INTESTINAL BILE ACID ABSORPTION

Ileal transport and the kinetics of ^{75}SeHCAT
in gastro-intestinal disease.

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Galzuurabsorptie vanuit de darm

Transport door het ileum en kinetiek van ^{75}SeHCAT bij maag- en darmziekten.

PROEFSCHRIFT

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OP GEZAG VAN DE Rector Magnificus
PROF.DR. C.J. Rijnvos
EN VOLGENS HET BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 24 APRIL 1991 OM 13.45 UUR

DOOR

ANTONIE JAN PIETER VAN TILBURG

Geboren te Rotterdam

2L Produkties BV - Rotterdam - 1991

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The publication of this thesis was supported by
Merck Sharp & Dome B.V., Haarlem, the Netherlands.

aan Dorothé

List of abbreviations

BAM	bile acid malabsorption
BBMV	brush border membrane vesicles
c-AMP	cyclic adenosine monophosphate
^{14}C-CAT	^{14}C-cholic acid taurine
eqt	equilibrium time
FBAL	faecal bile acid loss
HMG-CoA	3-hydroxy-3-methylglutaryl CoA
FL4D	fractional loss after 4 days
INBALTC	in vitro Na^+-dependent bile acid local transport capacity
INBAT	in vitro Na^+-dependent bile acid transport
k	fractional turnover
Na^+,K^+ -ATPase	Na^+,K^+-adenosine triphosphatase
ret	retention
^{75}SeHCAT	^{75}Se-homocholic acid taurine
TC	taurocholate
WBR_{50}	whole-body retention half-life

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CHAPTER 1

General introduction

1. INTRODUCTION

Bile acids are formed in the liver and excreted with other constituents into the bile duct system, flowing with the bile into the duodenum. Due to their detergent properties they play a major role in the absorption of dietary fat from the proximal small bowel. Bile acids are efficiently absorbed from the bowel. The distal ileum plays a major role as it is the only site in the intestine where bile acids are actively transported. After absorption from the intestine bile acids are transported by the portal venous system to the liver, where they are efficiently cleared from the portal blood. Subsequently they can be re-excreted into the bile. This cycle is termed the enterohepatic circulation of bile acids.
Due to the active bile acid absorption in the distal ileum, only a small amount of bile acids enters the colon. An increased spill-over of bile acids into the colon, which can occur in ileal disease, often results in diarrhoea due to the cathartic properties of bile acids. In a variety of gastrointestinal diseases characterized by diarrhoea, but also in other conditions such as constipation and colonic neoplasms the enterohepatic bile acid circulation is in some way altered or disturbed. The scope of this thesis is a study of aspects of the relation between bile acid absorption and various gastrointestinal diseases.

In this chapter the anatomic structures involved in the enterohepatic circulation of bile acids, as well as its physiology and pathophysiology will be described. Diagnostic methods which provide insight into specific areas of the enterohepatic circulation will be discussed. Specific attention will be paid to the ileal absorption of bile acids.

1.1 ANATOMY OF STRUCTURES INVOLVED IN THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS

Anatomy of liver and bile duct system

The liver is one of the largest organs in the body (1200-1500 grams in adults). It has a double blood supply. The portal vein brings blood from the intestines and spleen to the liver and the hepatic artery, coming from the coeliac axis of the aorta, supplies the liver with arterial blood. Venous blood flows via the left and right hepatic veins into the inferior vena cava just below the diaphragm. The right and left hepatic ducts emerge from the right and left hepatic lobes uniting in the porta hepatis to form the common hepatic duct. The cystic duct coming from the gallbladder joins to form the common bile duct, which runs caudally behind the duodenum to the pancreatic head where it usually joins the main pancreatic duct to form the ampulla of Vater.

Morphology of the liver

Rappaport (1) introduced the concept of a liver built up out of functional acini centred around a portal triad with a bile duct, terminal branches of the portal vein and of the hepatic artery and connective tissue. Blood flows from the portal triad to a terminal hepatic (central) vein through sinusoids. The hepatocytes are arranged in columns along the sinusoids. The sinusoidal wall consists of endothelial and phagocytic (Kupffer) cells, with the space of Disse between the sinusoidal lining cells and the hepatocytes. Blood reaches the hepatocytes via pores (fenestrae) between the lining of endothelial cells. Bile formed in the hepatocytes is secreted into small channels with a diameter of 1 μm (canaliculi) formed by a groove in the cell membranes of two adjacent hepatocytes. The canaliculi interconnect to form bile ductuli and subsequently lobular bile ducts running in the portal triads.

Anatomy of the intestine

The small bowel (duodenum, jejunum and ileum) as well as the colon are involved in the enterohepatic circulation. The small bowel has a length of 5-6 meters, the duodenum measuring 25-30 cm, the jejunum 2-2.5 m and the ileum 2.5-3 m and enters the colon through the valvula of Bauhini in the right lower abdominal quadrant. The large intestine, with a length of 1 m, consists of the caecum with appendix, the ascending, transverse, descending and sigmoid colon and the rectum terminating at the anus. The arterial blood supply to the small intestine and the colon proximal of the splenic flexure comes from the superior mesenteric artery and to the descending, sigmoid colon and rectum from the inferior mesenteric artery. Branches of the mesenteric veins join in the superior and inferior mesenteric veins, with the splenic vein forming the portal vein, which runs to the liver.

Morphology

The wall of the intestine comprises 4 main layers with on the inner side the mucosa, consisting of the epithelium, lamina propria and the muscularis mucosae. Outside the mucosa are present the submucosa, the muscularis externa and on the outer side the serosa or adventitia. The surface area of the small bowel is increased by circular concentric folds (valves of Kerckring), by crypts and villi, which are fingerlike 0.5 - 1 mm projections of the mucosa with cores of lamina propria and by microvilli (figure 1), present on the surface of the epithelial cells and forming the brush border, where enzymes and carriers are present. In the colon villi are absent and the crypts are deeper than in the small intestine.

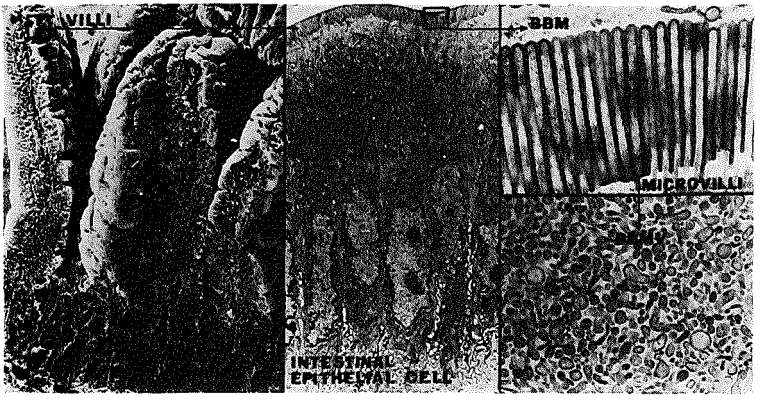

Figure 1: Scanning electron-micrograph of intestinal villi (left), electron-micrographs of an intestinal epithelial cell with its brush border (middle) (both kindly provided by Dr. J.M. van Dongen, Dept. Cellbiology & Histology, Erasmus University Rotterdam), detail of the brush border (upper right) and brush border membrane vesicles (BBMV) prepared in vitro (lower right) (both kindly provided by P.H. Cambier, University of Leiden).

1.2 PHYSIOLOGY OF THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS

1.2.1 Functions of bile acids

Bile acids (also termed bile salts) are considered to have three major functions (2). The first is **induction of bile flow.** The second is **lipid transport in bile and small intestinal content.** The third function is **promotion of catharsis.**

It has been known for at least a century, that bile acids **induce bile flow** (3), probably due to an osmotic effect of secreted bile acid anions (4). A linear relation exists between bile acid secretion and the secreted bile volume. At zero bile acid secretion the liver still secretes a watery bile - the bile acid-independent fraction, which is very small in man (5).

Since bile acids contain both hydrophilic and hydrophobic groups, they have **detergent and emulsifying properties.** The secretion of bile and pancreatic juice is mediated by the hormones cholecystokinin-pancreozymin (also capable of inducing gallbladder contraction), secretin and glucagon, which are released when food enters the duodenum. After secretion into the canaliculus bile acids form complexes with calcium ions and solubilize cholesterol and phospholipid-rich vesicles secreted by the liver, giving mixed micelles (6). Together, bile and pancreatic juice mediate the chemical hydrolysis and physical dispersion of nutrients - the process known as digestion. Bile acids disperse, in the form of mixed micelles, the fatty acid and 2-monoglyceride formed by the action of pancreatic lipase on dietary triglyceride. Micellar solubilization of fatty acid and monoglyceride increases their diffusion through the unstirred layer to the membrane of the enterocyte. It has long been recognized that micellar solubilization is essential for the absorption of fat soluble vitamins and cholesterol (7).

Bile acids have been used as **cathartics** for centuries, and it was generally assumed that bile acids increased intestinal propulsion. It was later shown that dihydroxy bile acids - chenodeoxycholic and deoxycholic acid, whether conjugated or unconjugated - inhibit water absorption and induce water and sodium secretion by the colon at concentrations above 3 mmol/l (8) by increasing intracellular cyclic adenosine monophosphate (c-AMP) levels (9,10).

1.2.2 Metabolism of bile acids

In 1943 Bloch et al. showed that bile acids are formed from cholesterol (11). In 1952 Siperstein et al. (12) and Bergström (13) identified this pathway as an important excretory route for cholesterol. The bile acids participating in the enterohepatic circulation are primary bile acids formed in the liver and secondary bile acids formed from primary bile acids by the action of intestinal bacteria. In man the two primary bile acids are cholic acid (3α,7α,12α-trihydroxy-5ß-cholanoic acid) and chenodeoxycholic acid (3α,7α-dihydroxy-5ß-cholanoic acid) (figure 2).

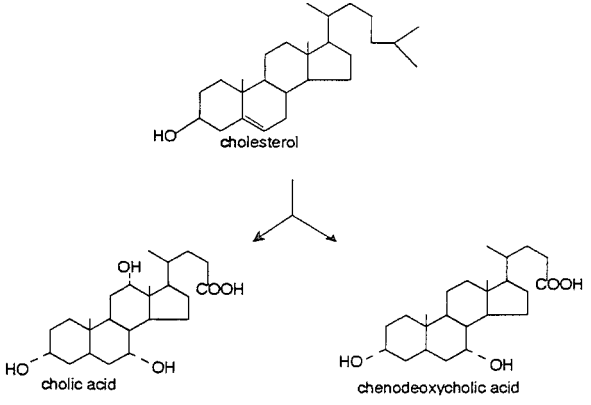

Figure 2: Structure of the primary bile acids cholic acid and chenodeoxycholic acid.

The conversion of cholesterol to bile acids involves saturation of the double bond, epimerization of the 3ß-hydroxyl group, reduction of the 5 double bond, introduction of hydroxyl groups at positions 7α and 12α, and cleavage of the C-27 side chain to C-24 carboxylic acid (14,15). The 7α-hydroxylation of cholesterol is the first step in the biosynthesis of both primary bile acids and considered to be rate-limiting. It has been demonstrated that the synthesis rate of cholic acid is about twice that of chenodeoxycholic acid (16). After formation the bile acids are amidated with glycine or taurine as N-acyl conjugates to form the four conjugated bile acids glycocholic acid, taurocholic acid, glycodeoxycholic acid and taurodeoxycholic acid. Conjugation is believed to be virtually complete in the hepatocyte. Taurine is the preferred amino acid for conjugation (17), but the concentration of taurine in the hepatocyte is usually so low that more bile acid is conjugated with glycine in a ratio of approximately 2 or 3:1 (18,19). Taurine administration expands the body taurine pools and results in bile acid-taurine conjugates as predominant conjugates. Conjugation has functional significance in that conjugated bile acids are more

resistant to precipitation in the presence of acid or calcium ions and are much more slowly absorbed by passive nonionic diffusion in the proximal small intestine than their unconjugated homologues. The cholyl and chenodeoxycholyl conjugated bile acids are excreted in the bile and most are reabsorbed in the small intestine without bacterial alteration. About one fourth of the primary bile acids are deconjugated by bacterial enzymes in the distal ileum (2). The majority of these bile acids are reabsorbed to return to the liver, where they are reconjugated with glycine or taurine. Each day one third to one fourth of the primary bile acid pool is converted by anaerobic bacteria to secondary bile acids and partially excreted (2). After deconjugation in the distal ileum or proximal colon 7-dehydroxylation converts cholic acid to deoxycholic acid (3α,12α-dihydroxy-5ß-cholanoic acid) and chenodeoxycholic acid to lithocholic acid (3α-hydroxy-5ß-cholanoic acid) (figure 3).

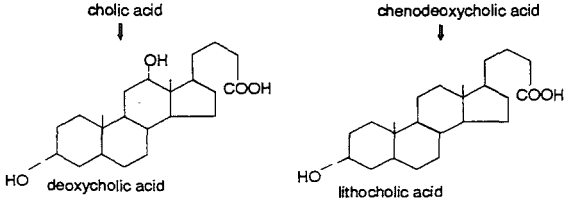

Figure 3: Structure of the secondary bile acids deoxycholic acid and lithocholic acid

One third to one half of the formed deoxycholic acid is absorbed and conjugated in the liver to glycodeoxycholic acid or taurodeoxycholic acid (2). After secretion into the bile it is reabsorbed with an efficiency similar to the conjugates of the primary bile acid chenodeoxycholic acid. Lithocholic acid is insoluble at body temperature and approximately one fifth is absorbed. Subsequently lithocholic acid is conjugated in the liver and the greater part is sulfated at the 3 position to sulfolithocholic acid glycine or sulfolithocholic acid taurine. Unsulfated lithocholic acid conjugates are probably largely reabsorbed from the small intestine and most sulfated in the liver (2). Sulfated conjugates of lithocholic acid are poorly absorbed and excreted via the faeces (2). Ursodeoxycholic acid (3α,7ß-dihydroxy-5ß-cholanoic acid), the 7ß-epimer of chenodeoxycholic acid is also present in human bile, formed partly by bacteria in the intestine from chenodeoxycholic acid and partly in the liver from the 7-keto derivative of chenodeoxycholic acid, which can be formed in the intestine by the action of bacterial 7-dehydrogenases on chenodeoxycholic acid. The formed keto-lithocholic acid is partly reduced in the liver to ursodeoxycholic acid, although the majority is converted to chenodeoxycholic acid. As it is formed in the liver from the secondary bile acid keto-lithocholic acid ursodeoxycholic acid is sometimes referred to as a tertiary bile acid. Other more uncommon bile acids are also present in minor quantities in human bile.

