$p<0.001$

FIGURE 10.2

EFFECT OF DEOXYCHOLIC ACID (7 mM) ON WATER ABSORPTION IN ONE PATIENT, AND RETURN TO NORMAL ON RE-INTRODUCING THE CONTROL SOLUTION



free or conjugated dihydroxy bile acids on small intestinal absorptive function is similar to that demonstrated in the colon by Mekhjian et al (1971). Although generally supporting the observations made on hamsters by Teem and Phillips (1972), these authors found that conjugates of the chenodeoxycholic acid did not inhibit absorption. Harries and Sladen (1972) found that conjugates of dihydroxy bile acids did not significantly inhibit absorption in rats, but the results of Wingate, Phillips and Hofmann agree with those of the present study. These authors also reported that the addition of lecithin to the bile acid solutions abolished the secretory effect of the dihydroxy bile acids. This finding suggests that bile acids may modify fluid movement in the small intestine after meals, when fat absorption has taken place. The addition of lecithin may decrease the critical micellar concentration of dihydroxy bile acids, thus decreasing the concentration of bile acid in molecular form, and probably reducing their activity (Small, 1971).

No information is available from these studies on the mechanism of action of dihydroxy bile acids. Unconjugated dihydroxy bile acids have been shown to cause morphological damage to the jejunal mucosa of hamsters in vivo (Low-Beer, Schneider and Dobbins, 1970; Teem and Phillips, 1972). This has not been shown with conjugated dihydroxy bile acids. Jejunal biopsies performed in four of the subjects at the end of the perfusion in this study were found to show no histological damage, and the rapid reversibility of the absorption inhibition suggests that no prolonged damage has occurred to the jejunal mucosa. There was no information from the present study concerning the absorption of bile acids during perfusion. Unconjugated bile acids are partly absorbed passively in the jejunum and partly actively in the ileum; conjugated bile

acids are absorbed actively, principally in the distal small intestine, and especially in the terminal ileum. (Small and Dietchy, 1968; Krag and Phillips, 1974). As the inhibiting effect of dihydroxy bile acids occurred whether they were in the free or conjugated form, it is unlikely that any absorption of unconjugated bile acids affecting the jejunal mucosa is responsible for the inhibiting effect. In addition there was no reason why deconjugation of the conjugated bile acids may have occurred during the perfusion and on several occasions this was checked by chromatography and no deconjugation was found.

The effect may be mediated by inhibition of sodium-potassium activated ATPase (Na-KATpase) (Katz and Epstein, 1968). Glycine conjugated dihydroxy and trihydroxy bile acids have been shown to influence intestinal ATPase in vitro, but the effects of individual bile acids on ATPase did not correlate well with their effect on water absorption in vivo. (Hepner and Hofmann, 1973). Bile acid anions are surface-active molecules, this being more marked in dihydroxy and trihydroxy bile acids (Dreher, Schulman and Hofmann, 1967). Active sodium absorption may be impaired by inhibition of Na-KATPase activity by dihydroxy bile acid; alteration of Na-KATPase by deoxycholate has been shown in animals (Rötgaard and Möller, 1971). Another possibility is that the dihydroxy bile acids may activate cyclic AMP. To date there is little positive evidence supporting this, although Binder and Rawlins (1973) have demonstrated an activation of adenyl cyclase in colonic mucosa in vitro by dihydroxy bile acids.

Unconjugated dihydroxy bile acids have also been shown to have effects on amino acid transport (Pope, Parkinson and Olsen, 1966), and they affect the re-esterification of fatty acids (Dawson and Isselbacher, 1960; Clark, Lanz and Senior, 1969), and the transport of glucose, Clark et al, 1969).

All these studies, however, were performed on animals.

The significance of the results of this study are considerable. Diarrhoea following states of bacterial overgrowth (as in the blind loop syndrome or small intestinal diverticulosis) may be accompanied by deconjugation of bile acids and followed by dehydroxylation. Thus, the total intraluminal concentration of dihydroxy bile acids may be considerably increased in such conditions. (Tabaqchali, et al, 1968). This may contribute to the diarrhoea experienced by these patients, although some degree of reabsorption of water and electrolytes may occur in the lower small intestine and colon. Other diarrhoeal conditions may also be due to increased concentration of dihydroxy bile acids present in the small intestine. The reported effect of lecithin reducing the inhibition of absorption which occurs with dihydroxy bile acids, does suggest that the correct balance of lipids, bile acids and formation of mixed micelles may be important in modifying fluid movement in the small intestine. Thus fat absorption may play a role in this control of fluid movement. Finally, the technique of small intestinal perfusion with bile acids has been shown to offer a suitable model for analysing solute/solvent flow relationships in differing bulk flow conditions with stable physicochemical parameters (Wingate, Krag and Mekhjian, 1973). This follows the observation from such studies that transmucosal bulk flow and ion exchange may be independent processes. This system may therefore offer a suitable model for further studies of these processes.

SUMMARY

The effect of conjugated and unconjugated bile acids in various concentrations on the absorption of water, sodium and chloride in the proximal jejunum in man has been studied using a perfusion system

incorporating a proximal occluding balloon.

Unconjugated cholic acid and its glycine and taurine conjugates had no significant effect on water and electrolyte absorption. Unconjugated deoxycholic acid significantly inhibited water and electrolyte absorption at 3 mM concentration and net secretion occurred at concentrations of 5 mM, 7 mM and 10 mM. Similar results were found when taurodeoxycholic and glycodeoxycholic acid solutions were used. A greater effect was noted when higher concentrations of bile acids were used.

Unconjugated chenodeoxycholic acid caused net water and electrolyte secretion at 3 mM concentration and this effect became more marked with higher bile acid concentrations. Inhibition of absorption also occurred with taurochenodeoxycholic acid in a concentration of 3 mM, and net secretion at a concentration of 10 mM.

The results show that dihydroxy bile acids, conjugated or unconjugated, inhibit water and electrolyte absorption in the proximal jejunum in low concentration and cause net secretion in high concentration.

These results may be important in the pathogenesis of diarrhoea associated with conditions of bacterial overgrowth as in the blind-loop syndrome and indicate the importance of bile acids and probably lipids in balancing fluid movement in the small intestine.

CHAPTER 11

EFFECT OF LIPIDS ON WATER AND ELECTROLYTE ABSORPTION IN THE JEJUNUM

INTRODUCTION

The findings reported in the previous chapter that dihydroxy bile acids, whether conjugated or deconjugated, significantly inhibit jejunal absorption of water and electrolytes, and the reported improvement of this inhibition by the addition of lecithin (Wingate, Phillips and Hofmann, 1973) raises the possibility that the balance of bile acids and lipids within the jejunum may have some effect on the normal water and electrolyte balance in the small intestine after a meal. The effect of lipids themselves on small intestinal water and electrolyte absorption is consequently of importance.

The relationship of some lipids to the pathogenesis of diarrhoea has been known for some time. Hydroxy fatty acids were identified initially in human faeces by James, Webb and Kellock (1961) who suggested that these compounds might act as cathartics in patients with steatorrhoea. Hydroxy fatty acids are formed within the intestine by bacterial hydration of unsaturated dietary fatty acids (Thomas, 1972). They are commonly found in the faeces in various diarrhoeal states (Wiggins, Pearson, Russell, et al, 1974). Hydroxy fatty acids have been shown to provoke water secretion in the rat colon (Bright-Asare and Binder, 1973) and to inhibit the absorption of water and electrolytes by the colon in man (Ammon and Phillips, 1973). In the ileum of dogs, water absorption was inhibited not only by hydroxy stearic acid and ricinoleic acid but also by oleic acid (Ammon and Phillips, 1974).