1.2.3 Dynamics of the enterohepatic circulation of bile acids

1.2.3.1 Bile acid pool size, composition and cycling frequency

Bile acid secretion can be measured by duodenal perfusion techniques and is 30-60 mmol per day depending on the dietary intake. The pool is 5-10 mmol and cycles 3-12 times daily. Roughly it consists of 35 % cholic acid, 35 % chenodeoxycholic acid, 24 % deoxycholic acid and 6 % lithocholic acid (20). Bile acid secretion is not related to bile acid pool size. Gastrin, secretin and glucagon stimulate the hepatocytes to secrete a bicarbonate rich watery bile (21,22), while gallbladder contraction is provoked by cholecystokinin-pancreozymin. The size of the bile acid pool can be decreased or increased by agents that accelerate or slow intestinal transit respectively (23). If bile acids are administered the pool expands until the maximal uptake rate in the intestine is exceeded. The limit for transport of bile acids in the enterohepatic circulation is the absorptive capacity of the intestine (2). Bile acids are absorbed solely into portal blood; there is no lymphatic absorption. Gallbladder bile has a bile acid concentration of 50 to 200 mmol/l and dilution in the small intestine lowers this concentration to 5 to 10 mmol/l. Portal blood has a concentration of 10 to 20 μmol/l and peripheral blood has a concentration of 2 to 6 μmol/l (2). The concentration in the canaliculus is unknown, but based on animal studies it is likely to be 5-100 mmol/l. Under normal conditions bile acids are barely detectable in urine due to efficient hepatic extraction, substantial binding to albumin and active tubular reabsorption. Therefore in healthy individuals daily faecal bile acid loss, mainly as deoxycholic acid, lithocholic acid and isolithocholic acid (the 3ß isomer of lithocholic acid), reflects hepatic synthesis of cholic and chenodeoxycholic acid respectively and does not exceed 1.2 mmol per day (24,25,26). This represents a daily faecal loss of 15 % of the bile acid pool and a loss per cycle below 5 % (27,28).

1.2.3.2 Regulation of bile acid synthesis

The liver has a great capacity for bile acid synthesis and normally bile acid synthesis may be considered to be "repressed" since it is one fifth to one tenth of it's maximal rate. It is known that the rate limiting enzyme in the synthesis of bile acids is cholesterol 7α-hydroxylase, but the mechanisms regulating bile acid synthesis are poorly understood. The simplest model is that bile acid synthesis is regulated directly or indirectly by the concentration of bile acids in the hepatocytes. When the enterohepatic circulation is interrupted surgically (bile fistula or ileal resection), pharmacologically (ingestion of bile acid binding agents such as cholestyramine), or pathologically (ileal disease), the concentration of bile acids in the hepatocyte falls and synthesis increases five to ten fold. In such situations the liver still makes twice as much cholic as chenodeoxycholic acid. Bile acid synthesis falls at ingestion of bile acids (29), during total fasting and in cholestasis (30).

1.2.3.3 Hepatic handling of bile acids

Bile acids are efficiently extracted by the liver, from the portal blood. A bile acid molecule resides in the systemic circulation for only a few minutes on average (31). Hepatic uptake of polar conjugated bile acids involves a saturable sodium

dependent process; uptake of lipophilic unconjugated bile acids is passive and sodium-independent.
The introduction of photoaffinity-labelling has set the stage for isolation of bile acid transport proteins (32). Cellular transport and secretion into the biliary canaliculi are rapid and result in low intracellular concentrations. Secretion of bile into the canaliculus involves an active sodium-independent transport process which is driven by the potential difference between hepatocyte and canaliculus (33).

1.2.3.4 Intestinal bile acid absorption

Absorption of bile acids may be passive, by diffusion from any part of the small or large intestine, or active, exclusively from the distal ileum (34).

Active ileal bile acid transport

In vitro and in vivo studies have demonstrated the presence of an active bile acid transport system in the distal ileum (35,36,37). This transport system is operative for all naturally occurring bile acids, conjugated and unconjugated. It is estimated that 70-82 % of the bile acid pool is reabsorbed each day as conjugated bile acid through active ileal transport (38). Transport is more rapid when the bile acid contains more hydroxyl groups (39). The uptake of trihydroxy bile acids is 6-8 times faster than the uptake of monohydroxy bile acids. Conjugation promotes active transport. Conjugated bile acids are transported 4-6 times faster than unconjugated bile acids and taurine conjugates are transported faster than glycine conjugates. Mutual inhibition of transport between the several types of bile acids has been demonstrated (40).

The data suggest that the ileal carrier has 2 recognition components for bile acids (41). One recognizes the steroid moiety. The other is suggested to be a cationic site on the membrane that has a coulombic interaction with the negatively charged bile acid.
Bile acids are absorbed against an electrochemical gradient and like other transport systems this requires sodium ions (42). Absorption is inhibited by ouabain, an inhibitor of Na^+,K^+-adenosine triphosphatase (Na^+,K^+-ATPase) (43) as the energy required for active transport is provided by the Na^+, K^+-ATPase localized in the basolateral membrane. Photo-affinity labelling has brought progress in the identification of the carrier protein (44).
Confirmation of the characteristics of this transport system has been made possible by in vitro experiments, allowing quantification of active bile acid transport in isolated brush border membrane vesicles, prepared from whole ileal tissue or ileal biopsies (45,46,47).

Passive bile acid absorption

The entire small and large bowel are capable of passive bile acid absorption. Passive absorption is influenced by the combined resistances of the unstirred water layer and the cell membrane. Passive diffusion may be in the ionized or in the non-ionized form depending on the prevailing pH and the dissociation constant (pKa) of the individual bile acid (48). Thus at normal intestinal pH

levels of 5.0 to 7.5 (49), a greater proportion of free bile acids with pKa's of 5.0 to 6.3 will be in the protonated (or nonionized) form than the glycine-conjugated bile acids (pKa's 4.3 to 5.2), while the stronger taurine-conjugated bile acids (pKa's 1.8 to 1.9) are almost entirely in the ionized form (50). Since passive bile acid absorption by nonionic diffusion is at least 5 to 6 times greater than diffusion of charged particles (51), free bile acids are absorbed more rapidly from extra-ileal sites than glycine-conjugates, while the ionized taurine-conjugates are almost totally dependent on the active transport site in the ileum for their reabsorption. The greater the number of hydroxyl groups, the lower the rate of membrane permeation. The orientation of the hydroxyl groups of bile acids also influences their passive absorption, 7α-hydroxy bile acids being better absorbed than their 7β-homologues. The length of the side chain seems unimportant (52).

The quantitative significance of passive absorption from extra-ileal sites is controversial (46). Although recent animal experiments have indicated that passive absorption in the jejunum at high bile acid concentrations might be more important than previously recognized (53,54), it is well known that ileal disease or resection frequently results in bile acid malabsorption and diarrhoea (55,56,57), while jejunal resection does not. During fasting, when bile acid secretion is occurring slowly but constantly due to 'incontinence' of the sphincter of Oddi, reabsorption might well be mainly jejunal (7). This could explain why fasting serum contains five more times conjugates of chenodeoxy cholic acid than of cholic acid (58), the latter being less easily diffusible and circulating less frequently (59). The absence of a gallbladder will make this mechanism more important and would also explain the major role of passive jejunal absorption of taurocholic acid in the rat (53).

1.3 PATHOPHYSIOLOGY OF THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS

1.3.1 Bile acid malabsorption

Symptomatic bile acid malabsorption occurs more frequently than is generally appreciated (60). The continuous painless watery diarrhoea usually said to be associated with this condition represents only one extreme of a spectrum of clinical disease (61).

1.3.1.1 Aetiology

Three types of bile acid malabsorption are recognized (62):
Type 1 follows resection or destruction of the ileal mucosa. Type 2, primary bile acid malabsorption, was hitherto considered rare. Type 3 is associated with cholecystectomy, vagotomy, some other gastro-intestinal conditions not involving the terminal ileum, and certain drugs.

Bile acid malabsorption type 1
As the ileum is the major site for bile acid reabsorption, ileal disease or resection causes bile acid malabsorption and in 1965 this was demonstrated in the dog (63). The most frequently encountered cause of ileitis is Crohns disease, but ileal pathology can also be found after radiotherapy, in amyloidosis, Reiter's Syndrome, coeliac disease, lymphoma, chronic use of nonsteroidal antiinflammatory drugs (64), lymphoma, tuberculosis and various other bacterial, viral and parasitic gastro-intestinal infections. Increased faecal bile acid excretion and/or markedly shortened half-lives of isotopic bile acids have been repeatedly demonstrated both in experimental animals and in man since (56,65,66,67,68). Symptoms may follow removal of as little as 15 cm (61).

Bile acid malabsorption type 2
The aetiology of abnormal bile acid loss in this condition, which was thought to be extremely rare, is unknown. It is also called primary or idiopathic bile acid malabsorption. In 1973 Hess Thaysen described three patients with chronic unexplained diarrhoea, bile acid malabsorption and a symptomatic response to the bile acid binding resin cholestyramine (69). He proposed a genetic defect of active ileal bile acid transport (70). Heubi demonstrated a decreased in vitro bile acid uptake into ileal biopsies of two boys with lifelong unexplained diarrhoea and bile acid malabsorption and stated that this supported Hess Thaysens hypothesis (71). Popovic described three adult patients with diarrhoea and bile acid malabsorption, who had villous atrophy in their terminal ileal biopsies and evidence of abnormal immune function, as demonstrated by the presence of auto-antibodies, complement activation and circulating immune complexes (72). He proposed an auto-immune disorder with the ileum as a target organ. However it is probably more correct to label these patients as suffering from type 1 bile acid malabsorption as their ilea are morphologically abnormal.

Several reports describing idiopathic bile acid malabsorption have followed since (60,73,74,75,76,77,78) suggesting that this condition is not as rare as previously thought (60,77,79,80). The mechanism of bile acid malabsorption has sofar not been elucidated.

Bile acid malabsorption type 3
Many conditions have been reported to be accompanied by increased bile acid loss, such as post-cholecystectomy diarrhoea (81), post-vagotomy diarrhoea (82), diabetes mellitus (83), medullary thyroid carcinoma (84,85), recovery from gastro-enteritis (85), cystic fibrosis (86), pancreatic insufficiency (60,87), coeliac disease (60), renal failure (88,89), 'food allergy' (85,90), secretory diarrhoea due to an apudoma (90), Zollinger-Ellison syndrome (91), fat hyperalimentation (92), bacterial overgrowth (79), lactose malabsorption (92) and various drugs, such as the bile acid binding cholestyramine and aluminium hydroxide, laxatives (85,90), neomycin and colchicine (93,94), phenphormin (95) and theophylline (85).
The mechanism of bile acid malabsorption in these conditions is unknown. It is speculated that ileal transit might be shortened, cycling frequency might be increased or the composition of fluids entering the ileum might be altered resulting in a lower bile acid uptake than normal (61).

Bile acid malabsorption in patients with colitis, either Crohn's disease apparently confined to the colon or ulcerative colitis has been reported (96,97,98) and attributed to the role of the ascending colon in the preservation of the enterohepatic circulation of bile acids (98,99). This mechanism might also be involved in collagenous colitis (85).

1.3.1.2 Clinical effects of bile acid malabsorption

Bile acid excess in the colon causing bile acid diarrhoea
Hofmann has introduced the term 'cholerheic enteropathy' to describe the syndrome of watery diarrhoea associated with the loss of bile acids into the colonic lumen when ileal reabsorption is insufficient (55). Inhibition of colonic salt and water absorption has been demonstrated in vitro and in vivo and has been attributed to dihydroxy bile acids (8).
Water and sodium secretion by the colon are induced at concentrations above 3 mmol/l (8) by a c-AMP mediated mechanism (9,10). Other mechanisms associated with the cathartic effect of bile acids are an increase in colonic motility (100,101), stimulation of defaecation (102), induction of mucus secretion, which has been documented in the dog (103) and mucosal damage and increased mucosal permeability (104,105,106). Bile acids also play probably a role in the regulation of small intestinal motility (107).

Bile acid deficiency in the small intestine causing maldigestion and malabsorption of fat and fatty acid diarrhoea
In patients with large ileal resections the increased bile acid synthesis cannot compensate for the increased loss. The concentration of bile acids in the proximal small intestine falls during the day - 1-2 mmol/l after meals in stead of the normal 4-12 mmol/l (108) - and is too low to efficiently solubilize in micellar form the products of fat digestion. Dietary fat is absorbed much more

slowly and throughout the length of the small intestine. Because of the loss of anatomic reserve the unabsorbed fatty acids pass into the colon and contribute to the diarrhoea by inducing water secretion (109).
The concentration of bile acids in stool water has been reported to be higher in patients with short ileal resections (up to 100 cm) and little or no steatorrhoea (57,110). The authors explain this finding by inhibition of the dehydroxylating enzymes of colonic bacteria by high cholic and chenodeoxycholic acid concentrations. These acids remain largely in solution. In contrast, after large resections dehydroxylation is for some reason unimpaired and the faeces have been reported to contain mainly the usual deoxycholic and lithocholic acids. These are more prone to precipitate than their parent acids, so the bile acid concentration in stool water is within normal limits at 1 to 3 mmol/l (57). The decreased bile acid pool size after large ileal resections contributes to this phenomenon. In these patients, who have gross steatorrhoea, diarrhoea seems mainly caused by unabsorbed fatty acids.
These findings have important **therapeutic implications**. With massive resections and gross steatorrhoea, the most effective anti-diarrhoeal treatment is restriction of dietary fat and replacement of the normal long-chain fatty acids by medium-chain fats. Although oral supplements of bile acids will decrease the degree of steatorrhoea, they also tend to increase the quantity of bile acids entering the colon and therefore may increase the diarrhoea. With shorter resections and bile acid diarrhoea, the most effective treatment is oral administration of a bile acid binding resin. In practice, ordinary antidiarrhoeal agents, designed to slow intestinal transit, such as loperamide, seem often to be as effective (111). Cholestyramine administration causes immediate improvement of the diarrhoea and the dose should be titrated since constipation may be induced (57). The use of cholestyramine increases faecal fat, but not enough to have nutritional significance. On the other hand, the absorption of fat soluble vitamins is seriously compromised, and cases of osteomalacia responding to vitamin D and of hypoprothrombinaemia responding to vitamin K have been reported (112,113). Patients on long term cholestyramine therapy should be given supplements of fat-soluble vitamins (7). These problems may be overcome by coating the resin with an indigestible material which is degradable by bacteria so that the resin is dispersed only where it is needed, in the colon (114). Less powerful bile acid adsorbents such as aluminium hydroxide can also sometimes be helpful in alleviating the diarrhoea (61).

Excessive oxalate absorption

The incidence of renal stones containing oxalate is higher in patients with ileal disease, bile acid malabsorption and steatorrhoea (2). In patients with steatorrhoea of any cause and an intact colon the quantity of urinary oxalate increases in direct proportion to the amount of faecal fat. Hyperoxaluria is usually not found unless fat excretion exceeds 15 g/day (115,116,117). Absorption of dietary oxalate is enhanced due to its increased solubility in faecal water in patients with steatorrhoea. Normally, oxalate is precipitated from solution by Ca^{2+} and thus made unavailable for absorption. In patients with steatorrhoea, excessive fatty acids are present to which Ca^{2+} preferentially binds (117), leaving oxalate in solution. Oxalate is absorbed passively from the small intestine. In healthy individuals the colon is considered to be

impermeable to oxalate anions (2). However bile acids (and fatty acids) increase the permeability of the colonic mucosa to small molecules, such as oxalate, so that oxalate is absorbed in greatly increased amounts from the colon (2).
Treatment consists of high fluid intake and dietary restriction of oxalate. In addition in some patients fat restriction, treatment with binding agents such as aluminium hydroxide and cholestyramine and supplemental calcium are necessary (2).

Gallstone formation
The depletion of biliary bile acids may jeopardize cholesterol solubility in bile (108,118,119,120) thereby predisposing to cholesterol gallstone formation (121,122).

1.3.1.3 Effects of bile acid malabsorption on the bile acid pool and cholesterol metabolism

Depending on the magnitude of bile acid loss, bile acid synthesis by the liver increases up to ten fold (2) by a similar increase in hepatic 7α-hydroxylase activity (123,124). Whether 12α-hydroxylase activity is suppressed resulting in a higher chenodeoxycholic acid:cholic acid ratio in both bile and blood (125) or normal with a normal chenodeoxycholic acid:cholic acid ratio (126) is disputed.

Depending on the cause of bile acid malabsorption, cholic acid does some times not come in contact with intestinal bacteria resulting in the disappearance of the secondary bile acid deoxycholic acid from the entero-hepatic circulation (125,127). Finally the glycine:taurine bile acid ratio increases from 2 or 3:1 to between 12 and 20:1 (66,67,128). There are two mechanisms for the increase in the glycine:taurine ratio. Firstly, the increased hepatic bile acid synthesis rate rapidly exhausts the limited stores of taurine, while the ubiquitous glycine is freely available. Secondly, in ileal dysfunction intestinal reabsorption of taurine conjugated bile acids is impaired to a greater degree, as absorption of taurine conjugated bile acids is almost entirely dependent on active ileal transport.

Since bile acid synthesis represents the major catabolic pathway for cholesterol (11,13) an increased bile acid synthesis leads to lower serum cholesterol levels. However, as interruption of the enterohepatic circulation also stimulates hepatic cholesterol synthesis by increasing HMG-CoA reductase activity, this effect is not always maintained (129).

1.3.2 Constipation

It has been suggested that constipation is associated with a low faecal bile acid loss (130). Constipation is a well known side-effect of bile acid binding agents. Faecal weight has been reported to correlate with faecal bile acid excretion (131). A few cases of severe, lifelong constipation with extremely low faecal bile acid content have been reported (132,133). Bile acids have been used to treat constipation (134,135). It is not clear whether constipation is the result or the cause of decreased bile acid excretion. It is tempting to speculate that small

bowel motility and therefore ileal bile acid transport plays an important role, as in patients with the irritable bowel syndrome small bowel transit was prolonged in the constipation subgroup and shortened in the diarrhoea subgroup (136).

1.3.3 Colonic neoplasmata

It has been proposed that colonic adenoma and carcinoma are related to alterations in the enterohepatic circulation of bile acids (137,138,139,140). Colonic absorption of deoxycholic acid and the proportion of secondary bile acids in duodenal bile have been shown to be elevated in patients with adenomatous colonic polyps (140). Increased faecal bile acid concentrations have been found in patients with colonic cancer (138,139) as well as a different faecal bacterial spectrum (137), with bacteria able to desaturate the bile acid nucleus (138).

1.3.4 Bacterial overgrowth (stagnant loop) syndrome

When normal propulsion in the small intestine is impaired because of anatomic or muscular abnormalities, or when there is continuous contamination of a region of small intestine by gastro- or entero-colonic fistula, anaerobic bacteria may proliferate, creating a "stagnant loop" syndrome. As a consequence bile acid deconjugation is greatly increased and unconjugated bile acids precipitate, since they are much less soluble at a given pH than are conjugated bile acids. Subsequently bile acid concentrations fall below their critical micellar concentrations, causing fat maldigestion and steatorrhoea. The unconjugated bile acids are eventually redissolved and reabsorbed so that there is little true interruption of the enterohepatic circulation in such patients (2).

1.3.5 Hepatobiliary disease: cholestasis, cirrhosis, hepatitis

In **cholestasis** bile flow is obstructed physically or ceases because of biochemical events in the liver and the major lipid constituents of bile - bile acids, lecithin, cholesterol, and bilirubin-diglucuronide - increase in blood. For bile acids, the increase will continue until a new steady state is obtained, when loss becomes equal to input. Hepatic synthesis falls by more than 50% (2). The major new conjugates formed are the sulphates and glucuronides of the conjugated primary bile acids (30,141,142). Sulphation greatly decreases tubular reabsorption of bile acids and may cause some decrease in albumin binding of bile acids. The net result is a great increase in renal excretion of sulphated bile acids (30), especially the sulphates of chenodeoxycholic acid. Serum bile acids remain mostly unsulphated because renal clearance of sulphated bile acids is so high. Increased sulphation seems a protective response by the liver, designed not only to promote renal excretion of bile acids, but also to prevent toxicity of accumulated bile acids within the liver cell, which can contribute to the pathogenesis of cholestasis (143,144), as sulphation abolishes their detergent properties (145,146).

The pruritus of cholestasis is probably caused by retention of bile acids in the skin, since itching patients have high concentrations of bile acids in their skin and the levels tend to fall to normal on the same day that the pruritus is relieved (147). This has been supported by the observation that application of all three major bile acids to blister bases induced itching (148).

In **cirrhosis** the total bile acid pool is decreased with a marked lack of cholic acid due to reduced synthesis (149). Synthesis of chenodeoxycholic acid is reduced to a lesser extent, which suggests a specific deficiency of the enzyme 12α-hydroxylase (150). Due to less dehydroxylation of cholic acid then normal (151) the deoxycholic acid content of bile is markedly reduced (152). Reduced uptake of bile acids by the liver (153,154) and porto-systemic shunting (2) result in increased serum bile acid levels. It has repeatedly been shown that there is relative excess of dihydroxy bile acids and of taurine conjugates in the blood (155,156,157). The urine contains increased amounts of sulphated bile acids (158,159). Cirrhotics secrete large amounts of diluted bile (160,161).

In chronic active **hepatitis** and acute hepatitis B serum bile acid levels are elevated early in the course of the disease (162,163).

1.4 DIAGNOSTIC METHODS FOR INVESTIGATION OF THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS

1.4.1 Analytical chemistry of bile acids

Bile salt concentration can be measured in faeces or bile enzymatically, by gas-liquid chromatography, by immunoassay or by high-pressure liquid chromatography (164). The bile acid concentration in plasma can be measured by a variety of enzymatic- or immuno-assays (164,165).

Enzymatic analysis based on the 3α-hydroxy steroid dehydrogenase (E.C.1.1.1.50) produced by Pseudomonas testosteroni is widely used (166,167) in a simple, moderately sensitive, rather specific measurement of all 3α-hydroxy bile acids, whether conjugated or unconjugated.

Gas-liquid chromatography, gas chromatography mass spectrometry, enzyme immunoassay, radioimmunoassay and high pressure liquid chromatography are more complex methods of measuring certain bile acid groups or individual bile acids and will not be dealt with in this chapter as they are not used for routine clinical purposes.

1.4.2 Serum bile acid measurements: principles and practice

Plasma bile acids fluctuate widely, being lowest in the fasted state and maximal about 2 h post-prandially. Concentrations in peripheral blood represent an instantaneous balance between absorption from the intestine and hepatic uptake and therefore reflect enterohepatic integrity. Due to the very efficient hepatic extraction of bile acids from the portal blood - more than 90 % on a single pass - blood levels are low (61). Hepatic clearance of a bile acid is not influenced by portal blood levels (168). It should be borne in mind, that differences in hepatic uptake and cycling rates for the various individual bile acids exist and are reflected by the corresponding serum levels. Intestinal absorption in the fasting state is determined by the amount of bile acids released in the intestine by the sphincter of Oddi and by intestinal transport, which in the fasting state is controlled by the interdigestive complex (2).

Whether **fasting-state serum bile acid measurements** actually provide clinically useful information remains unestablished. Some, but not all, studies have suggested that the level of serum bile acids is helpful in distinguishing chronic persistent from chronic active hepatitis (169). The sensitivity of serum bile acid levels appears to be superior to most other tests for detecting cirrhosis because possibly no other test is as sensitive as serum bile acid levels for the detection of porto-systemic shunting (2).

The use of an **oral tolerance test** has been explored by several groups, in which an unconjugated bile acid, such as ursodeoxycholic acid, is administered orally and the increase in its level in serum monitored (170). Although inconvenient, this test appeared to have excellent specificity and some sensitivity (2).

The **two-hour postprandial serum bile acid level** has been suggested to be a sensitive indicator of liver disease (171), but other studies have failed to confirm this observation (2).

As the postprandial increase in cholyl conjugates occurs because of active ileal transport, the postprandial elevation of cholyl conjugates has been shown to be much lower in patients with ileal dysfunction (168,172,173,174). However the clinical utility of this test has not been established (2).
In principle, measurement of **bile acid clearance after intravenous injection** is the best way of characterizing hepatic uptake of bile acids. In practice, however, mixing and distribution problems complicate the problem of unravelling the plasma disappearance curve to obtain an accurate estimate of hepatic uptake kinetics; thus, intravenous bile acid clearance tests are likely to have little utility (175).
In conclusion elevated serum bile acid levels indicate either cholestasis or liver cell injury. Although bile acid measurements have been shown to be excellent tests (176,177), other tests provide almost as much information and are less time consuming and less expensive. Nonetheless fasting-state serum bile acid measurements appear to have value for the prediction of the outcome of severe cirrhosis (178) and, in principle, are extremely sensitive indicators of portal systemic shunting (179).

1.4.3 ^{14}C-glycocholic acid breath test : principles and clinical utility

The ^{14}C-glycocholic acid breath test was the first test involving bile acids to become widely used in clinical practice. Measurement of the concentration of $^{14}CO_2$ in multiple aliquots of expired air, collected in the 5 h following the oral administration of [1-^{14}C]-glycocholic acid (figure 4), was originally introduced for the detection of bacterial colonization of the small intestine (180,181). Not the cholic acid moiety, but the glycine is labelled.

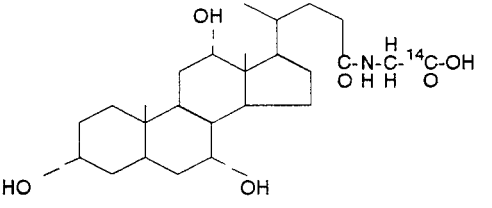

Figure 4: Structure of [1-^{14}C]-glycocholic acid

After encountering anaerobic bacteria, glycocholic acid is hydrolysed, the ^{14}C-glycine is oxidized with the release of $^{14}CO_2$, which is absorbed and detected in expired air.
Unfortunately, rapid intestinal transit cannot be differentiated from bacterial colonization (182,183), although an 'early' $^{14}CO_2$ maximum (within 4 hours) suggests proximal intestinal bacterial colonization and a 'late' maximum overspill of bile acids into the colon (184). A false-negative rate of 20 % in detecting bile acid malabsorption has been reported (185,186,187), possibly by the replacement of anaerobic gut flora by micro-aerophilic organisms (61).

Therefore modifications have been proposed such as increasing the period over which samples are collected to 24 h (188) and supplementing the breath measurements with direct assays of radioactivity in stool (180).
In conclusion, the ^{14}C-glycocholic acid breath test is moderately useful in the detection of bacterial colonization and bile acid malabsorption (189). Bile acid malabsorption can more directly be diagnosed by other techniques such as measurement of faecal bile acids or measurement of abdominal retention or faecal loss of a labelled bile acid. With the glucose H_2 breath test bacterial colonization of the small bowel can be measured equally well, with a high specificity (190), avoiding the use of radioactivity (191).

1.4.4 Faecal bile acid excretion and its clinical applications

Faecal bile acid excretion is the net result of hepatic secretion and passive and active absorption in the small and large bowel. As urinary excretion can be neglected except in cholestasis, under steady state conditions faecal bile acid excretion equals hepatic synthesis. Measurement of bile acid excretion has been reported to be superior to the Schilling test in diagnosing ileal malfunction (186,192).

1.4.4.1 Measurement of faecal bile acids

Individual and total faecal bile acids can be measured with chromatographic techniques, but the enzymatic 3α-OH steroid dehydrogenase assay is most frequently used in clinical practice.
Measurement of 3α-OH bile acids in faeces tends to underestimate total faecal bile acid content slightly (166,167) as sulphated lithocholic conjugates, as well as 3-oxo or 3-ester bile acids are not measured by this method. It is estimated that 75-95 % of faecal bile acids contain a free 3α-OH group (193). Although it has been reported, that determination of bile acids in a single morning stool sample is closely correlated with 3-day faecal bile acid excretion (194), it is usual to pool or average all stool passed for at least 3 days in order to minimise the effects of day-to-day variations (61).
Total faecal bile acid content measured with the 3α-OH steroid dehydrogenase method did not exceed 1.2 mmol/day in normals in several studies (25,26,78,195). Comparable values for the normal range of bile acid excretion measured with various methods are reported in some reviews by experts in the bile acid field (2,7,24,38,48).

1.4.4.2 Measurement of excretion of [24-^{14}C]-cholic acid taurine

At the introduction of the [1-^{14}C]-glycocholic acid breath test, supplemental measurements of excretion of ^{14}C radioactivity in stools were advocated in order to differentiate between bacterial colonization of the small bowel and bile acid malabsorption (180). However this makes the test much more complicated and less elegant. In addition ^{14}C-glycine formed by bacterial deconjugation is partly lost from the gut and expired, resulting in a consequent underestimation of faecal bile acid loss (84). [24-^{14}C]-cholic acid taurine (^{14}C-CAT), introduced as a tool for measurement of faecal bile acid loss, is at least as sensitive in detecting ileal pathology as [1-^{14}C]-glycocholic acid and more specific, as after bacterial deconjugation the ^{14}C label is attached to the steroid moiety (84) (figure 5).

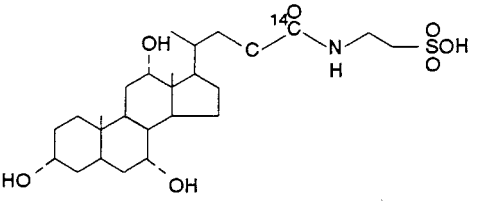

Figure 5: Structure of [24 - ^{14}C]-cholic acid taurine

Faecal ^{14}C-cholic acid activity can be determined by a whole-faeces combustion method (84,183). Excretion of ^{14}C-CAT is usually expressed as its whole-body retention (biological) half-life. Based on results in the literature (56,196) and results in our laboratory a ^{14}C-CAT half-life of 3 days has been chosen as the lower limit of normal (84). A curvilinear regression and a good mathematical correlation between the individual results of ^{14}C-CAT half-life measurement and total faecal bile acid excretion has been found (78,84). However the necessity of collection and subsequent combustion of stools have prevented this labelled bile acid from gaining widespread popularity.