AIM OF STUDY

The importance of lipids in altering water and electrolyte absorption in the jejunum in man thus requires to be investigated and the aim of this study is to investigate the effect of lipid administered in the form of an emulsion on water and electrolyte absorption in the jejunum in man.

SUBJECTS AND METHODS

Subjects

Six normal control subjects with no evidence of gastro-intestinal disease, and two patients known to have chronic pancreatitis of some years' duration took part in the study. The diagnosis of chronic pancreatic insufficiency had been diagnosed positively by a secretin-pancreazymin stimulation test.

Composition of perfusing solutions

The initial control perfusate consisted of iso-osmotic glucose-saline with a glucose concentration of 56 mM, and an osmolality of 300 m. osmol/l.

The lipid perfusate consisted of glucose-saline, to which was added glycerol trioleate (Sigma Chemicals, 99% pure) to a final concentration of 5 mM, and sodium taurocholate, to a final concentration of 2.5 mM. After addition of triglyceride and bile acid to the solution, emulsification was assisted by insonation for 15 minutes. (MSE 100 watt ultrasonic disintegrator). The resultant emulsion was stable throughout the study, had a final pH of 7.4 and an osmolality of 308 m. osmols/l.

Phenolsulphonphthalein (PSP) in a concentration of 5 g/l and ^{51}Cr EDTA were added as aqueous phase markers.

Experimental procedure and analytical methods

The perfusion technique used was a triple-lumen tube system as described in Chapter 4. No occluding balloon was used in this study. The experimental procedure and analytical methods were as previously described in Chapter 9. Each subject and patient was perfused initially with the control glucose-saline solution and after an equilibration period of 45-60 minutes, 3 ten minute collections were made. The lipid emulsion was then perfused and, after a further period of equilibration, 7-8 ten minute collections were made. Each subject thus acted as his own

control in this study.

RESULTS

The results of perfusing the lipid emulsion compared with control solutions in the normal subjects and two patients with chronic pancreatic insufficiency are shown in Table 11.1. The absorption figures for the control solution are the mean of 3 ten minute collection periods for each patient prior to the introduction of the lipid emulsion. The absorption figure during perfusion of the emulsion is the mean of 7 or 8 ten minute collection periods. In the normal control subjects the mean water absorption in the control period was 256 $\pm$ 21.7 ml/hr. When the lipid emulsion was perfused the mean water absorption in the control subjects was 45.6 $\pm$ 4.0, statistically significantly less than the absorption when the control solution was perfused ($p < 0.001$). The mean sodium absorption of the group in the control period was 24.3 $\pm$ 0.5 m.mol/hr which was statistically significantly greater than the mean sodium absorption when the lipid emulsion was introduced (15.8 $\pm$ 1.5 m.mol/hr (($p < 0.05$). A similar significant difference was observed with the chloride absorption during the control period. In all cases a statistically significant reduction of absorption of water, sodium and chloride was observed in these control subjects when the lipid emulsion was perfused into the jejunum.

In the patients with chronic pancreatitis, the water absorption in the control period in the two patients studied was 334 ml/hour and 220 ml/hour respectively. These figures did not appear to differ from the equivalent figures for water absorption when the lipid emulsion was introduced in the same two patients (361 ml/hour and 194 ml/hour respectively). The mean water absorption in these two patients in the control period

TABLE 11.1

MEAN WATER, SODIUM AND CHLORIDE ABSORPTION IN CONTROL SUBJECTS AND PATIENTS WITH CHRONIC PANCREATIC INSUFFICIENCY PERFUSED WITH GLUCOSE-SALINE SOLUTION AND WITH LIPID EMULSION

NORMAL CONTROLS	MEAN WATER ABSORPTION (ml/hr./segment)		MEAN SODIUM ABSORPTION (m.mol/hr/segment)		MEAN CHLORIDE ABSORPTION (m.mol/hr/segment)	
	Control Solution	Lipid Emulsion	Control Solution	Lipid Emulsion	Control Solution	Lipid Emulsion
1	168	32.9	23.2	10.3	21.9	12.9
2	243	40.8	24.8	12.7	23.3	14.1
3	308	53.9	23.8	17.4	24.0	18.2
4	314	59.4	26.1	20.3	25.3	22.8
5	260	47.0	25.2	16.3	24.9	17.2
6	243	39.6	22.9	17.8	22.3	21.4
MEAN ± SEM	256 ± 21.7	45.6 ± 4.0	24.3 ± 0.5	15.8 ± 1.5	23.6 ± 0.6	17.8 ± 1.6
CHRONIC PANCREATITIS						
1	334	361	30.6	32.4	21.6	18.9
2	220	194	23.3	22.1	20.1	17.2
MEAN	277	278	26.9	27.2	20.3	17.8

and during lipid emulsion perfusion was therefore 227 ml/hour and 278 ml/hour. The mean sodium absorption during the control period was 26.9 m.mol/hour in these two patients and 27.2 m.mol/hour during lipid emulsion. No marked difference was observed also with chloride absorption in the two subjects.

DISCUSSION

The results of this study demonstrate a statistically significant reduction in absorption of water, sodium and chloride in normal subjects perfused with a lipid emulsion compared with the absorption calculated in the same subjects perfused with a glucose-saline solution. However, in two patients with chronic pancreatic insufficiency the mean water, sodium and chloride absorption was of the same order of that found during perfusion of the standard glucose-saline solution.

Ammon, Thomas and Phillips (1974) demonstrated that oleic acid and ricinoleic acid significantly reduced the absorption of water and electrolytes in the human jejunum and indeed in some instances induced fluid secretion. The effect was noted to be rapidly reversible. They found that the addition of lecithin and monolein did not diminish the secretory effect of ricinoleic acid. These authors were interested in the fact that 10-dihydroxystearic acid (a bacterial metabolite of oleic acid), structurally resembles ricinoleic acid. Ricinoleic acid is a known cathartic implicated in the pathogenesis of diarrhoea associated with steatorrhoea (Webb, James and Kellock, 1961; Kim and Spritz, 1968).

In the normal control group, perfused with a lipid emulsion, lipolysis of glycerol trioleate probably occurred, resulting in the release of oleic acid which has appeared from our studies to be absorbed almost immediately. This information is based on absorption data based on studies using 14C oleic acid and 3H glycerol triether (Gerskowitch and Russell, 1974). It is

considered that the reduction in normal absorption of water and electrolytes in these normal subjects may be caused by oleic acid. Ricinoleic acid (12 hydroxyoctadeconic acid) is the active principle of castor oil. The mechanism of the diarrhoea caused by this compound may be partly due to an effect on water and electrolyte absorption in the upper small intestine as demonstrated in this paper, although other factors may well be involved. Oleic acid may also alter intestinal motility (Iwao and Terada, 1962), increase mucus secretion (Schmid, 1952) and may also decrease sodium transport in vitro (Phillips, Love and Mitchell, 1965). This possibility is further supported by the finding of a normal range of water and electrolyte absorption in the patients with chronic pancreatic disease. These patients had reduced lipolysis due to the chronic pancreatic deficiency and following this, presumably reduced absorption of triglyceride from the oil phase. Thus less oleic acid is released which therefore will be unable to cause damage to the absorption mechanisms.

From the results of this study it would appear that dietary lipids in addition to bile acids have an important part to play in regulating fluid and electrolyte balance in the upper small intestine. Should an imbalance occur for any reason (bile acid deficiency or impaired fat absorption) then an imbalance of the bile acids and lipids will occur and some inhibition of water and electrolyte absorption either from dihydroxy bile acids or fatty acids may follow.

When lipolysis is normal but fat absorption is impaired, a longer area of intestine is exposed to fatty acids, and in steatorrhoea, the whole small intestine and colon may be exposed to excess fat. Thus, marked inhibition of water and electrolyte absorption is likely to follow and secretion of fluid may occur.