1.4.4.3 The ^{75}Se-homocholic acid taurine (^{75}SeHCAT) test

The development and subsequent introduction in the early eighties of [23-^{75}Se]-25-homocholic acid taurine (^{75}SeHCAT) (197) has facilitated the measurement of bile acid excretion considerably (figure 6).

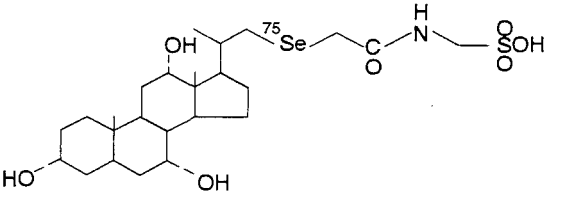

Figure 6: Structure of [23-^{75}Se]-25-homocholic acid taurine (^{75}SeHCAT)

^{75}SeHCAT is the taurine conjugate of a trihydroxy bile acid, selenahomocholic acid. This is an isomer of cholic acid which has been further modified by the incorporation of selenium into the side chain in the 23 position. At tracer levels this has little effect on its biological behaviour (197), which resembles that of taurocholic acid (74,90,198). The selenium can be labelled with the gamma-ray-emitting isotope selenium-75, which has a physical half-life of 121 days, decaying by electron capture. In the ionizing radiation emitted during the decay, gamma photons with energies of 120 keV (15%), 140 keV (54%), 270 keV (56%), 280 keV (23%) and 400 keV (12%) are of importance. Depending on the sensitivity of the counter an oral dose of 37-370 kBq (1-10 μCi) is administered. The largest dose usually given (370 kBq) results in a total absorbed dose of 0.2 μGy/kBq and a critical organ dose (small intestine and gallbladder) of 3.2 μGy/kBq (199). This implies that this dose is much lower than the skin doses received routinely during gastrointestinal fluoroscopy (199).
As the absorption of taurine conjugates of trihydroxy bile acids is almost exclusively due to active transport and as ^{75}SeHCAT was selected for its resistance to bacterial deconjugation, on theoretical grounds it is a specific marker for terminal ileal function.
Although the elimination of ^{75}SeHCAT is biexponential in man, the second phase of excretion is only detected after excretion of 96% of the administered dose (74). This implies that during the first two weeks, provided that there is no significant retention of activity in the colon, excretion is effectively monoexponential (61). The rate of excretion can therefore be expressed as the whole-body retention half-life, fractional turn-over, or the percentage retained or lost at any arbitrary time (61).
It has been claimed, that correction for colonic retention by $^{51}CrCl_3$ as a non-absorbable marker improves the accuracy of the ^{75}SeHCAT test (200). However appreciable colonic retention is unlikely in diarrhoea as was recently demonstrated by Smith et al. (201) and correction for retention by non-absorbable markers has not been generally accepted (61).

Two methods of measuring ^{75}SeHCAT excretion are applicable: measurement of faecal gamma activity in collected stools (198) or measurement of retained radioactivity with an uncollimated gamma camera (90) or a whole body counter (74). The correlation between ^{75}SeHCAT excretion and ^{14}C-CAT excretion has been shown to be excellent (198). Retention measurements with a whole body counter correlate well with abdominal gamma camera measurements (192,202). The ^{75}SeHCAT test based on retention measurements has been evaluated using the Schilling test (74,192,203) and faecal [1-^{14}C]-glycocholic acid measurements (90,203). ^{75}SeHCAT excretion has been shown to correlate with faecal bile acid excretion (74,80,204,205). However, the reference values for the ^{75}SeHCAT test, set by Nyhlin et al. (74), have been established on clinical grounds (i.e. presence or absence of ileal pathology). Based on this study the following reference values are still recommended by the manufacturer of ^{75}SeHCAT (Amersham International Ltd) -^{75}SeHCAT retention after one week: abnormal < 15% ; normal > 20% - and have been widely used in the literature (192, 98, 206). In other studies, usually involving small patient numbers and sometimes using different parameters for ^{75}SeHCAT excretion, different reference values have been

reported (60,90,207,208). It is surprising that reference values were never primarily based on faecal bile acid excretion, as it is now widely recognized, that bile acid malabsorption can occur in many other conditions than ileal disease (see 1.3.1) and the occurrence of diarrhoea is quantitatively related to the exposure of the colon to (dihydroxy) bile acids (8).
The ^{75}SeHCAT test is nowadays widely performed with retention measurement after one week, which has been reported to be superior to retention measurement after 4 days (74). Other protocols of execution of the ^{75}SeHCAT test have also been applied, with more than two retention measurements (200,204,207,209), or abdominal scanning on 4 occasions within 48 hours after intravenous administration of ^{75}SeHCAT (205). It has not been established, whether the ^{75}SeHCAT test protocol with measurement of gamma activity on only two occasions gives equally reliable results as the protocol with more frequent measurements. One of the factors probably determining the number of countings required is the sensitivity of the counter (61).

On theoretical grounds ^{75}SeHCAT is the optimal marker for ileal dysfunction as it is not subject to bacterial deconjugation and therefore exclusively actively absorbed in the distal ileum. In fact, reference values for the ^{75}SeHCAT test, set in initial clinical studies, have been largely based on the presence or absence of ileal disease (74,90) and it is not surprising, that many authors report a high **sensitivity** of the ^{75}SeHCAT test for ileal dysfunction (60, 85, 203-205, 207). One study reported a false-negative rate of 40 % in ileal Crohn's disease (208), but it should be borne in mind that the reference values applied for the ^{75}SeHCAT test in a particular study directly determine its sensitivity (208). Normal ^{75}SeHCAT excretion in ileal Crohn's disease can be encountered in limited ileal involvement (< 30 cm) (98), a low disease activity (192) or ileal stricture (208). Whether ^{75}SeHCAT can provide additional information about the **length of non-functioning ileum** is disputed. Some authors report a correlation between ^{75}SeHCAT test outcome and the length of the diseased or resected ileum (98,207,209), but this has not been confirmed by others (60,203).
^{75}SeHCAT excretion has been compared with faecal bile acid excretion with reference to the detection of ileal disease in only two studies (204,205). In one study some overlap was found in ^{75}SeHCAT whole-body retention half-life as well as in total faecal bile acid excretion between normal controls and patients with ileal disease, while in the second study no overlap was found in ^{75}SeHCAT whole-body retention half-life and faecal total 3α-OH bile acid and cholic acid excretion. These limited data suggest that determination of total faecal bile acid content is not inferior to the ^{75}SeHCAT test in detecting ileal disease.

As bile acid malabsorption can be associated with a variety of gastrointestinal conditions other than ileal malfunction (see 1.3.1), it is questionable whether the ^{75}SeHCAT test is a test with a high **specificity** for the diagnosis of ileal disease. Reviews of conditions associated with an abnormal ^{75}SeHCAT test, other than ileal pathology, are scarce (60,80,85). In none of these reports has the ^{75}SeHCAT test been compared with faecal bile acid measurements, which on theoretical grounds might be superior to the ^{75}SeHCAT test in detecting bile

acid malabsorption not due to ileal disease. Abnormal ^{75}SeHCAT test results have been reported in primary bile acid malabsorption (72), the irritable bowel syndrome (60,85), postgastrectomy diarrhoea (60,85,90), postcholecystectomy diarrhoea (76), drug-induced diarrhoea (85,90), aluminium hydroxide ingestion (60), diarrhoea due to food allergy or food intolerance (85,90), fat hyperalimentation (85,90), medullary thyroid carcinoma (85), secretory diarrhoea due to an apudoma (90), coeliac disease (60,76), recent proximal jejunal resection (76), pancreatic insufficiency (60), ulcerative colitis (85), Crohn's colitis apparently confined to the colon (192), and collagenous colitis (85). This listing closely resembles the conditions found in association with increased faecal excretion of ^{14}C-labelled or chemically measured bile acids and suggests that the ^{75}SeHCAT test has not the alleged high specificity for detection of ileal disease.

The **clinical utility** of the ^{75}SeHCAT test is disputed. Some authors prefer a therapeutic trial with cholestyramine as the ^{75}SeHCAT test is an expensive test (6,85,210) and bile acid malabsorption is thought to be rare (6). They accept the clinical response to cholestyramine as a valid indicator of a causal role for bile acids in unexplained diarrhoea. Others judge that the ^{75}SeHCAT test is very useful (61,76) and propose that a therapeutic trial with cholestyramine is more costly than the ^{75}SeHCAT test, less reliable ($\geq$ 50 % false-negative results) and takes longer, while bile acid malabsorption is less uncommon than previously thought (61).

1.4.5 Bile acid secretion, synthesis and pool measurements

Bile acid **secretion** can be measured only in its accessible part, the small intestine, by perfusion techniques (2) and occasionally in operated patients by a T-drain placed in the common bile duct.
Bile acid **synthesis** can be measured by quantitating faecal bile acid output or by isotope dilution techniques (16,211,212).
The total quantity of a bile acid, the '**pool size**', is calculated from the dilution of a ^{14}C-labelled bile acid in duodenal bile by simultaneous chemical assay of the individual bile acids and measurement of the concentration of radioactivity either in consecutive duodenal aspirates (213) or a single sample of bile (214). The combination of chemically measured faecal bile acid content and the calculated half-life of a labelled bile acid also allows for an approximation of the pool size (84). Duodenal sampling has the advantage that there has been no selective reabsorption or deconjugation and dehydroxylation of bile acids in the intestine, but is an invasive procedure. Compartmental analysis assumes a steady state in which the time taken for the tracer to be completely mixed in any compartment is much less than the turnover time. Because of the complex anatomy of the 9 bile acid containing compartments (215), namely the lumen of the small intestine, that of the large intestine, the gall bladder, and bile ducts, the liver, plasma and probably other tissues including fat, it is difficult to ensure that the fundamental conditions are properly satisfied (61). All estimates of pool size should therefore be treated with some reserve. A specific bile acid gives only information about itself. In the presence of malabsorption, deviations from the ideal rapid mixing become increasingly greater and estimates of pool size progressively less accurate.

The **composition** of the bile acid pool can only be determined with gas-liquid chromatography (216) or high-pressure liquid chromatography (217) of bile obtained with duodenal intubation or the Entero-Test, an easily applicable, alternative method for sampling of duodenal bile (218).
These methods are largely research tools and have little routine clinical applicability.

1.4.6 Micro-assays quantitating active ileal bile acid transport

Active ileal Na^+-dependent bile acid transport can be quantified in brush border membrane vesicles, prepared from ileal biopsies or whole ileal tissue.
In our laboratory brush border membrane vesicles are prepared (figure 1) using a modification of the method of Kessler et al. (219) based on a precipitation of divalent cations. Uptake of ^3H-taurocholic acid, present in a very low concentration, into brush border membrane vesicles in the presence of a 100 mM NaCl gradient is followed during 60 seconds and is related to the amount of brush border membrane protein. The microassay has been evaluated using human donor ileum and rabbit ileum (47) and specifically represents active Na^+-dependent bile acid uptake.

Active ileal bile acid transport is reduced in foetal dogs (220) and neonatal rats and dogs (221,222), and could explain considerably lower post-prandial duodenal bile acid concentrations found in near-term foetuses than in adults (223).
In cystic fibrosis, a condition often accompanied by bile acid malabsorption and steatorrhoea a specific active transport defect could not be demonstrated with this technique (46).
The mechanisms of diarrhoea with or without bile acid malabsorption and many other conditions, possibly associated with altered bile acid handling, such as postcholecystectomy or postvagotomy diarrhoea, colitis, colonic neoplasmata, constipation and the irritable bowel syndrome (see 1.3.1) are unknown. It is not known whether ileal active bile acid transport in these conditions is altered. It might be expected that active ileal bile acid transport is reduced in ileal disease or after ileal resection, but this has never been documented in vitro.
Hess Thaysen, who first described the clinical entity 'primary bile acid malabsorption', proposed a defective active bile acid transport (carrier defect) as the pathogenesis of bile acid malabsorption in this rare condition. Only two studies, in which three children are described with lifelong diarrhoea and unexplained bile acid malabsorption, have supported this hypothesis, although the technique used - measurement of the uptake of labelled taurocholic acid from a bile acid containing medium into whole ileal biopsies - could not discriminate between active or passive absorption or binding (75,224). In vitro measurement of active ileal bile acid transport could elucidate the pathogenesis of primary bile acid malabsorption in adults as well as the role of active ileal bile acid transport in relation to various gastrointestinal diseases.

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CHAPTER 2

Scope of the thesis

SCOPE OF THE THESIS

The work described in this thesis deals with the enterohepatic circulation of bile acids in relation to intestinal disease.
Active bile acid transport in the intestine, exclusively occurring in the distal ileum and playing a major role in the enterohepatic circulation, was specifically studied.

The aims of this study were:

- to characterize active bile acid transport in the distal ileum in various intestinal diseases affecting the enterohepatic circulation of bile acids by means of a microassay capable of quantifying active bile acid uptake into brush border membrane vesicles prepared from terminal ileal biopsies
- to evaluate whether bile acid malabsorption in idiopathic bile acid diarrhoea is due to an ileal bile acid carrier defect using the microassay, mentioned above
- to evaluate the utility of the gamma-emitting bile acid analogue ^{75}Se-homocholic acid taurine (^{75}SeHCAT) in predicting faecal bile acid excretion and to evaluate the reference values applied in the literature
- to evaluate which conditions give rise to an abnormal ^{75}SeHCAT and/or faecal bile acid excretion and to demonstrate possible relations between faecal weight, oro-anal transit time, Na^+ and K^+ excretion, bile acid pool size and bile acid excretion parameters in these conditions
- to evaluate the role of the ^{75}SeHCAT test in detecting ileal disease and predicting the length of inflamed or resected distal ileum.

CHAPTER 3

Na^+-dependent bile acid transport in the ileum: the balance between diarrhea and constipation.

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SUMMARY

Ileal Na^+-dependent bile acid transport was quantified in vitro as the uptake of ^3H-taurocholate into brush border membrane vesicles. Vesicles were prepared from ileal biopsies of 158 patients placed in 10 diagnostic categories. Active bile acid transport (expressed as pmoles taurocholate uptake per mg brush border membrane protein per 15 s, median and interquartile ranges indicated) did not differ significantly in 6 categories: irritable bowel syndrome (71, 35-97; n=21), colon polyps (42, 30-89; n=29), colitis (62, 33-91; n=31), postvagotomy or postcholecystectomy (69, 37-97; n=11), diarrhea without increased bile acid loss (58, 48-85; n=12) and lack of gastrointestinal pathology (74, 45-103; n=22). A decreased active bile acid transport was found in 3 categories: ileal disease (4, 1-36; n=11), partial ileal resection (5, 1-35; n=5) and constipation (41, 22-50; n=8). Bile acid transport was increased in patients with bile acid-losing diarrhea with endoscopically and histologically normal ilea (111, 94-135; n=8). These findings indicate that a low fecal bile acid loss, presumed to be present in constipated patients, is associated with a low Na^+-dependent ileal bile acid transport and a high bile acid loss with a high active bile acid transport. Ileal bile acid transport might be regulated by the availability of bile acids to the ileal enterocytes.