SUMMARY

The effect of a lipid emulsion on jejunal water and electrolyte

absorption has been determined in six normal control subjects and in two patients with chronic pancreatic insufficiency, each patient acting as his own control.

A statistically significant reduction of jejunal absorption of water and electrolytes was observed in all control subjects in which the emulsion was perfused compared with the control period in which glucose-saline solution was perfused. The two patients with chronic pancreatic insufficiency who had impaired lipolysis had normal absorption of water and electrolytes before and during perfusion of the emulsion.

These results suggest that a correct balance between bile acids and lipids is essential for correct fluid and electrolyte balance in the upper jejunum.

CHAPTER 12

THE EFFECT OF THE DIURETIC FRUSEMIDE ON WATER AND ELECTROLYTE ABSORPTION FROM THE JEJUNUM

INTRODUCTION

The effect of chemical stimulants such as dihydroxy bile acids, hydroxy fatty acids and oleic acid on water and electrolyte transport in the jejunum leads to a consideration of the effect of various drugs on jejunal water and electrolyte absorption. Ricinoleic acid is the active principal of castor oil. Cathartics of the anthraquinone group may also stimulate intestinal secretion. (Forth, Rummel and Baldauf, 1966).

A large number of other drugs may however, cause diarrhoea. The administration of frusemide is occasionally associated with diarrhoea as an unwanted side effect (Cooperman and Rubin, 1973; Kennedy, 1974). As frusemide is considered to be an inhibitor of renal transport mechanisms (Knox, Wright, Howards et al, 1969), it is possible that intestinal transport mechanisms may also be altered by the drug. The principal action of frusemide on the kidney is similar to that of ethacrynic acid, namely inhibition of sodium reabsorption in the ascending loop of Henle (Cannon and Kilcoyne, 1969). Amongst other actions, ethacrynic acid has been shown to inhibit water absorption by hamster gut sacs, secretion occurring with higher dosage (Binder, Katz, Spencer et al, 1966). A reduction of sodium absorption has also been noted in isolated rabbit ileum after mucosal application of ethacrynic acid (Al-Awquati, Field and Greenough, 1974). If frusemide were to alter the water absorption mechanism in this way in the human jejunum, a possible explanation of frusemide-induced diarrhoea would be found.

The aim of this investigation was thus to study the effect of frusemide on jejunal absorption of water and electrolytes using the technique of small-intestinal perfusion.

SUBJECTS AND METHODS

Subjects

The study was performed on twelve normal volunteer subjects who gave informal consent for the procedure to be performed.

Composition of perfusing solutions

For this study, the perfusing solution contained sodium (140 m.mol/l), potassium (5 m.mol/l), chloride (100 m.mol/l), bicarbonate (45 m.mol/l) and glucose (11.2 m.mol/l). The osmolality was 300 m.osmol/l.

Phenolsulphonphthalein (PSP: 150 mg/l), and in some subjects, ^{14}C-polyethylene glycol 4000 (1g/l),(Wingate, Sandberg and Phillips, 1972) were used as water-soluble, non-absorbable markers.

Technique, experimental procedure and analytical methods.

The study was performed by the technique of small intestinal perfusion with a double-lumen tube with proximal occluding balloons and a mixing segment 30 cm long, (based on that of Phillips and Summerskill, 1966 and described in Chapter 9). Immediately proximal to this were two occluding balloons and above this was a further collection point for removal of the fluid collecting above the occluding balloons. The tube was passed under X-ray control, with an image intensifier, until the balloons were at the ligament of Treitz.

Lack of contamination by the perfusate of fluid aspirated above the balloons was demonstrated in the ensuing 10-15 min. by measuring marker concentration. After a 50-60 minute equilibration period, three separate ten-minute collections were taken, frusemide (20 mg or 40 mg), administered intravenously, and ten-minute collections continued for 50 or 60 minutes. Each subject in the study thus acted as his own control.

5 subjects were given frusemide 20 mg and 7 were given frusemide in a dose of 40 mg. intravenously.

Water, sodium, potassium and chloride absorption was calculated by measuring the change in concentration of the non-absorbable markers and using the formula for the double-lumen tube perfusion system.

Statistical methods

The statistical significance of the results was assessed by the paired t-test.

RESULTS

The effect of 20 mg frusemide on water and electrolyte absorption from the jejunum in 5 subjects is shown in Table 12.1. No significant change in transport was observed. A satisfactory diuresis occurred in all 5 subjects.

The mean net water and electrolyte absorption after 40 mg of frusemide is shown in Table 12.2. The reduction in net absorption of water is statistically significant at 20, 30, 40, and 50 min. The reduction in net sodium absorption is significant at 30, 40 and 50 min and the reduction in net potassium absorption significant at 40 and 50 min. There was a net secretion of chloride in some subjects and a rather low absorption (in relation to the sodium absorption) in the remainder - the effect of frusemide in a dose of 40 mg. is to increase the net secretion significantly at 40 and 50 min.

The mean urinary water excretion ($\pm$ S.E.M.) before and after 40 mg of frusemide was 137.6 ± 28.5 ml/hr and 1378 ± 176.1 ml/hr respectively, and sodium excretion before and after the same dose of frusemide was 10.9 ± 3.1 mmol/hr and 156.3 ± 21.2 mmol/hr respectively.

The change in absorption after frusemide was statistically significant whether polyethylene glycol or the Phenol Red data were used to calculate the results. It was noted, however, that the water absorption

TABLE 12.1

MEAN ELECTROLYTE AND WATER ABSORPTION (± SEM) BEFORE AND AFTER 20 mg FRUSEMIDE

	Before Frusemide (mean of 3 collections)	After Frusemide (minutes after I.V. injection)					
		10	20	30	40	50	60
Mean sodium absorption (m.mol/hr)	16.98 ± 12.17	13.89 ± 5.43	2.78 ± 19.2	2.62 ± 3.42	15.4 ± 7.21	14.04 ± 5.66	3.81
Mean potassium absorption (m.mol/hr)	0.55 ± 0.46	0.53 ± 0.26	0.10 ± 0.89	0.08 ± 0.58	0.51 ± 0.51	0.41	1.32
Mean chloride absorption (m.mol/hr)	7.41 ± 6.0	4.76 ± 4.48	-10.40 ± 15.2	-3.35 ± 8.89	7.50 ± 8.78	9.70 ± 10.90	16.90
Mean water absorption (ml/hr)	188.9 ± 60.5	162.50 ± 33.50	83.52 ± 118.3	120.75 ± 50.63	181.35 ± 40.90	174.00	300.0

TABLE 12.2

MEAN ELECTROLYTE AND WATER ABSORPTION (± S.E.M.) BEFORE AND AFTER

40 mg. FRUSEMIDE

		After frusemide					
	Before Frusemide	10 min	20 min	30 min	40 min	50 min	60 min
Mean sodium absorption (mmol/hr)	14.80 ± 4.83	11.13 ±5.17	9.06 ±5.55	2.32* ±7.57	−1.89* ±7.57	−7.35* ±8.25	−1.72 ±8.19
Mean potassium absorption (mmol/hr)	0.73 ±0.23	0.53 ±0.23	0.49 ±0.27	0.37 ±0.35	0.16** ±0.36	0.07* ±0.37	0.37 ±0.32
Mean chloride absorption (mmol/hr)	−0.92 ±4.63	−1.73 ±4.43	−3.36 ±4.72	−5.89 ±5.42	−9.42* ±6.24	−12.45* ±7.37	−8.14 ±6.07
Mean water absorption (ml/hr)	140.41 ±39.09	111.39 ±40.5	94.87* ±42.19	56.2* ±52.03	18.17** ±53.53	15.50** ±61.91	-

* $p < 0.05$

** $p < 0.005$

calculated from polyethylene glycol data was consistently less than when PSP data were used.

DISCUSSION

The results of this study indicate that frusemide, administered intravenously in a dose of 40 mg. significantly alters the transport mechanisms of water and electrolytes in the jejunum. No significant effect is found with 20 mg. frusemide. Inhibition of water and electrolyte absorption occurred in all subjects, and mean net secretion of sodium occurred 40, 50 and 60 minutes after the injection. A mean net chloride secretion was also observed in most subjects and was present in some in the control period. This is probably related to the composition of the bicarbonate-containing perfusate.

The mechanism of action of frusemide in inhibiting jejunal absorption of electrolytes and water, warrants further investigation. The diuretics frusemide and ethacrynic acid are considered to act on the kidney in a similar way, and ethacrynic acid inhibits water absorption in hamster gut sacs and may cause secretion. (Binder, Katz, Spencer et al, 1966). Both these diuretics are potent inhibitors of Na/K ATPase enzyme system in vivo and in vitro (Knox, Wright, Howards et al, 1969; Duggan and Noll, 1965). Ethacrynic acid, however, has also been shown to inhibit cholera toxin induced intestinal secretion, and reverses cyclic AMP - mediated intestinal secretion.(Al-Awqati, Field, Greenough, 1974). Cyclic AMP probably stimulates intestinal secretion by its effect on chloride movement, and this appears to be specifically inhibited by ethacrynic acid. It does not result from ethacrynic acid inhibition of cyclic AMP itself. These reports suggest that the effect of ethacrynic acid and frusemide in inhibiting electrolyte and water absorption in

the jejunum may be related to their effect on the Na/K ATPase system, rather than to any relationship to cyclic AMP.

Chlorothiazide has been shown to inhibit water and electrolyte absorption from the human colon (Levitan, 1972), and inhibition of sodium and potassium has been demonstrated in the rat ileum by chlorothiazide, acetazoleamide and frusemide. (Strombeck and Ingraham, 1972).

The results of the study may explain the diarrhoea which may occur when frusemide is administered in large doses, as in some patients with renal disease. They also raise important questions relating to the absorption of drugs (such as digoxin) which may be given with a diuretic such as frusemide. Reduced absorption of such drugs, if given with frusemide, may necessitate an alteration of dosage regimes and requires further investigation to determine whether frusemide has such an effect on digoxin absorption, if these two drugs are administered together.

SUMMARY

Frusemide, in a dose of 40 mg administered intravenously, significantly reduced the absorption of water and electrolytes from the human jejunum, a double-lumen perfusion system with proximal occluding balloons being used. Net secretion of water and electrolytes occurred in some subjects.

No significant change in water and electrolyte absorption was observed with 20 mg of frusemide.

These findings may explain the diarrhoea which may be induced by frusemide in some patients and may be of importance in relation to the absorption of drugs such as Digoxin, which may be administered at the same time as frusemide.

CHAPTER 13

CONCLUSIONS

The results of the studies reported in this section are of importance in gaining a further knowledge of the physiological mechanisms involved in water and electrolyte balance in the jejunum and also in explaining the pathogenesis of some forms of diarrhoea.

The dihydroxy bile acids deoxycholic and chenodeoxycholic acid, whether conjugated or unconjugated, were found to significantly inhibit water and electrolyte absorption in the jejunum and cause secretion of fluid in higher concentration. This effect has been shown to be modified by the addition of the phospholipid, lecithin.

The introduction of a lipid emulsion also appears to inhibit water and electrolyte absorption in the jejunum and this is attributed to the release of oleic acid following lipolysis. Further evidence supporting this comes from the fact that perfusion in patients with chronic pancreatitis has no significant inhibitory effect on water and electrolyte absorption in the jejunum. In such patients lipolysis is inefficient because of pancreatic enzyme insufficiency.

The results of these two studies taken together emphasise that bile acid and lipids have an important part to play in normal water and electrolyte balance during digestion and absorption of lipids in the proximal jejunum. Thus, the trihydroxy bile acid cholic acid, has no effect on water and electrolyte absorption, but the dihydroxy bile acids, deoxycholic and chenodeoxycholic acid (whether conjugated or unconjugated) do significantly inhibit water absorption and may cause secretion of fluid into the jejunal lumen. This inhibitory effect can be abolished if the dihydroxy bile acids are present in the jejunum in conjunction with lecithin. Lecithin thus appears to have an important function in preventing dihydroxy

bile acids from interfering with normal absorption of water and electrolytes. Lipid, however, in the upper small intestine is generally in the form of an emulsion consisting of long chain lipids, shorter chain lipids undergoing lipolysis to monolein and eventually fatty acids such as oleic acid. An emulsion containing glycerol trioleate and sodium taurocholate was perfused into the proximal jejunum, and was found to significantly inhibit water and electrolyte absorption, probably due to the production of oleic acid. Normally, however, these fatty acids are absorbed rapidly after their production in the jejunal lumen. The balance of water and electrolyte absorption and secretion in the proximal jejunum is therefore likely to be normally adjusted by the occurrence of lipolysis of ingested lipids and rapid fat absorption in the proximal jejunum. Bile acids and lipids thus appear to have an important role to play in this mechanism.

These findings are also of importance in various disease states. Watery diarrhoea may accompany states of bacterial overgrowth in the small intestine as in the blind loop syndrome or small intestinal diverticulosis. In such conditions deconjugation of bile acids may be followed by dehydroxylation with the production of an increased concentration of dihydroxy bile acids in the small intestine. If this occurs then inhibition of water and electrolyte absorption may follow and indeed net secretion may result if these bile acids are present in high concentration. This finding may be the explanation for the watery diarrhoea which occurs in states of bacterial overgrowth, and may also be important in other diarrhoeal conditions. In conditions where lipolysis of lipids is normal but fat absorption is impaired, as in mucosal forms of malabsorption (eg coeliac disease), a longer area of intestine is exposed to the released fatty acids. When steatorrhoea is present the entire small intestine and colon

may be exposed to excess fat. Thus inhibition of water and electrolyte absorption is likely to occur in these conditions and the diarrhoea which may be present in conjunction with steatorrhoea may in part be due to the effect of fatty acids on the jejunal absorptive mechanisms.

The effect of drugs on small intestinal absorption of water and electrolytes and on other aspects of intestinal absorption is also likely to be important. This work leads on directly from studies of fatty acids such as oleic acid or ricinoleic acid on water and electrolyte absorption in the small intestine, as ricinoleic acid is considered to be the active principle of castor oil. Cathartics of the anthraquinone group have also been shown to stimulate intestinal secretion as have other laxatives such as bisacodyl (Ewe 1972). Many drugs may cause diarrhoea as a side-effect,, one such being frusemide. The effect of such drugs on small intestinal absorption mechanisms is clearly important and the diuretic drug frusemide has been studied using the small intestinal perfusion technique. The results demonstrate that 40 mg of frusemide administered intravenously significantly reduce the absorption of water and electrolytes from the human jejunum, although no effect is observed if a 20 mg dose is used. These results are of significance in possibly explaining the diarrhoea which may occur when frusemide is administered in large doses, and has important implications in relation to the absorption of drugs such as digoxin, which may be administered with frusemide on a regular basis. Some inhibition of the absorption of digoxin may possibly follow the administration of the drug at the same time as frusemide is given. This aspect requires further investigation.

The title of this section is the study of various individual factors in influencing absorption of water and electrolytes in the human small intestine using the intestinal perfusion technique. Following the results of these studies, a better classification of the causes of diarrhoea due

to intestinal secretion can be formulated. The principal causes of intestinal secretion can be classified as follows:

(a) Abnormal physical conditions, such as mechanical bowel obstruction, low pH and after irradiation.

(b) The effect of chemical stimulants such as dihydroxy bile acids on the small intestine and colon, hydroxy fatty acids, and drugs such as anthraquinone cathartics, frusemide and possibly other compounds.