INTRODUCTION

Bile acids are absorbed in the small intestine with an efficiency of more than 95% per cycle (1,2). Although recent animal experiments have indicated that passive bile acid absorption in the jejunum at high bile acid concentrations might be more important than previously recognized (3,4), it is well known that ileal disease or resection frequently results in bile acid malabsorption and diarrhea (5-7). Therefore under normal conditions, with low luminal bile acid concentrations, active Na^+-dependent transport, which has the highest activity in the most distal part of the ileum (8-11), probably plays a dominant role in the intestinal reabsorption and the enterohepatic circulation of bile acids (1,12,13). Active ileal transport of various bile acids is probably mediated by a common carrier protein localized in the brush border (8-11,14-16). Ileal disease can result in decreased bile acid absorption and spill-over into the colon (5-7,17), where dihydroxy bile acids can stimulate secretion by increasing intracellular cyclic adenosine monophosphate (c-AMP) levels (18-19). It has also been proposed that colonic adenoma and carcinoma are related to altered ileal bile acid absorption (20-23). Increased fecal bile acid loss can occur in post-cholecystectomy or postvagotomy diarrhea (24-29), essential bile acid diarrhea (30-34) and various other conditions (35-42). The mechanisms of bile acid malabsorption in these situations are not well understood.

We have developed a microassay by which it is possible to measure in vitro Na^+-dependent bile acid transport (INBAT) using brush border membrane vesicles (BBMVs) derived from ileal biopsies (43).

Under in vivo conditions, Na^+-dependent uptake of conjugated bile acids is driven by a Na^+-gradient of about 125 mM across the brush border of the ileocytes (44,45), maintained by the Na^+,K^+-adenosine triphosphatase (Na^+,K^+-ATPase) localized in the basolateral membrane of the enterocytes. In vitro, because of the lack of this ATPase in the BBMVs after the initial rapid

increase of the intravesicular taurocholate (TC) concentration to several times the extravesicular concentration, the Na^+-gradient dissipates in about 1 min. With diminishing intravesicular sodium concentration the accumulated TC diffuses slowly out of the vesicle (15). The in vitro uptake velocity thus has to be determined within this first 1-min period. This INBAT parameter actually represents the rate of active bile acid transport across the brush border membrane under standard conditions.
The microassay has been evaluated using human, rat, and rabbit ilea (46). We measured INBAT in ileal biopsy specimens taken at colonoscopy in patients without gastrointestinal disease known to affect bile acid kinetics and in patients with conditions presumably associated with changes in intestinal bile acid handling.

MATERIALS AND METHODS

Patients

One hundred fifty-eight patients undergoing colonoscopy for diagnostic purposes were studied. According to their final diagnoses, they were assigned to 1 of the following 10 diagnostic categories:

1. irritable bowel syndrome (n=21), diagnosed by a compatible history and the absence of abnormal findings (apart from diverticulosis coli) on endoscopy or barium investigations.

2. colon polyps (n=30), identified at colonoscopy and histologically classified as hyperplastic or adenomatous polyps. This group also included 4 patients with familial polyposis coli and 4 patients with a history of colonic cancer.

3. ulcerative colitis or Crohn's colitis (n=31), diagnosed endoscopically and confirmed histologically, without evidence of ileal involvement.

4. history of a truncal or selective vagotomy or/and a cholecystectomy (n=11) without obvious diarrhea.

5. diarrhea (fecal wet weight > 250 g/day) with increased fecal bile acid loss (> 1.2 mmol/24 h) (n=8) caused by laxative abuse (1 patient), neuropathic diarrhea (1 patient), alcohol abuse (1 patient), persisting diarrhea after treatment of a Giardia lamblia infection (1 patient). In 4 patients no cause for the diarrhea was found.

6. diarrhea (fecal wet weight > 250 g/day) with normal fecal bile acid loss (≤ 1.2 mmol/day) (n=12) attributable to various causes.

7. ileal disease (n=11), identified endoscopically as ileitis and confirmed histologically.

8. ileal resection (n=5). Resection range 20-80 cm in 3 patients, unknown in 2 patients.

9. constipation (n=8), defined as complaints of infrequent and often difficult passage of hard stools.

10. miscellaneous (n=22), a group of patients not fitting any of the above categories. This group consisted of 13 patients with unexplained abdominal pain and/or occult intestinal blood loss, 2 patients with a lactase deficiency, 2 patients without diarrhea who were recovering from an infectious enteritis, 2 patients with solitary rectal ulcers and 1 patient with an adenomatous jejunal polyp resulting in an iron-deficient anemia, 1 patient with Menetrier's disease and 1 patient with a postoperative enterovesical fistula.

Normal values for fecal bile acid loss have been obtained from the literature (47-49) and from healthy volunteers.

Biopsy procedure
Ileal biopsies were taken during colonoscopy for histological studies, and 5-7 tissue samples (25-50 mg) were pooled and directly frozen and stored at -70 °C for subsequent INBAT measurements.

Human donor ileum preparation
The entire ileum of one renal allograft donor was used for INBAT measurements. After resection the intraluminal contents were directly removed by thorough washing with ice-cold saline (150 mM NaCl). The ileum was then cut into 10-cm segments and rapidly frozen in liquid nitrogen. For the INBAT measurements, ileal segments frozen in liquid nitrogen, were crushed in a mortar.

Preparation of brush border membrane vesicles
Brush border membrane vesicles were prepared using a modification of the method of Kessler et al. (50) based on a precipitation by divalent cations. Frozen (-70 °C) ileal specimens (25-50 mg) were thawed in ice-cold isotonic buffer (300 mM mannitol, 12 mM Tris-HCl, pH 7.1) at a final tissue concentration up to 250 mg/ml.
The pooled samples were disrupted in a minimum volume of 150 µl of isotonic buffer in an Eppendorf tube for two 45-s periods, using a Vibro-mixer (Model E1; ChemAp, Volketswil, Switzerland) fitted with a glass rod. Any small pieces of muscle and connective tissue, present only in the case of human ileal segments, were then removed using a microliter pipette. The suspension was diluted with 5 volumes of ice-cold water, and $MgCl_2$ was added to a final concentration of 10 mM. After standing for 40 min at 0 °C the suspension was centrifuged at 500 x g for 15 min. The supernatant was decanted and stored at 0 °C. The pellet was resuspended in buffer (50 mM mannitol, 10 mM $MgCl_2$, 2 mM Tris-HCl, pH 7.1) and the suspension was centrifuged as before. The pellet was discarded. The two supernatant fractions were combined and centrifuged for 30 min at 27,000 x g to spin down the BBMVs. The BBMV pellet was then resuspended in 34 µl buffer (100 mM mannitol, 10µM $MgSO_4$, 1 mM HEPES-Tris, pH 7.5) using a Hamilton syringe. Protein content was determined in duplicate on 6-µl samples (51).

Transport measurements

The BBMV suspension (17µl) was preincubated for 5 min at 25 °C. The incubation was started by the addition of 204 µl of transport buffer (100 mM mannitol, 108 mM NaCl, 10µM $MgSO_4$, 1 mM HEPES-Tris, pH 7.5, 25 °C) containing 4 µM ^{3}H(G)-TC (244 GBq/mmol, New England Nuclear, Boston, Mass.). The quantity of pooled ileal biopsy tissue permitted only a single 3-point INBAT determination. Samples (50 µl) of the incubation mixture were taken at 15, 30, and 45 s, diluted in 4 ml ice-cold stop buffer (100 mM mannitol, 100 mM NaCl, 10µM $MgSO_4$, 1 mM HEPES-Tris, pH 7.5, saturated with lithocholate (approximately 50µM lithocholate)), filtered over a 0.45-µM pore size membrane (SM 11306, Sartorius, Göttingen, Germany) on a filtration unit (Amicon Manifold, Amicon BV, Oosterhout, Netherlands; pressure difference 650-700 mmHg) and washed 4 times with 4 ml of the same stop buffer. Lithocholate was added to the stop buffer because it was found to reduce nonspecific binding of TC to the membrane filters. Radioactivity on the filter in the absence of BBMVs was subtracted from the total activity on the filter in the presence of BBMVs to correct for nonspecific binding. With human ileal biopsy specimens, the increase in TC uptake from 15-30 s was chosen as the optimal uptake parameter because it combines uptake quantification, minimal nonspecific binding and low intraassay variation.

Each membrane filter was dissolved in 10 ml scintillation liquid (Instagel, Packard Instruments, Groningen, Netherlands). Radioactivity was measured in a liquid scintillation counter (Packard Minaxi Tri-carb 4000).

Microassay evaluation

Unfortunately, pooled human ileal biopsy specimens do not yield enough BBMVs to measure brush border enzyme activities, brush border membrane enrichment, or passive TC uptake (NaCl vs. KCl) in individual patients. Human donor ileum and rabbit ileum were therefore used for evaluation of the microassay. Rabbit ileum was also used as reference material for INBAT measurements in all human ileal biopsy assay series.

Ileal biopsies contain only superficial mucosal tissue and can therefore be compared with mucosal scrapings. Kessler et al. (50) found no essential differences in characteristics of BBMVs prepared either from jejunal scrapings or from frozen whole jejunal tissue. We found similar results for INBAT measurements using rabbit ileal mucosal scrapings and frozen whole ileal tissue (data not presented), suggesting that INBAT data obtained from ileal biopsies are comparable to data obtained on BBMVs from whole ileal tissue. Uptake was expressed per mg BBMV protein rather than in terms of brush border enzyme activity because disease may have unpredictable effects on brush border enzymes.

The procedure of brush border membrane vesicle preparation in human donor and rabbit ileum was evaluated by maltase studies; 53% of maltase activity in the ileal homogenate was recovered in the BBMVs with an increase in specific activity from 102 to 1410 mU/mg protein (enrichment factor 13.8). The purity of the BBMVs is demonstrated by electronmicroscopy (Figure 1).

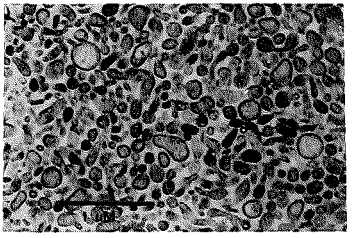

Figure 1: Electron-micrograph of rabbit BBMVs (original magnification x 23,000).

The rapid taurocholate uptake in the presence of a 100-mM Na^+-gradient compared with the minimal uptake in the presence of a 100-mM K^+-gradient (Figure 2) confirms the specificity of the active Na^+-dependent transport. The low uptake in the presence of a 100-mM K^+-gradient implies that passive diffusion as well as the osmotic effect of a 100-mM gradient is negligible under our microassay conditions because of a very low concentration of TC with a high specific activity (4 µM, 244.2 GBq/mmol ^{3}H-TC). Binding is also low, as shown by the low values of accumulated TC after 10 min, representing binding and intra-vesicular bile acid accumulation, compared with the high values at the moment of overshoot (first minute) (Figure 2).

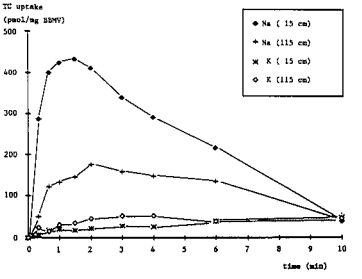

Figure 2: Taurocholate uptake in BBMVs from proximal (115 cm from the ileocecal valve) and from distal (last 15 cm) human ileal segments in the presence of a NaCl or KCl gradient (100 mM).

The distribution of Na^+-dependent bile acid uptake along the distal ileum was determined using 10-cm segments (0-130 cm from the ileocecal valve). Comparison of the transport curves obtained with BBMVs from these segments shows that active transport is present in the distal 60-90 cm of the ileum with the highest activity in the last 20 cm before the ileocecal valve (Figure 3).

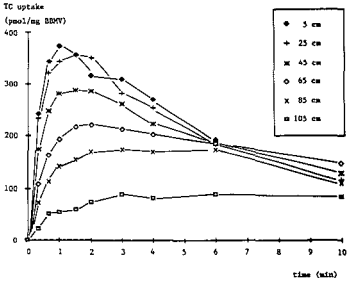

Figure 3: Taurocholate uptake in BBMVs from 10-cm segments of human ileum. The mean distance from the ileocecal valve is indicated for each segment. Incubation was in the presence of 4 μM TC and 100 mM NaCl .

Taurocholate uptake was found to be linear with the BBMV protein content (range, 2-20 μg/filter; Figure 4) and TC concentration (range, 2-10 μmol TC/l) (data not presented). The INBAT values were corrected for TC concentrations in the individual assays.

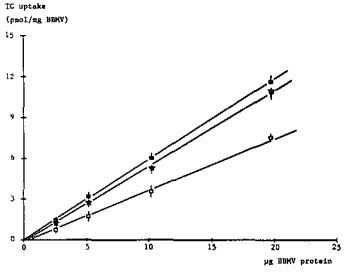

Figure 4: Taurocholate (mean ± S.D.) uptake by various amounts of BBMV (μg membrane protein) at 15 s (❑), 30 s (•), 45 s (■) after initiation of the uptake. Incubation was in the presence of 4 μM TC and 100 mM NaCl.

The coefficient of variation for the INBAT determination in reference rabbit ileal tissue was 23%-27% for TC uptake at 15, 30, and 45 s in 20 separate INBAT determinations over a period of 2 years (Figure 5). Individual human INBAT values were used only when the reference INBAT values were within the normal day-to-day assay variations. Determinations based on BBMV samples containing less than 2 μg protein were discarded.

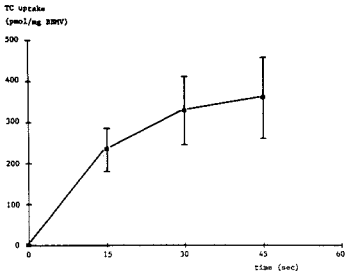

Figure 5: Mean TC uptake (mean ± S.D.) by equal amounts of BBMVs from reference rabbit ileum at 15, 30, and 45 s after initiation of uptake in 20 successive series of INBAT determinations. Incubation was in the presence of 4 μM TC and 100 mM NaCl.

Statistical analysis

The INBAT values of the various categories were compared using a Wilcoxon signed-rank test. The INBAT values (Figure 6) are presented with median values and interquartile ranges.

RESULTS

The mean BBMV yield per ileal biopsy (μg BBMV protein/mg wet tissue) for each of the various diagnostic categories is presented in Table 1. The BBMV yield did not differ significantly for most diagnostic groups. Only the constipation group showed an increased yield (Wilcoxon, $p < 0.05$) compared with all other groups except the diarrhea and postvagotomy/ postcholecystectomy groups.

The mean TC uptake values after 15, 30, and 45 s, corrected for binding to the filters and the TC uptake between 15 and 30 s, are presented in Table 1. Unless specified otherwise the term **INBAT** is preserved for the TC uptake between 15 and 30 s.

A wide interindividual variation was found in the INBAT values (Δ15-30) in the various categories (Figure 6). INBAT values were found in the same range in 6 of the diagnostic categories: irritable bowel, colon polyps, colitis, diarrhea without abnormal bile acid loss, cholecystectomy or vagotomy, and rest. Mean INBAT was reduced in ileitis, ileal resection, and constipation, and increased in bile acid-losing diarrhea (Tables 1 and 2).

As is illustrated in Figure 3 the time course of the TC uptake into BBMVs of various areas of the ileum is different: the rapid initial increase in TC uptake is more pronounced in the distal than in the proximal ileum, resulting in a higher fractional TC uptake in the first 30 s relative to the maximal uptake. Because differences might exist in this respect between the various diagnostic categories, the fractional TC uptake in the first 30 s at the TC concentration used is indicated as 30/max in Table 1. The mean 30/max value found for the ileal resection category is lower than the 30/max values of all other categories and corresponds with the lower fractional TC uptake (not indicated) for the proximal donor ileum. However, due to the wide variation and small number of patients in this group, this decrease was not significant (Wilcoxon test). In all other diagnostic categories, the fractional uptake in the first 30 seconds was

found to be in the same range, with the exception of the constipation group, when a significant increase in fractional TC uptake was found (Wilcoxon, $p < 0.05$). The mean 30/max value of 0.99 indicates that the maximal TC uptake is reached earlier in this category.

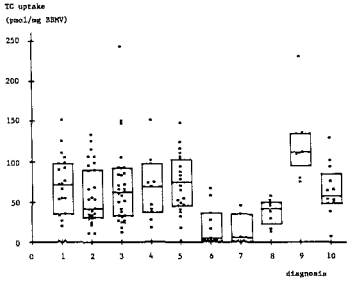

Figure 6: In vitro Na^+-dependent bile acid transport values expressed as picomoles TC uptake per mg membrane protein per 15 s. The median, 25th, and 75th percentiles are indicated. 1, irritable bowel syndrome; 2, colon polyps; 3, colitis; 4, postvagotomy and postcholecystectomy; 5, miscellaneous; 6, ileitis; 7, ileal resection; 8, constipation; 9, diarrhea, bile acid loss > 1.2 mmol/day; 10, diarrhea, bile acid loss ≤ 1.2 mmol/day.

Table 1: Taurocholate uptake of brush border membrane vesicles prepared from ileal biopsies of patients with various gastrointestinal diseases after 15, 30, and 45 s incubation, uptake of taurocholate between 15 and 30 s, fractional taurocholate uptake in the first 30 s, and brush border membrane vesicle yield per diagnostic category

Diagnostic	uptake (pmol/mg protein)					BBMV yield	
categories	15s	30s	45s	Δ15-30s	30/max	(μg/mg ±SD)	n
Irritable bowel	134	200	230	68	0.87	1.27± 0.55	21
Colon polyps	124	177	192	58	0.94	1.30± 1.15	29
Colitis	109	175	199	68	0.88	1.51± 1.05	31
Postvag/postchol	154	220	262	67	0.84	1.75 ±1.14	11
Miscellaneous	142	212	255	71	0.84	1.34± 0.70	22
Ileitis	26	43	48	19	0.92	1.16± 0.55	11
Ileal resection	23	40	100	17	0.40	1.05± 0.25	5
Constipation	76	111	113	37	0.99	2.07 ±1.05	8
Diarrhea							
FBAL > 1.2mmol/d	205	329	395	124	0.82	1.46 ±1.13	8
FBAL ≤ 1.2mmol/d	137	201	249	66	0.81	1.54± 0.62	12

Postvag/postchol: Postvagotomy/postcholecystectomy; FBAL: fecal bile acid loss.

Table 2: Levels of significance of the differences in values of in vitro Na^+-dependent bile acid transport between diagnostic categories

Diagnostic categories	Ileitis	Ileal resection	Consti-pation	Diarrhea, FBAL > 1 .2
Irritable bowel	0.01	0.05	0.05	0.01
Colon polyps	0.01	0.05	NS	0.01
Colitis	0.01	0.01	0.05	0.01
Postvagotomy/Post-cholecystectomy	0.01	0.05	NS	0.01
Miscellaneous	0.01	0.01	0.05	0.01
Diarrhea,FBAL ≤1.2	0.01	0.01	0.05	0.01
Constipation	NS	NS		0.01

NS, not significant ($p > 0.05$); FBAL, fecal bile acid loss (mmol/day). Statistical significance expressed as p value of Wilcoxon signed-rank test. The 4 categories with decreased or increased INBAT values are indicated horizontally; the other 6 categories are indicated vertically.

DISCUSSION

The microassay developed in our laboratory enables us to quantify active bile acid transport across the ileal brush border in patients in a relatively simple way. The characteristics of active ileal bile acid transport using our microassay on human donor ileum are in keeping with the literature (15,16,45) and previous studies by our department (43,46). Uptake of TC takes place via an active, Na^+-dependent transport system, with the highest activity in the distal ileum (Figure 3) (8,10). The yield of BBMVs from ileal tissue (μg BBMV protein/mg wet tissue) was similar for most diagnostic categories. An increased BBMV yield was found in the constipation group only, a finding we cannot readily explain.

INBAT is expressed as pmoles TC per mg BBMV per 15 s (Δ TC uptake, 15-30 s). INBAT values found in individual patients only reflect a single observation in an ileum that along its course has a bile acid absorption gradient of unknown shape. These values do not necessarily represent the overall active transport capacity of the ileum. This means that individual INBAT values from one single ileal site have limited diagnostic value.

Analyzing the INBAT results in the various diagnostic categories, the rest group, consisting of various patients without gastrointestinal disease known to affect the enterohepatic bile acid cycle, might serve as a control group. Healthy individuals were not included in our study because colonoscopy and ileal biopsy is an invasive procedure. Five categories did not differ significantly from

the rest group: irritable bowel, colon polyps, colitis, postcholecystectomy or postvagotomy, and diarrhea without increased bile acid loss. Therefore, these findings fail to provide an explanation for the altered bile acid metabolism which has been reported in colon polyps and after vagotomy or cholecystectomy (20-29).

INBAT determinations in ileal biopsies of the diagnostic categories show a wide scatter (Figure 6). This suggests a wide biologic variation in the INBAT values of individuals within these categories because the interassay variation of the INBAT contributes only partly to this scatter (Figure 5).

In 4 groups INBAT determinations differ from the first 6 groups. As might be expected, decreased INBAT was found in ileal disease and after distal ileal resection, conditions in which increased bile acid spillover into the colon is found because of impaired ileal absorption. Surprisingly, INBAT was also reduced in patients with constipation. Conversely, increased INBAT was found in bile acid-losing diarrhea. Decreased INBAT values in the constipation group were found, together with an increased BBMV protein yield and a higher fractional TC uptake in the first 30 s. All these changes can be a result of a regulatory or an adaptive mechanism. Whether these findings are somehow interrelated will be subject of further study.

If we assume that INBAT results reflect the number of carriers per cell and that a regulatory mechanism is present for the number of bile acid carriers per ileocyte in a fashion similar to that described for vitamin B_{12} (52) then the results in constipation and bile acid-losing diarrhea could be explained by motility-induced changes in luminal bile acid concentrations. Diarrhea with increased fecal bile acid loss was associated with increased INBAT. In this condition the luminal concentration of bile acids in the last centimeters of the ileum will presumably be higher than normal as a result of inefficient absorption higher up, probably secondary to a short ileal transit time. This might give rise to up-regulation of the number of bile acid carrier molecules. After distal ileal resection, frequently leading to bile acid-losing diarrhea, low INBAT values are found. This demonstrates the lower bile acid transport capacity in this part of the ileum (Figure 3), which apparently is not capable of a marked adaptation (53).

In patients with irritable bowel syndrome, small bowel transit has been reported to be prolonged in the constipation subgroup and shortened in the diarrhea subgroup (54). Dowling suggested that constipation is associated with a low fecal bile acid loss (55) because constipation can be induced by bile acid-binding agents, fecal weight correlates with fecal bile acid content (56), a few patients with severe constipation and very low fecal bile acid losses have been reported (57,58), and bile acids have been used to treat constipation (59,60). In constipation, a slower ileal transit could result in more efficient bile acid absorption in the upper part of the actively transporting ileum, leaving only a low concentration of bile acids available to the more distal ileocytes in the biopsy area, where low INBAT values are found. In conclusion, a change in local bile acid concentration might trigger up- or down-regulation of the number of bile acid carriers in the distal ileum.

Table 3: Concept of the relationship between ileal transit and in vitro Na^+-dependent bile acid loss in normal individuals and in patients with bile acid-losing diarrhea or constipation

	BA-losing diarrhea	Normal	Constipation
Small intestinal transit	Rapid	Normal	Slow
Luminal BA concentration in:			
First part of the distal ileum	High	High	High
Last part of the distal ileum (biopsy area)	High	Intermediate	Low
INBAT (biopsy area)	High	Intermediate	Low
Net ileal BA resorption	Decreased	Normal	Increased

BA:bile acid

The concept that the availability of conjugated bile acids to the ileocytes stimulates Na^+-dependent bile acid uptake is illustrated in Table 3. An important consequence of this hypothesis is that bile acid malabsorption in patients with morphologically normal ilea is probably caused by motor abnormalities resulting in decreased ileal transit time. Further experiments are needed to support this proposition.

ACKNOWLEDGEMENT

The authors thank prof. J.H.P. Wilson and dr. G.R. Swart for critical reading of the manuscript and Gardi Voortman, Jeannette Touw-Blommesteyn for their expert laboratory assistance, and P.H. Cambier of the Laboratory for Electron Microscopy of the University of Leiden for his technical assistance in the electron microscopy on the brush border membrane vesicle pellets.

Parts of this work have been presented as posters at the Autumn Meeting of the British Society of Gastroenterology, 1985 (Gut, 1985;26:A1113-4) and the Meeting of the American Gastroenterological Association 1987 (Gastroenterology, 1987;92:1681).

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CHAPTER 4

Primary bile acid diarrhoea without an ileal carrier defect. Quantification of active bile acid transport across the ileal brush border membrane.

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SUMMARY

Unexplained bile acid malabsorption associated with diarrhoea responding to cholestyramine was first described by Hess Thaysen in 1973. Convincing evidence of the proposed mechanism of increased bile acid loss - a defective active ileal bile acid transport - has never been reported.
Active bile acid transport was quantified in vitro using brush border membrane vesicles prepared from terminal ileal biopsies of 10 patients fulfilling the criteria of idiopathic bile acid diarrhoea, recruited out of a group of 181 patients with bile acid malabsorption due to various causes.
Transport was quantified as in vitro Na^+-dependent bile acid transport (INBAT) (expressed as pmoles taurocholate/mg brush border membrane protein/15 seconds), and in vitro Na^+-dependent bile acid local transport capacity (INBALTC) (expressed as pmoles taurocholate/gram ileal biopsy tissue/15 seconds).
The lowest INBAT and INBALTC values found in the 10 patients with idiopathic bile acid diarrhoea were well above the 10^{th} percentile values of a control group of 132 patients.
Both INBAT (mean 88, range 30-136) and INBALTC (mean 158, range 85-268) were significantly higher in the 10 patients than in the control group (INBAT: mean 63, range 1-244, INBALTC: mean 98, range 1-408).
Quantification of active ileal bile acid transport in these 10 patients with idiopathic bile acid malabsorption suggests that a genetic (carrier) defect is rare in adults.

INTRODUCTION

Increased faecal bile acid loss occurs in the majority of patients with terminal ileal disease and after ileal resection. This is called bile acid malabsorption type 1 (1,2).
Hess Thaysen described three patients with chronic diarrhoea of unknown aetiology, increased faecal bile acid loss and a response to cholestyramine treatment (3,4). Several more patients with idiopathic or primary bile acid malabsorption (type 2) have now been described (5-7), as determination of bile acid excretion has been facilitated by the introduction of ^{14}C- and later ^{75}Se-labelled bile acids and the $^{75}SeHCAT$ test has gained wide use in the analysis of chronic diarrhoea.
Various other conditions have been reported to be accompanied by increased faecal bile acid loss (bile acid malabsorption type 3), such as post-cholecystectomy diarrhoea (8,9), post-vagotomy diarrhoea (10), diabetic diarrhoea (11,12), medullary thyroid carcinoma (13), cystic fibrosis (14,15) and use of various drugs such as colchicine and neomycin (16,17), theophylline (18), biguanides (19), mannitol (20) and various laxatives (18).
The aetiology of primary bile acid malabsorption is unknown. In 1973 Hess Thaysen proposed a genetic defect of active ileal bile acid transport, but was unable to confirm this hypothesis in his (adult) patients. Recently in three patients with primary bile acid malabsorption distinct morphological changes were described in terminal ileal biopsies, consisting of crypt hyperplastic villous atrophy, colonic metaplasia and increased mononuclear infiltration of

the lamina propria (21). These patients also showed evidence of abnormal immune function, as demonstrated by the presence of auto-antibodies and complement activation. The authors therefore postulated an auto-immune disorder with the ileum as a target organ resulting in bile acid malabsorption. These findings have not been confirmed by other authors. Heubi et al. reported a defective in vitro ileal bile acid uptake in two boys with lifelong diarrhoea and steatorrhoea, who had an ultrastructurally normal terminal ileum (22). However the technique used in this study, based on an incubation of whole ileal biopsies in a bile acid containing medium for 2 minutes, could not discriminate between active absorption, passive absorption or binding. Recently we described the results of an in vitro assay performed in brush border membrane vesicles derived from terminal ileal biopsies, allowing the selective quantification of active Na^+-dependent bile acid transport. As a genetic carrier defect would be expected to be present anywhere in the distal ileum, we used this technique to investigate the role of the brush border membrane in primary bile acid malabsorption. Bile acid transport was quantified as in vitro Na^+-dependent bile acid transport (INBAT) and as in vitro Na^+-dependent bile acid local transport capacity (INBALTC) in brush border membrane vesicles derived from terminal ileal biopsies obtained at colonoscopy with retrograde ileoscopy in 10 patients with bile acid malabsorption of unknown cause. Results were compared with a group of 132 patients without disorders known to affect active bile acid transport in the distal ileum (23).