(c) The effect of bacterial toxins such as Vibrio cholerae, Staphylococcus aureus, Clostridium perfringens, Shigellae and Escherichia coli.

(d) Stimulation by hormonal factors. A number of hormones have now been demonstrated to have an effect on intestinal absorption.

Thus, the prostaglandins E_1 and F_2 induce a net jejunal secretion of water and electrolytes. (Cummings, Newman, Misiewicz et al, 1973; Matuchansky and Bernier, 1973). Prostaglandins, like cholera exotoxin, stimulate intestinal mucosal cyclic AMP activity. (Kimberg, Field, Gershon et al, 1974). A secretory effect has also been demonstrated with secretin and cholecystokinin. (Hicks and Turnberg, 1973; Bussjaeger and Johnson, 1973; Moritz, Finkelstein, Meshkinpour et al, 1973; Turnberg and Grahams, 1974), vasoactive intestinal polypeptide, (Desbukuois, Lowdet, Lowdet, 1973) and alterations of intestinal absorption have also been reported with gastrin (Hubel, 1972a, Modigliani, Mary and Bernier, 1973), glucagon (Hubel, 1972b) and anti-diuretic hormone (Soergel, Whalen, Harris et al, 1968). It may be that normal balance of these hormones is required for normal intestinal absorption to occur in the jejunum. Imbalance of the hormones may lead to inhibition of absorption or the production of net secretion. Further information is required about these hormonal influences on intestinal absorption.

No mention has been made in this section of the effect of disease processes on intestinal absorption and the possible production of intestinal secretion in some conditions. These points will be discussed at greater length in the next section.

SECTION IV

APPLICATION OF THE SMALL INTESTINAL PERFUSION TECHNIQUE TO THE STUDY OF JEJUNAL ABSORPTION ABNORMALITIES IN COELIAC DISEASE AND CROHN'S DISEASE

INTRODUCTION

This section is devoted to the application of the small intestinal perfusion technique to study the specific absorption abnormalities in the jejunum in adult coeliac disease and Crohn's disease.

Coeliac disease, or gluten-induced enteropathy, is characterised by severe damage to the villi of the small intestinal mucosa, giving the characteristic histological appearance of sub-total villous atrophy (Shiner and Doniach, 1960). The condition is accompanied by actual or potential malabsorption of many nutrients following the small intestinal mucosal damage. The jejunum is involved extensively (although there is some evidence to suggest that patchy damage may occur in some patients), but the ileum is often spared or only affected to a minimal extent. Correction of malabsorption and a return of the abnormal morphology of the jejunum towards normal follows exclusion of gluten from the diet. The clinical and histological response to a gluten-free diet provides essential confirmation of the diagnosis.

Coeliac disease is characterised by symptoms of diarrhoea and sometimes steatorrhoea, and occasionally abdominal pain, abdominal distension and vomiting. Multiple deficiencies may follow malabsorption. Steatorrhoea may occur in coeliac disease but is rarely severe, and may not be present at all in many patients. Diarrhoea is common and may occur in the absence of steatorrhoea. In such patients the diarrhoea is usually of a watery nature. The mechanism of this watery diarrhoea is not fully understood; it is possible that a number of mechanisms may be involved. The nature of the absorption abnormalities in the jejunum in coeliac disease requires further investigation and the small intestinal perfusion technique may offer further information on this problem.

Crohn's disease, or regional enteritis, also poses an absorption problem in the small intestine. Although the terminal ileum is most often involved, the disease may affect any part of the alimentary canal, and is usually discontinuous, with diseased segments of bowel separated by apparently healthy areas. The principal symptoms of Crohn's disease are diarrhoea and abdominal pain. The diarrhoea is usually of watery nature and steatorrhoea may occur in some patients. (Raffensberger, D'Agostino, Manfredo et al, 1967). The jejunum may be directly affected in Crohn's disease, but the histological appearances of partial villous atrophy may occur in the jejunum although no Crohn's lesion may be present in that area. Diarrhoea in Crohn's disease is almost certainly of multiple origin and absorption abnormalities in the upper small bowel and particularly in the jejunum may play a part in its production. Further information is required onabsorption abnormalities in the jejunum, and the small intestinal perfusion technique seems suited to provide such information.

CHAPTER 14

ABSORPTION ABNORMALITIES IN THE JEJUNUM IN ADULT COELIAC DISEASE

INTRODUCTION

It has been considered for some time that some of the symptomatology common in coeliac disease, such as diarrhoea, may be related to malabsorption of water and electrolytes by the damaged jejunal mucosa.(Hotz, 1940; Wollaeger and Scribner, 1951; Comfort, Wollaeger, Taylor et al, 1953; Taylor, 1955; Cooke, 1957). Confirmation of inhibition of absorption of water and electrolytes was provided by the early intubation studies of Schedl andClifton (1963) and Fordtran, Rector, Locklear et al (1957) and a suggestion of net accumulation of fluid in the jejunum was found by Holdsworth and Dawson (1965). Undirectional fluxes of deuterium oxide and of 24 Na from jejunum to blood have also been found to be reduced (Higgins, Lee, Schoeler et al, 1957; Newsholm and French, 1964). Further information is required regarding specific absorption abnormalities which may be present in coeliac disease together with the response of these abnormalities to withdrawal of gluten from the diet.

AIMS OF STUDY

The aim of this investigation is to apply the technique of small intestinal perfusion to patients suffering from untreated adult coeliac disease to determine the absorption abnormalities which may be present, and to repeat these studies in the same patients after an adequate clinical and histological response to gluten-free diet.

PATIENTS AND METHODS

Patients

Jejunal perfusion was performed on 14 patients with untreated adult coeliac disease and in 15 normal control volunteers. The mean age of the coeliac disease group was 33 years (range 21-60) and in the control group 49 years (range 28-60). The coeliac group consisted of 5 males and 9 females and the control group 9 males and 6 females.

The criteria for the diagnosis of adult coeliac disease were as follows: a typical history of diarrhoea with the passage of pale, foul-smelling stools and often watery diarrhoea, abdominal discomfort and distension, and weight loss. In all patients the faecal fat excretion was greater than 5 g per day, and the d-Xylose excretion was decreased. Jejunal biopsy, using the Crosby-Kugler capsule (Crosby and Kugler, 1957) showed evidence of sub-total villous atrophy in all patients. Clinical remission occurred on withdrawing gluten from the diet in all these patients and improvement was noted in the histology of the jejunal mucosa within 3 months of starting a gluten-free diet. In all patients diarrhoea was present at the time of the study. The normal control patients had no history of gastro-intestinal symptomatology.

In 8 patients the perfusion was repeated 3-4 months after starting the gluten-free diet. In two of these patients a third perfusion was performed one year after gluten withdrawal. In the patients who had three perfusions, jejunal biopsy on each occasion performed at the time of perfusion showed some improvement of histological change and on the third occasion was reported as having mild partial villous atrophy.

Methods.

A triple-lumen perfusion system was used in the study, using the tube described in Chapter 4. The perfusate consisted of iso-osmotic glucose-saline with a glucose concentration of 56 mM. The final osmolality of the perfusing solution was 300 m.osmol/l. The experimental technique and analytical methods were as described in Chapter 9.