METHODS

Patients

Ten patients referred to our hospital between 1981 and 1988 for analysis of chronic diarrhoea were found to have bile acid malabsorption, defined as 3α-hydroxy bile acids ≥ 1.2 mmol/d (24-27), for which extensive analysis failed to show a cause. There was no history of food intolerance, significant weight loss, alcohol abuse, use of laxatives or other medication, possibly related to the diarrhoea. Routine blood tests including erythrocyte sedimentation rate, full blood count, creatinine, urea, electrolytes, total protein, albumen, bilirubin, alkaline phosphatase, alanine aminotransferase, and glucose were normal.

Patient characteristics, duration and nature of the diarrhoea, faecal weight, faecal bile acid excretion, and the ^{75}SeHCAT test results including recovery of radio-opaque markers, administered to 5 patients, are presented in table 1. Simultaneous administration of these markers with the ^{75}SeHCAT dose as a control on the accuracy of faecal collection was routinely performed from 1983 onwards. The age and sex distribution of this group of 10 patients (mean age 44 ±9 yrs, m/f ratio 1.0) was not different from the group of 132 control patients (mean age 39 ± 15 yrs, m/f ratio 0.69). All patients were thoroughly investigated and other causes of diarrhoea were excluded. Results of the investigations, performed in the analysis of diarrhoea, are presented in table 2. Small bowel X-rays, colonoscopy with retrograde ileoscopy and microscopy of terminal ileal biopsies were normal in every patient.

Table 1 : Clinical data of patients with primary bile acid malabsorption in relation to bile acid excretion

patient: ident., sex(m/f)	age (yrs)	duration (yrs) character of diarrhoea	faecal weight (g/d)	FBAL	^{75}SeHCAT WBR$_{50}$ (days)	FL4D (%)	recovery of 25 markers on days: 1	2	3	effect of chol
1. RK,f	37	> 7 / cont	290	3.3	1.3	79	23	2	0	++
2. AK,m	39	5/ int	290	3.7	1.1	83	24	0	1	+
3. BN,m	34	2/ int	250	1.5	1.6	77	7	18	0	+
4. NP,m	56	> 5/ cont	.	3.2	1.0	96	.	.	.	+
5. HR,f	38	5-10 / cont	280	3.3	0.1	99	.	.	.	++
6. AS,f	31	4 / cont	.	3.3	1.5	85	7	18	0	+
7. CS,f	55	5-10 / int	.	1.3	1.7	77	.	.	.	++
8. BV,m	55	> 10 / cont	270	1.8	1.8	78	.	.	.	+
9. MW,m	53	> 10 / int	240	2.2	2.1	75	20	4	0	+
10. AW,f	44	> 20 / cont	260	2.7	1.2	89	.	.	.	+

chol: cholestyramine; marked response (+), complete response (++), FBAL: faecal bile acid loss in mmol/d (normal < 1.2), WBR50: whole-body retention half-life (normal > 2.8 d, abnormal < 1.8 d), FL4D: fractional loss in 4 days (normal < 58 %, abnormal > 72%), cont: continuous, int: intermittent, (.) not done.

Control group

INBAT values of 132 patients undergoing colonoscopy for conditions not associated with altered active ileal bile acid transport (23) were used as control values. INBAT values of normal persons were not available, owing to the invasive character of a colonoscopy with terminal ileal biopsy.

Chemical bile acid analysis

Total 3α-hydroxy bile acids were determined enzymatically in duplo in pooled faeces, collected during 4 or 5 days (24-26).

Table 2 : Laboratory investigations related to the exclusion criteria for secondary bile acid malabsorption

patient	blood		urine		faeces					intestinal funktion test			jejunal biopsy
ident., sex(m/f)	T4	vit	5-HIAA	lax	biochem. fat	α-ch	ob	microb. cult	par	LB	ST	GC	
1. RK,f	n	n	n	—	n	n	—	—	—	n	•	n	n
2. AK,m	n	•	•	•	•	n	—	—	•	•	•	n	
3. BN,m	n	n	•	—	•	n	•	—	—	•	•	•	n*
4. NP,m	•	•	•	•	•	•	•	—	—	n	•	•	n*
5. HR,f	n	n	•	•	•	•	—	—	•	•	•	•	
6. AS,f	n	n	n	•	•	•	•	•	•	n	n	n	n
7. CS,f	•	•	•	•	n	n	—	—	•	n	•	•	n*
8. BV,m	•	•	•	•	•	•	—	—	•	•	•	•	
9. FW,m	•	n	•	•	n	•	—	—	—	•	n	n	
10. AW,f	n	n	n	—	n	n	—	—	—	n	•	n	n*

(n) normal result, (—) negative result, (•) not done
vit: serum vitamin levels (folic acid, Vit. B_{12}, iron, Vit. D_3 and Vit. A), lax: laxatives, fat: fat excretion on a 70 g fat containing diet (n < 7 g), cult: culture for salmonella, shigella, yersinia, campylobacter, par: repeated microscopic examination for parasites, α-ch: α-chymotrypsin, ob: occult blood, LB: H_2-breath test after 50 g lactose, ST: Schilling test, GC: ^{14}C-glycocholate breath test. •:including establishment of normal disaccharidase activities.

Biopsy procedure

In all 10 patients a colonoscopy with retrograde ileoscopy was performed. The terminal ileum was endoscopically normal in all patients. Biopsies were taken 5-10 cm proximal of the ileo-coecal valve for light microscopical investigation and five additional specimens (25-50 mg) were directly frozen for bile acid transport studies.

INBAT determinations

Determination of active bile acid transport quantified as INBAT was performed as described in detail previously (23). The two main steps of the procedure are the isolation of brush border membrane vesicles and bile acid transport measurements in the isolated vesicles. These steps will now be described in summary:

Brush border membrane vesicle isolation
Frozen ileal biopsies, thawed in isotonic buffer, were disrupted using a Vibro-mixer. After addition of $MgCl_2$ the suspension was centrifuged twice at 500 x g and the two combined supernatant fractions were centrifuged at 27000 x g to spin down the brush border membrane vesicles and the pellet was resuspended in buffer.

Transport measurements
Incubation of the brush border membrane vesicle suspension was performed with a transport buffer containing 4 μM ^{3}H(G)-taurocholate in the presence of a 108 mmol/l Na^+ gradient. Incubation was stopped by addition of samples of the incubation mixture at fixed time intervals (after 15, 30 and 45 seconds) to ice cold lithocholate-saturated stop buffer. Samples were filtered and washed 4 times with the same stop buffer. Radioactivity on the filter was measured in a liquid scintillation counter and corrected for non-specific binding. The increase in TC uptake from 15-30 seconds was used as uptake parameter, combining uptake quantification, minimal non-specific binding and a low intra-assay variation. INBAT was expressed as pmoles taurocholate per mg brush border membrane protein per 15 seconds as described previously (23).

The bile acid transport capacity of the ileal tissue (INBALTC), which is not represented by the calculated INBAT values, was determined by quantifying taurocholate uptake velocity per unit ileal biopsy tissue (and not per unit brush border membrane vesicle protein). INBALTC could therefore easily be calculated from INBAT (INBALTC = INBAT x brush border membrane vesicle yield) and was expressed as pmoles taurocholate per g ileal biopsy tissue per 15 seconds.

Cholestyramine treatment
All patients were treated with cholestyramine. In general the starting dose was 2 x 2 g and the dose was increased up to 3 x 4 g depending on its effect and acceptance by the patients. All patients reported either a marked decrease in defaecation frequency and improvement in consistency of stools or normalization of defaecation and even constipation (scored as marked or complete response in table 1).

RESULTS

Bile acid malabsorption was considered to be present when total faecal bile acid excretion was $\geq$ 1.2 mmol/d (24-27). The ^{75}SeHCAT whole-body retention half-life was abnormal (< 1.8 d) in 8 and equivocal (1.8-2.8 d) in 2 patients, while the fractional ^{75}SeHCAT loss after 4 days was abnormal (> 72 %) in all patients. The 5 patients given radio-opaque markers excreted virtually all their markers within 48 hrs. In a sixth patient (No. 10) carmine red was discovered in the faeces 21 hrs after administration.
The results of active bile acid transport studies in our patients are presented in table 3 and compared with the transport values obtained in controls.

In none of the patients were values suggestive of a defect transport found. The lowest individual values found for INBAT and INBALTC corresponded with the 19th resp. 52nd percentiles of the range of INBAT and INBALTC values found in the control group. Moreover mean INBAT ($p < 0.05$) as well as mean INBALTC ($p < 0.05$) were significantly increased (Wilcoxon signed rank test) in comparison to the group of 132 control patients. The brush border membrane vesicle yield in the 10 patients with idiopathic bile acid malabsorption did not differ significantly from the brush border membrane vesicle yield in the control group.

DISCUSSION

Primary bile acid malabsorption, as described by Hess Thaysen in 1973 and 1976 (3,4), is characterized by chronic diarrhoea with increased bile acid loss, not caused by any of the conditions known to be associated with abnormal bile acid loss, and responding to cholestyramine. Supplying bile acid binding agents such as cholestyramine is an effective anti-diarrhoeal treatment in most conditions associated with bile acid malabsorption. A lack of response to cholestyramine by definition excludes bile acid malabsorption as the cause of the diarrhoea, but not the presence of increased bile acid excretion (28). A positive response however provides no information about the cause of bile acid malabsorption. As dihydroxy bile acids can induce electrolyte and water secretion by increasing cyclic adenosine monophosphate (29,30), measuring faecal aqueous dihydroxy bile acid concentrations can provide additional information about the role of bile acids in the aetiology of the diarrhoea (31). However these measurements are technically more difficult to perform and might not reflect the actual concentrations in the proximal colon as water and bile acid absorption are different processes and bacterial dehydroxylation continues after defaecation.

Merrick and coworkers (5) suggested that primary bile acid malabsorption is probably not as rare, as originally thought (3,4). In 7 years after evaluating almost twohundred patients with bile acid malabsorption we encountered ten patients fulfilling the criteria of primary bile acid malabsorption. None of these patients had any histological abnormalities on examination of their terminal ileal biopsies distinguishing them from the three cases published by Popovic et al. (21). In our opinion the three patients described by Popovic strictly do not fulfil Hess Thaysens criteria (4) and should in fact be labelled as suffering from type I bile acid malabsorption, as their ilea were morphologically abnormal.

Hess Thaysen has proposed a genetic defect in active bile acid transport as the cause of the unexplained bile acid malabsorption in his patients. Active bile acid transport in the ileum is a Na^+-dependent process which can be demonstrated and quantified in brush border membrane vesicles derived from ileal biopsies (23,32). We did not encounter a defective active ileal bile acid transport in any of our patients. In fact if anything, active bile acid transport quantified as INBAT and INBALTC was increased in comparison with the control group. These findings confirm earlier results in 8 patients with bile acid-losing diarrhoea due to various causes (23). The present results support our hypothesis that active bile acid transport is regulated by the availability of bile acids to the ileal enterocytes (23). As a defect in active bile acid transport

Table 3: Active ileal bile acid transport parameters and brush border membrane vesicle yield found in patients with primary bile acid malabsorption (n=10) in comparison to controls (n=132)

patients: ident., sex	*INBAT (pmol/mg prot)	*INBALTC (pmol/g biopsy)	*BBMV yield (mg prot/g biopsy)
1. RK,f	85	183	2.15
2. AK,m	30	229	7.63
3. BN,m	74	268	3.62
4. NP,m	43	117	2.71
5. HR,f	111	109	0.98
6. AS,f	40	120	2.99
7. CS,f	136	91	0.67
8. BV,m	135	134	0.99
9. FW,m	117	243	2.08
10. AW,f	112	85	0.76
total (n=10):	88 (30-136)	158 (85-268)	2.46 (0.67-7.63)
controls (n=132):	63 (1-244)	98 (1-408)	1.69 (0.45-7.61)

*INBAT: in vitro Na^+-dependent bile acid transport (pmol taurocholate uptake per mg brush border membrane protein per 15 seconds),
*INBALTC: in vitro Na^+-dependent bile acid local transport capacity (pmol taurocholate uptake per g ileal biopsy tissue per 15 seconds),
*BBMV yield: brush border membrane yield in mg per g ileal biopsy tissue

cannot be demonstrated, the aetiology of primary bile acid malabsorption remains unclear. The net amount of bile acids escaping ileal reabsorption is probably the resultant of the amount and concentration of bile acids entering the terminal ileum, the presence of other intraluminal contents such as fiber (33), non-absorbed fat (34), the intraluminal pH (35), the frequency of entero-hepatic cycling, the ileal transit time (20) (availability factors) and the number of bile acid carriers per ileal surface area and the total ileal surface containing these carriers (capacity factors). Although no information is available about the length of distal ileum capable of active bile acid transport in primary bile acid malabsorption, it seems unlikely, that this segment would be effectively shorter than in normals. No data are available on the composition and size of the bile acid pool in this condition, which on theoretical grounds

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CHAPTER 5

The ^{75}Se-homocholic acid taurine (^{75}SeHCAT) test re-evaluated: combined measurement of fecal ^{75}Se activity and 3α -OH bile acids in 211 patients.

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The Journal of Nuclear Medicine, accepted December 1990

ABSTRACT

The recommended reference values for the ^{75}Se-homocholic acid taurine (^{75}SeHCAT) test, used in the analysis of chronic diarrhea, were evaluated in 211 patients by comparing simultaneous measurements of 3α -OH bile acids and ^{75}Se activity in daily collected stools.
An initial evaluation in 11 patients showed that the fecal collection method, which allows inspection and additional analysis of stools, was equivalent to the abdominal retention method.
As indicated by marker recovery, fecal collection was adequate in 195 (92%) of the 211 patients.
Application of the reference values recommended by the manufacturer (7-day retention: > 20% normal, < 15% abnormal) to our patient data resulted in a 51% false-positive and a 10% false-negative score for the ^{75}SeHCAT test in predicting fecal 3α-OH bile acid excretion (normal < 1.2 mmol/d).
^{75}SeHCAT whole-body retention half-life (WBR_{50}) was > 2.8 d in < 10% of the patients with bile acid malabsorption and < 1.7 d in < 10% of the normals. We recommend that a ^{75}SeHCAT WBR_{50} < 1.7 d is abnormal, a WBR_{50} > 2.8 d is normal and a WBR_{50} in the range 1.7-2.8 d is equivocal, which was the case in 48% (94/195) of the patients in this study.

INTRODUCTION

Detection of bile acid malabsorption is of diagnostic and therapeutic importance. Chemical methods for determining fecal bile acid content are cumbersome (1,2). [24-^{14}C]-Cholic acid taurine (^{14}C-CAT) has been used as a bile acid marker, but still requires time consuming fecal analysis (3-6). Bile acid malabsorption is nowadays simply detected using the gamma emitter [23-^{75}Se]-25-homocholic acid taurine (^{75}SeHCAT) (7,8). Excretion of ^{75}SeHCAT can be expressed as its whole-body retention half-life (WBR_{50}) or as the fraction retained or excreted after 3-7 days (9). The ^{75}SeHCAT test can be performed by measuring ^{75}Se excretion in stools (7) or by measuring ^{75}Se retention by abdominal gamma camera scanning or whole body counting (8,10). Although both methods should produce similar results, we are not aware of any such comparative study in the literature.
Reference values for the ^{75}SeHCAT test (table 1) (3,8,11-13) have largely been based on clinical grounds and never on chemically measured fecal bile acid loss (FBAL), although a correlation between ^{75}SeHCAT excretion and FBAL has been reported by several authors (3,14-16). It was our impression that the reference values propagated by the manufacturer of ^{75}SeHCAT gave rise to a high number of false-positive tests. This motivated us to evaluate whether these reference values reflected chemically measured FBAL in 211 patients, in whom combined data on excretion of ^{75}SeHCAT and 3α-OH bile acids were available. In these patients recovery of radio-opaque markers served as a control on the accuracy of fecal collection.