RESULTS

The absorption of water and electrolytes in each study was calculated as the mean of the results of 10-12 ten-minute collection periods, and was expressed as ml/hour/30 cm segment(for water) and m.mol/hour/30 cm

segment (for sodiumand chloride). The mean water, sodium and chloride absorption in coeliac disease and control groups is shown in Table 14.1. The mean water absorption ($\pm$ SEM) in the coeliac disease group was -17.2 ± 10.0 ml/hour, compared with 220.8 ± 2.1. ml/hour in the control group. The difference between these means is statistically significant ($p<0.001$). The mean sodium absorption in coeliac patients (-5.2 ± 1.3 m.mol/hour) was statistically significantly less than that of the control group (25.3 ± 1.8 m.mol/hour) ($p<0.001$). A similar statistical difference was also obtained with chloride absorption. Each individual figure in Table 14.1 in the coeliac disease column refers to the mean absorption calculated over 10-12 ten-minute collection periods. The mean of the group as a whole is at the bottom of each column. These figures demonstrate that 10 of the 14 patients with adult coeliac disease had a mean net secretion of water, but in 3 of the 4 patients recorded who had mean reduced absorption but not secretion, one or two negative values were obtained at some time during the study. Thus, net secretion of water did occur at some point during the study in 13 of the 14 patients with untreated adult coeliac disease. Similar results were obtained with respect to mean sodium and chloride absorption.

The effect of gluten withdrawal on the absorption abnormalities in the 8 patients in which perfusion was repeated 3 to 4 months after starting a gluten-free diet as shown in Table 14.2. No significant improvement in mean water, sodium and chloride absorption was found in these 8 patients, in spite of the fact that all had undergone satisfactory clinical remission during that period.

In one of the two patients who underwent a third perfusion a year after starting a gluten-free diet, some improvement of water, sodium and chloride absorption appeared to have occurred although this had not yet

TABLE 14.1

MEAN WATER, SODIUM AND CHLORIDE ABSORPTION IN ADULT COELIAC DISEASE AND NORMAL CONTROLS

Mean water absorption (ml/hr)		Mean sodium absorption (m.mol/hr)		Mean chloride absorption (m.mol/hr)	
Coeliac disease	Normal controls	Coeliac disease	Normal controls	Coeliac disease	Normal controls
20	159	1.6	15.9	0	14.6
-2	224	-1.5	27.1	-0.3	22.7
50	195	-3.2	22.9	-0.5	30.1
-29	238	-3.8	30.2	-1.7	18.8
-2	200	5.1	19.1	3.4	37.3
-27	158	-10.1	32.4	-6.2	30.3
-58	259	-2.4	16.9	-5.7	15.8
-29	361	-6.7	29.0	-6.3	19.6
-91	142	-10.7	38.6	-9.5	20.2
16	172	-8.3	29.1	-1.8	32.1
-47	224	-9.8	31.4	-6.5	15.6
25	151	0.8	23.5	-1.2	23.8
-26	243	-8.4	18.5	-6.2	25.3
-41	154	-14.6	17.6	-10.1	20.5
	432		22.8		25.3
Means $\pm$ SEM					
- 17.2 $\pm$ 10.0	220.8 $\pm$ 21.1	-5.2 $\pm$ 1.3	25.3 $\pm$ 1.8	-3.1 $\pm$ 0.9	22.9 $\pm$ 1.5
$P<0.001$		$P<0.001$		$P<0.001$	

TABLE 14.2

CHANGES IN ABSORPTION OF WATER, SODIUM AND CHLORIDE

AFTER GLUTEN WITHDRAWAL (3-4 MONTHS)

Patient No.	Mean Water Absorption (ml/hr)		Mean Sodium Absorption (mmol/hr)		Mean Chloride Absorption (mmol/hr)	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
1	-27	-4	-2.4	-0.6	-1.2	-0.4
2	16	31	1.6	2.0	0	0.8
3	-26	15	-8.4	-2.1	-6.2	-1.0
4	-47	-16	-3.8	0.2	-1.7	-0.8
5	- 2	18	-3.2	1.6	-0.3	1.5
6	-41	-16	5.1	8.3	-1.8	-2.3
7	50	52	-10.7	-3.3	-6.3	-1.0
8	-29	- 8	0.8	-1.2	-0.5	2.2
Means ± SEM	-13.3 ± 11.6	9.0 ±10.6	-2.6 ± 1.8	0.4 ± 1.3	-2.3 ± 0.9	-0.1 ± 0.5

reached the normal range. This patient (Number 3 in Table 14.2) showed a mean water absorption of -26 ml/hour initially, 15 ml/hour 3 months after starting the gluten-free diet, and one year later the mean water absorption level was 103 ml /hour. Similar results were obtained with mean sodium and chloride absorptions in this patient. This patient had remained clinically well during this period. In the other patient perfused on three occasions (number 8 in Table 14.2), the mean water absorption was initially -29 ml/hour, -8 ml/hour (at 3 months) and was still abnormal at -14 ml/hour at one year. This patient, after initial improvement, had again developed diarrhoea and his weight had not risen satisfactorily. Subsequent questioning of the patient revealed that he was not keeping strictly to his gluten-free diet.

DISCUSSION

The results of this study indicate that in untreated adult coeliac disease a net secretion of water, sodium and chloride may occur into the lumen of the jejunum. The results are similar to those reported by Schmid, Phillips and Summerskill (1969). It seems possible that the watery diarrhoea often seen in coeliac disease, together with the abdominal distension and vomiting, may be related to the presence of large amounts of fluid in the upper small intestine. This, in turn, is due not only to reduced absorption of water and electrolytes, but also to net secretion. Some of this excess fluid may be reabsorbed in the distal jejunum and ileum where the mucosal damage is less severe. Normal absorption of water and glucose and an increased absorption of sodium and chloride have been demonstrated in the ileum in untreated coeliac disease (Silk, Kumar, Webb et al, 1975). Absorption of some of the excess water and sodium may also occur in the colon (Devroede and Phillips, 1969).

Jejunal absorption of glucose is also inhibited in adult coeliac disease (Schedl and Clifton, 1963), and reduction of absorption of amino acids and peptides have also been shown (Schedl, Pierce, Rider et al, 1968; Silk, Kumar, Perrett et al, 1974). Malabsorption of folic acid has also been shown in untreated coeliac disease using a jejunal perfusion system (MacKenzie and Russell, 1974). A similar secretory state has been found in perfusion studies of patients with tropical sprue (Corcino, Maldonado and Klipstein, 1973).

There is evidence that the sodium secretion in coeliac disease appears to be against an electro-chemical gradient; the negative mucosal electro-potential difference in coeliac disease is no different from that of a control group. (Schmid et al, 1969; Cochran and Russell, personal observation). Damage to the active sodium pump may allow leakage of sodium and water but active secretion of sodium also appears to occur. Water loss may follow the sodium movement and chloride movements may also be similar. There also appears to be a loss of the active absorption mechanism for bicarbonate (Schmid et al, 1969). The possible place of cyclic AMP in the production of these abnormalities in coeliac disease also requires to be studied.

One of the most interesting results of this study is the lack of significant improvement in the absorption of sodium, chloride and water after gluten withdrawal, up to one year after a gluten-free diet was started. In each of the patients, clinical remission had occurred and repeat jejunal biopsy performed at the same time as the perfusion study showed improvement in the histological appearance of the jejunal mucosa although none were regarded as entirely normal. This probably reflects both persistence of functional abnormalities in spite of some morphological

improvement, and the fact that studies were performed in the proximal jejunum, the site of maximal involvement in coeliac disease. There may be improvement in the extent of the disease and in function in more distal areas. A functional improvement in the absorption mechanism may be more gradual and this is suggested by the trend to an improved (although still abnormal) water absorption in one patient one year after the start of the gluten-free diet. This may fit in with the hypothesis that the part of the small intestine studied in the perfusion investigation is the worst affected in coeliac disease and consequently the slowest to recover to normal when gluten withdrawal occurs.

SUMMARY

The transport of water and electrolytes across the jejunal mucosa was studied in 14 patients with untreated adult coeliac disease using a triple-lumen tube perfusion system and the results compared with those obtained in 15 normal control subjects.

The results indicate that not only was there an inhibition of water and electrolyte absorption in patients with adult coeliac disease but that in most of these patients a secretion of water and electrolytes occurred into the jejunum. Secretion was found in 13 of the 14 patients at some point during the study.

Three months after the introduction of a gluten-free diet, and with clinical remission, no significant improvement had occurred in water and electrolyte absorption in the jejunum. There was a trend towards some improvement in one patient re-perfused one year after gluten withdrawal.

The results of this study may explain some of the symptomatology of coeliac disease although other secondary factors may also be involved.

CHAPTER 15

ABSORPTION ABNORMALITIES IN THE JEJUNUM IN CROHN'S DISEASE

INTRODUCTION

Diarrhoea is a common symptom in patients with Crohn's disease and is probably of multiple aetiology in most cases. Steatorrhoea may occur and other forms of malabsorption may be present and excessive faecal excretion of bile acid giving rise to cholerheic diarrhoea may also occur in Crohn's disease if severe damage has occurred to the terminal ileum. Little is known, however, of the function of the proximal jejunum in Crohn's disease in patients in which the jejunum may or may not be affected by the disease as judged by radiological means. Absorption abnormalities of the proximal jejunum, if present, may be a further factor in the causation of diarrhoea in Crohn's disease.

AIM OF STUDY

The aim of this study is to apply the technique of small intestinal perfusion to patients with Crohn's disease who have not undergone surgery, in an attempt to determine if jejunal absorptive function is abnormal in such patients.

PATIENTS AND METHODS

Six patients with Crohn's disease were studied. They consisted of 4 females and 2 males with an age range of 22 to 46. The results were compared with a group of 15 volunteer subjects (9 males and 6 females) with a mean age of 49 years (range 28–60).

All the patients with Crohn's disease suffered from diarrhoea and all had been recently diagnosed, 4 by a positive histological diagnosis either by jejunal biopsy or following laparotomy and biopsy, and the remaining two by the presence of severe radiological involvement of the small intestine. All the patients had diarrhoea at the time of the perfusion study. None had any medical treatment at the time of the perfusion, and no patient had undergone surgery at that time. Full clinical details of

these 6 patients are shown in Table 15.1.

Methods

The method used was that of the triple-lumen perfusion technique, as described in Chapter 9. The perfusate consisted of an iso-osmotic glucose-saline solution containing glucose in a concentration of 56 mM and with a final osmolality of 300 m.osmols/l. The experimental procedure and analytical methods were as previously described in Chapter 9.

RESULTS

The results of the mean water, sodium and chloride absorption in the six patients with Crohn's disease are shown in Table 15.2. Only one, or possibly two, of these subjects can be considered to have normal water and electrolyte absorption in the proximal jejunum (numbers 4 and 5). In two subjects (numbers 1 and 2) a positive absorption was observed but a considerable inhibition was present compared with normal subjects. In two other subjects (number 3 and 6) a net secretion of water, sodium and chloride was observed. Of the patients considered to have partial villous atrophy on jejunal histological examination, two exhibited a net secretion of water and electrolytes. This included one patient (number 3), in which evidence of Crohn's disease was found histologically on jejunal biopsy.

Patients 3 and 6, the two with the most marked absorption abnormality, were also found to be deficient in folic acid.

DISCUSSION

The results of this study indicate that many patients with Crohn's disease are likely to have inhibition of water and electrolyte absorption in the proximal jejunum and some may have a net secretion of water, sodium and chloride into the lumen of the jejunum. This suggests that the intestinal mucosa is involved to a much greater extent in Crohn's disease than generally appears from the degree of radiological involvement and

TABLE 15.1

CLINICAL DETAILS OF PATIENTS WITH CROHN'S DISEASE

Patient No.	Age	Sex	Duration of diarrhoea	Radiological involvement	Jejunal histology	Means of diagnosis
1	36	F	6 months	Mid and terminal ileum	Normal	Laparotomy
2	22	F	2 months	Mid-ileum: probably ascending colon	Partial villous atrophy	Radiology
3	28	M	12 months	Distal jejunum: most of ileum	Partial villous atrophy and Crohn's disease	Jejunal biopsy
4	46	M	18 months	Terminal ileum	Normal	Laparotomy
5	32	F	3 months	Mid and terminal ileum transverse colon	Normal	Radiology
6	31	F	6 months	Mid ileum: possibly ascending colon	Early partial villous atrophy	Laparotomy

TABLE 15.2

MEAN WATER, SODIUM AND CHLORIDE ABSORPTION IN PATIENTS WITH CROHN'S DISEASE

Patient No.	Mean Water absorption (ml/hr/30 cm segment)	Mean Sodium absorption (m.mol/hr/30 cm segment)	Mean Chloride absorption (m.mol/hr/30 cm segment)
1	35	3.1	1.9
2	68	13.6	11.8
3	-16	-2.4	-3.6
4	231	23.3	21.6
5	186	20.2	18.4
6	-10	-1.0	-1.8
Normal Control Subjects (mean levels)	220.8 ± 21.1	25.3 ± 1.8	22.9 ± 1.5

the jejunal histology. In only one patient of the group, radiological damage to the jejunum was present, although it is of interest that this patient did have a positive histological diagnosis of Crohn's disease in the jejunal biopsy, and had net secretion of water and electrolytes. Ileal involvement was present radiologically in all the patients studied but it does appear likely that involvement of the jejunum was present in these subjects, although no radiological damage is apparent at this stage.

Phillips and Schmid (1969) found a significant reduction in jejunal absorption of water and electrolytes in 4 cases of Crohn's disease but no information was available in this series about the jejunal mucosal histology. A significant reduction in bidirectional sodium flux was found in patients with Crohn's disease who had previously undergone panproctocolectomy with ileostomy (Allan, Steinberg, Dixon et al, 1975). Using radio-isotope techniques the bidirectional transport rates of water, sodium and potassium across isolated loops of small intestine was found to be abnormal by Atwell and Duthie (1964). Net water and sodium secretion was also observed in this study.

In another series no change in jejunal absorption was observed in a group of patients with Crohn's disease (Binder and Ptak, 1970). The variability of the results in Crohn's disease probably reflects the variability in the extent and severity of the disease. This is indicated by the wide range of water and electrolyte absorption results obtained in this series.

These results indicate the variability of jejunal involvement in Crohn's disease, but emphasise the probability of significant jejunal involvement in this condition although there may be no radiological evidence of this. These results provide more evidence that Crohn's disease is a diffuse rather than a focal lesion of the gastro-intestinal tract.

SUMMARY

Jejunal absorption abnormalities in 6 patients with Crohn's disease have been studied using the triple-lumen tube perfusion technique. Only two of the 6 patients could be considered to have normal water and electrolyte absorption. Two others had inhibition of absorption, and in two patients net secretion of water and electrolytes was observed.

The results emphasise the diffuse nature of Crohn's disease and indicate that the intestinal mucosa is involved to a much greater extent than can be judged by radiological appearances alone in this condition.

CHAPTER 16

CONCLUSIONS

In this section the technique of small intestinal perfusion has been applied to two conditions affecting the small-intestine - adult coeliac disease and Crohn's disease.

In adult coeliac disease it was found that all the patients had an inhibition of jejunal absorption of water and electrolytes and almost all exhibited net secretion of water, sodium and chloride during the study. In Crohn's disease the results were variable but in 4 of the 6 patients studied, an inhibition of water and electrolyte absorption was observed in the jejunum and in two subjects secretion of water, sodium and chloride was found.

No significant improvement was found in the absorption of water and electrolytes in patients with adult coeliac disease 3 to 4 months after starting a gluten-free diet, during which time all the patients studied had improved clinically and to some extent histologically. One patient showed an improvement of absorption one year after gluten withdrawal was started but one further patient showed no improvement at this time although it was subsequently found that this patient had not been keeping strictly to his gluten-free diet.

The results have considerable clinical relevance in that they offer an explanation for the watery diarrhoea which frequently occurs both in coeliac disease and Crohn's disease, and in coeliac disease may also account for the abdominal distension, pain and vomiting which are common features of this condition. This inhibition of water and electrolyte absorption, and secretion of fluid into the intestinal lumen in coeliac disease, may be part of the explanation for the symptomatology in this condition, but it is likely that multiple factors are involved. Thus when steatorrhoea is present, excessive faecal excretion of hydroxy fatty acids which are chemically similar to ricinoleic acid, may irritate the colonic mucosa and

cause secretion of fluid into the lumen of the colon, thus resulting in diarrhoea. Increased motility within the colon may also follow excessive faecal excretion of hydroxy fatty acids. Other factors may also follow failure of small intestinal function in coeliac disease. Brush border digestive enzyme failure (Welsh et al, 1969) impaired cholecystokinin release with secondary pancreatic insufficiency (DiMagno, Go and Summerskill, 1969), defective micelle formation (Low-Beer, Heaton, Heaton et al, 1971) and exudation (Waldmann, 1966) may all contribute to water malabsorption in the jejunum together with this intestinal secretion. Inhibition of water and electrolyte absorption and secretion, however, may be a major factor in this diarrhoea. In coeliac disease the proximal jejunum is the most markedly damaged part of the small intestine and some degree of reabsorption of water and electrolytes may occur in the ileum and to some extent in the colon.

In Crohn's disease, other factors may also be involved in causing diarrhoea in addition to secretion of water and electrolytes into the jejunum. Thus, breaking of the enterohepatic circulation of bile acids may cause cholerheic diarrhoea, and fat malabsorption with excretion of hydroxy fatty acids may also occur. However, inhibition of jejunal absorption of water and electrolytes and secretion in the jejunum, may have a greater effect in causing diarrhoea in Crohn's disease than in coeliac disease. The ileum and colon may be affected by the Crohn's disease and be unable to absorb any fluid which may have been secreted in the jejunum. There is thus less scope for adjusting to this jejunal abnormality, and symptoms may well be related to what is happening in the jejunum. In Crohn's disease a further clinical relevance of the findings is that the disease is likely to be more widespread in the small intestine than is often suspected by radiological examination. It seems likely that in Crohn's disease there is a diffuse abnormality which is widespread in the bowel

rather than focal lesions of the disease. Thus it appears that untreated adult coeliac disease and Crohn's disease may fall also into the category of secretory diarrhoea.

Little information is available from these studies as to the mechanism of the jejunal absorption abnormalities in coeliac disease and Crohn's disease. Inhibition of active sodium absorption by damage to the sodium pump seems likely and some loss of sodium following this may well occur. In addition the presence of a normal EPD across the jejunal mucosa in coeliac disease suggests that there is active secretion of sodium in addition to a permeability leak. Damage to the sodium pump may explain the inhibition of fluid absorption, but it cannot by itself explain the development of actual secretion. Stimulation of secretion is known to occur in conditions in which there is a release of cyclic AMP as in cholera. To date there is no evidence suggesting that there is stimulation of the cyclic AMP mechanism in these conditions but this requires to be excluded. Other factors must be also considered and investigated.

The lack of response of the absorption abnormality to exclusion of gluten from the diet in patients with adult coeliac disease is of considerable interest. This may indicate a slow response and indeed in one patient there was some trend towards improvement of absorption one year after starting gluten-free diet. On the other hand, it is possible that the absorption abnormality may remain for some time and may only improve in the proximal jejunum (where damage is most marked) much later in the response to gluten-free diet. The clinical response which occurred may be due to improvement in distal jejunal and ileal function, so that a greater degree of reabsorption of the fluid secreted in the proximal

jejunum may occur shortly after starting a gluten-free diet. The response of Crohn's disease to treatment either with sulphasalasine or corticosteroid therapy has not yet been studied, but the relative lack of the clinical response to these forms of treatment suggests that little change in the absorption abnormalities in the jejunum is likely to occur.

These studies have concentrated on only one possible cause of the diarrhoea which frequently occurs in coeliac disease and Crohn's disease and studies only one part of the alimentary tract - the proximal jejunum. As previously mentioned, multiple factors are likely to be involved in the pathogenesis of the diarrhoea in individual patients with these conditions. Each patient requires to be studied independently and individually to determine the degree to which each separate factor is contributing to the causation of the diarrhoea. Each factor should then be treated independently if at all possible.

These studies demonstrate that the small intestinal perfusion technique may be of value in determining the specific absorption abnormalities in various disease states. This allows a greater understanding of the pathogenesis of the symptoms associated with these diseases, and in turn is likely to lead to more effective forms of therapy for these symptoms.

SECTION V

CONSIDERATIONS ON FUTURE APPLICATIONS OF THE SMALL INTESTINAL PERFUSION TECHNIQUE

In the 15 years since the small intestinal perfusion technique was first described, considerable advances have been made in developing and understanding the technique, and a wide range of applications have appeared. With a greater understanding of the problems of the methodology, the way seems clear for further advances in the development of the system.

The present basic technique can be further applied to the study of the absorption of compounds other than water, electrolytes, glucose and amino acids. Thus, by using 3H - pteroylglutamic acid, the various influences on folic acid absorption can be studied using the intestinal perfusion system (MacKenzie and Russell, 1974). This has been applied to the study of folic acid deficiency in various disease processes such as coeliac disease and to investigate the importance of intestinal pH in the absorption of folic acid. The same basic intestinal perfusion system can be applied to study the effectiveness of acid neutralisation in the duodenum. The study of factors influencing this neutralisation may possibly shed more light on the pathogenesis of duodenal ulceration.

The application of the small intestinal perfusion technique to the study of fat digestion and absorption has long been sought, and the validation of 3H glycerol triether as an adequate lipid phase marker has made this feasible (Gerskowitch and Russell, 1974). The problems of applying the technique to the study of fat absorption are considerable but the availability of an adequate lipid phase marker opens up a new range of applications of the system.

The technique also has a potential wide range of applications in the study of absorption of various drugs and the influence of various factors on drug absorption. Standard methods for studying the absorption of drugs have always been indirect and to some extent unsatisfactory. Additional

valuable information can be obtained by applying the perfusion technique to drug absorption.

The system can also be applied to study the activity of membrane digestive enzymes by measuring the disappearance of oligosaccharides and oligopeptides from the perfusion solution, (Soergel, 1971). It is also possible to study the output of pancreatic juice and bile quantitatively by a modification of the perfusion method (Go, Hofmann and Summerskill, 1970). The technique can also be used to study electrical potential differences across the intestinal mucosa during intestinal absorption, by introducing a probe electrode into the jejunal lumen as part of the perfusion tube system. This technique may be of immense value in investigating the influence of various factors on intestinal absorption in the jejunum.

Further advances in the technique of intestinal perfusion can be expected. The use of isotopes such as 24 Na in conjunction with a perfusion system allows the measurement of bidirectional fluxes across the intestinal mucosa. The use of a "slow marker perfusion" (Soergel, 1971) in which an inert marker is added to the existing intestinal contents distal to the duodeno-jejunal junction, allows evaluation of intestinal contents during fasting, after test meals, after stimulation of pancreatic secretions, and after injections of hormones and drugs. The advantage of this system is that the technique interferes only minimally with intestinal motor function, bacterial flora and digestive secretions. It is thus much more physiological than the standard small intestinal perfusion technique.

Further advances can be expected in the application of the technique to many other disease processes, particularly in the study of the response of diseases to various forms of therapy.

The trend in the future of the small intestinal perfusion system is likely to be towards the development of more physiological systems which will be aimed at providing more specific information about the physiology

and pathophysiology of small intestinal function. The development and current application of the technique has provided much valuable, practical information which will in time help to develop more effective systems.

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