Table 1: Reference values for the ^{75}SeHCAT test in the literature and reference values recommended by the manufacturer

Author	(ref)	pat.no.	parameter	normal	abnormal
Thaysen	(8)	38	5d retention	> 35 %	< 30 %
Nyhlin	(3)	63	7d retention	> 19 %	< 12 %
Merrick	(11)	169	7d retention	> 15 %	< 8 %
Scheurlen	(12)	64	WBR_{50}	> 1.2 d	< 1.2 d
Schroth	(13)	16	WBR_{50}	2.5 ± 0.5 d	
manufacturer			7d retention	> 20 %	< 15 %

ref: reference number, pat.no.: number of patients investigated with ^{75}SeHCAT, d: days.

Preceding this evaluation we performed a small study comparing the fecal collection and abdominal retention methods in 11 patients with a wide range of bile acid loss to see whether both methods produced similar results and whether abdominal scanning on two occasions was as accurate as more frequent measurements.

METHODS

Patients

^{75}SeHCAT test, initial methodological evaluation

Eleven patients admitted to the hospital with Crohn's disease limited to the colon (n=4), Crohn's disease of the colon and terminal ileum (1), ulcerative colitis (1), carcinoid syndrome (1), post-cholecystectomy diarrhea (1), diarrhea with alcohol abuse (1), abdominal pain (1) and idiopathic bile acid diarrhea (1) were investigated. In these patients ^{75}SeHCAT WBR_{50} values were calculated using the fecal collection method as well as using various abdominal retention measurement protocols. ^{14}C-CAT WBR_{50} and chemical fecal bile acid loss were determined simultaneously with the ^{75}SeHCAT test.

^{75}SeHCAT test, evaluation and assessment of reference values

In the past 9-10 years over 600 ^{75}SeHCAT tests were performed in our laboratory for clinical reasons (diarrhea analysis, suspected ileal disease etc.) using the fecal collection method. In many patients the accuracy of fecal collection was controlled by the recovery of radio-opaque markers administered together with the ^{75}SeHCAT dose. Patients who were not given radio-opaque markers were not included in the study. No clinical data were used for selection of the patients.

The fractional ^{75}SeHCAT loss after 4 days (FL4D) was measured in all patients as this was the simplest way of quantifying ^{75}SeHCAT excretion and fractional ^{75}SeHCAT loss or retention after 3-7 days is the most frequently used ^{75}SeHCAT test parameter in the literature. In addition ^{75}SeHCAT WBR_{50} and/or FBAL were measured in case of an equivocal or abnormal ^{75}SeHCAT FL4D, when fecal collection was known to be incomplete on one or two days of the test or in case of special interest in a particular patient for clinical or research purposes.
This resulted in 211 patients, in whom both ^{75}SeHCAT WBR_{50} and FL4D were available and fecal bile acid loss was also measured chemically. In 195 of these 211 patients, at least 90 % of the administered markers were recovered, indicating accurate fecal collection.

Measurement of bile acid isotope excretion

^{75}SeHCAT test, fecal collection method.
(performed in the initial evaluation (n=11) and in the evaluation and assessment of reference values (n=195)).
The patients ingested a ^{75}SeHCAT capsule (370 kBq, specific activity 481 GBq/mmol) with a standard Lundh meal after an overnight fast. Stools were then collected in 24 hr portions for 5 days. Gamma emitting activity of the 24 hr stool portions collected in plastic pots (diameter 9 cm, height 20 cm) was measured at least two times during 60 seconds with a collimated 5 cm NaI crystal (length 15 cm, diameter 9 cm). The distance between the cover of the pot and the diaphragm of the collimator was 20 cm, combining optimal counting efficiency with a minimal influence of the fecal volume. Background activity (< 2 % of the administered dose) was always subtracted.

^{75}SeHCAT test, abdominal retention measurements
(only performed in the initial evaluation (n=11))
Gamma camera scanning of the abdomen was started 3-4 hrs. after ingestion of the ^{75}SeHCAT capsule (day 0) and was repeated daily for 4 days and again at day 7. The scanning was done without a collimator, with a patient-scanner distance of 90 cm in anterior-posterior and posterior-anterior views for the 280 keV peak, which is one of the major energy peaks, comprising 23 % of the total energy, with a 20 % window and for an integral set up with a threshold of about 50 keV, as all energy peaks of ^{75}Se are well above this peak. For calculation of the counting data the geometric mean formula ($\sqrt{APxPA}$) was used. Background activity was always subtracted.

^{14}C-CAT excretion measurement
(only performed in the initial evaluation (n=11))
^{14}C-CAT (185 kBq, specific activity 2 GBq/mmol) was ingested simultaneously with the ^{75}SeHCAT-capsule. ^{14}C-CAT activity in the faeces was determined according to Hörchner et al. (5) and van Blankenstein et al. (6) by oxidation of the ^{14}C-CAT to $^{14}CO_2$ by burning in a stream of oxygen, collection of the $^{14}CO_2$ in hyamine and determination of the ^{14}C activity in a liquid scintillation counter. WBR_{50} was calculated using the least squares method.

Chemical fecal bile acid determination
In both the 11 patients in the initial evaluation and in the 195 patients in the evaluation and assessment of reference values, total 3α-hydroxy bile acids were enzymatically determined in duplicate in pooled and mixed stools, collected during 4-5 days (1,17,18). A mean 24 hr FBAL < 1.2 mmol was defined as normal based on observations in our laboratory and literature data (1,17-20).

Marker administration and recovery
Twentyfive radio-opaque markers were administered with the ^{75}SeHCAT dose. Their recovery was checked by making an X-ray of the 5 plastic pots with the 24 hr stool collections. Fecal collection was considered accurate when at least 23 of the 25 administered markers (> 90 %) were recovered.

^{75}SeHCAT WBR_{50} calculation
As the elimination of bile acids follows first order kinetics (7,13,21), the turnover can be expressed as the time necessary for excretion of 50 % of the administered dose (WBR_{50}). Although the elimination of ^{75}SeHCAT follows a biexponential curve (3,22), the first component comprises 96 % of the administered radioactivity and the second part of the curve is only reached within one week in case of substantial bile acid malabsorption, as is encountered after ileal resection (9,16). We therefore regarded the elimination of ^{75}SeHCAT as following a monoexponential curve for our calculation of WBR_{50} (14):

1. $$WBR_{50} = \ln 2 / k$$

(k is fractional ^{75}SeHCAT turnover)
As a linear relation is found between ln(^{75}SeHCAT retention) and the expired time after ^{75}SeHCAT administration after an equilibrium time interval (representing the time necessary for the bile acid tracer to mix with the bile acid pool as well as the time necessary for transit through the colon), this relation can be characterized by:

2. $$\ln(ret) = \ln 100 - k \,.\, (t - eqt)$$

(ret is % ^{75}SeHCAT retention after a time interval (t),
eqt is equilibrium time)
from 2. follows: $$k \,.\, (t - eqt) = \ln 100 - \ln(ret)$$

$$k = (\ln 100 - \ln(ret))/(t - eqt)$$

combined with 1.: $$WBR_{50} = \ln 2 \,.\, (t - eqt)/(\ln 100 - \ln(ret))$$

or: $$eqt = t - (WBR_{50} \,.\, (\ln 100 - \ln(ret))/\ln 2)$$

These equations, characterizing the relation between ^{75}SeHCAT WBR_{50}, the equilibrium time and the fractional ^{75}SeHCAT retention after a certain time interval, can be applied in order to calculate the equilibrium time or ^{75}SeHCAT WBR_{50} when the values of the other two parameters are known. For ^{75}SeHCAT WBR_{50} calculation based on more than two measurements, the

fractional turnover was determined with the least squares approximation method.

RESULTS

Initial evaluation: comparison of fecal collection and abdominal retention measurements.

The individual WBR_{50} values varied widely in the 11 patients (table 2). The coefficients of correlation between the WBR_{50} calculations are presented in table 3. A very good correlation was found between the WBR_{50} calculations with the ^{14}C-CAT and $^{75}SeHCAT$ collection methods ($R = 0.97$; $p < 0.01$). $^{75}SeHCAT$ WBR_{50} was slightly lower in most patients than ^{14}C-CAT WBR_{50} ($^{75}SeHCAT$ $WBR_{50} = 0.6 \cdot {}^{14}C\text{-CAT}\ WBR_{50} + 0.8$ (days)), in agreement with other studies in which $^{75}SeHCAT$ WBR_{50} was either slightly lower than or similar to ^{14}C-CAT WBR_{50} (7,23). The results of all other abdominal scanning protocols correlated well with ^{14}C-CAT WBR_{50} (R: 0.80-0.97;$p < 0.01$) .

Table 2: WBR_{50} of ^{14}C-CAT and $^{75}SeHCAT$ calculated according to the various scanning protocols and chemically measured fecal bile acid loss in 11 patients

	FECAL COLLECTION		$^{75}SeHCAT$ WBR_{50} with ABDOMINAL SCANNING						FBAL	Diagnosis
	^{14}C-CAT WBR_{50}	$^{75}SeHCAT$ WBR_{50}	daily x5 int	daily x5 280	d 0 + d 7 int	d 0 + d 7 280	d 0 + d 4 int	d 0 + d 4 280	mmol/d	
1.	24.8	15.6	13.0	14.0	13.9	12.9	13.4	9.9	0.7	Colitis C
2.	8.9	6.6	5.0	5.0	4.8	4.6	4.6	4.4	0.8	Carcinoid
3.	8.3	3.0	3.2	3.0	3.5	3.1	3.4	3.8	0.5	U colitis
4.	4.8	3.8	4.5	4.6	5.2	5.3	6.0	6.1	0.9	Colitis C
5.	1.7	1.4	2.1	1.6	2.4	1.7	2.0	2.0	1.7*	Post-chol
6.	6.4	5.5	3.8	3.8	3.8	3.8	6.6	6.8	0.9	Colitis C
7.	4.1	3.6	3.3	3.0	3.7	3.3	4.2	3.6	1.2*	Abd pain
8.	4.3	4.4	5.4	5.6	5.2	5.4	5.2	6.4	1.0	Ileocol C
9.	3.5	3.1	2.8	2.4	4.1	2.5	6.4	3.3	1.1	Colitis C
10.	5.0	3.8	3.7	3.4	2.7	2.4	4.0	3.7	0.7	Alcohol A
11.	0.6	0.8	0.9	1.1	1.2	1.3	0.9	1.0	3.2*	Idiop BAD

d: day, int: integral set up, 280: 280 keV, FBAL: fecal bile acid loss in mmol/day, *: abnormal result (≥ 1.2 mmol/d), Colitis C: colitis Crohn, Carcinoid: carcinoid syndrome, U Colitis: ulcerative colitis, Post-chol: post-cholecystectomy diarrhea, Abd pain: unexplained abdominal pain, Ileocol C: ileocolitis Crohn, Alcohol A: diarrhea presumed due to alcohol abuse, Idiop BAD: idiopathic bile acid diarrhea.

Table 3: Coefficients of correlation between the various methods of WBR_{50} calculations

			FECAL COLLECTION		^{75}Se ABDOMINAL SCANNING daily(x5)	
			^{14}C-CAT	^{75}SeHCAT	int	280
FECAL COLLECTION	^{14}C-CAT					
	^{75}SeHCAT		0.97			
^{75}Se ABDOMINAL SCANNING	daily (x5)	int	0.95	0.98		
		280	0.95	0.97		
	days 0+7	int	0.94	0.96	0.98	
		280	0.93	0.96		0.99
	days 0+4	int	0.87	0.92	0.92	
		280	0.80	0.86		0.88

int: abdominal scanning at integral spectrum, 280: scanning at 280 keV.

With regards to the ^{75}SeHCAT test, no differences between the fecal collection and the abdominal scanning methods or between scanning at 280 keV and at an integral set up could be demonstrated in these 11 patients.

Evaluation and assessment of reference values of the ^{75}SeHCAT test.
Reference values for the ^{75}SeHCAT test were determined in 195 patients with proven accurate collection by establishment of 90 % confidence limits for WBR_{50} and FL4D, for both patients with normal FBAL (< 1.2 mmol/d) and bile acid malabsorption (≥ 1.2 mmol/d).
In 47 patients with FBAL < 1.2 mmol/day, we found a mean ^{75}SeHCAT WBR_{50} of 2.6 days (10-90% range: 1.7-3.9) and a mean ^{75}SeHCAT loss after 4 days (FL4D) of 61 % (10-90% range: 47-73).
In 148 patients with FBAL ≥ 1.2 mmol/day we found a mean WBR_{50} of 1.7 days (10-90% range 0.6-2.8) and a mean FL4D of 75 % (10-90 % range: 58-92) (table 4).
Using these 90 % confidence limits for ^{75}SeHCAT excretion, patients can be placed in one of three categories:

-normal bile acid loss: WBR_{50} > 2.8 days or FL4D $< 58\%$
-abnormal bile acid loss: WBR_{50} < 1.7 days or FL4D $> 73\%$
-equivocal bile acid loss: WBR_{50} 1.7-2.8 days or FL4D 58-73%

Table 4: Relation between ^{75}SeHCAT test parameters WBR_{50} and FL4D and chemically measured FBAL

		FBAL(mmol/d)		
		≥ 1.2	< 1.2	
FL4D	> 73 %	82	4	86
	equiv	53	27	80
	< 58 %	13	16	29
		148	47	195

		FBAL(mmol/d)		
		≥ 1.2	< 1.2	
WBR_{50}	< 1.7 d	68	3	71
	equiv	66	28	94
	> 2.8 d	14	16	30
		148	47	195

	FBAL(mmol/d)		
	≥ 1.2	< 1.2	
WBR_{50} < 1.7 d and FL4D > 73%	66	2	68
WBR_{50} or FL 4D equivocal	74	34	108
WBR_{50} > 2.8 d and FL4D < 58%	8	11	19
	148	47	195

The data represent numbers of patients for each category. WBR_{50}: whole-body retention half-life, FL4D: fractional loss after 4 days, FBAL: fecal bile acid loss, d: days, equiv: equivocal.

Application of these reference values implicated that 41% of the patients had an equivocal FL4D and 48% had an equivocal WBR_{50}. Of the 94 patients with an equivocal WBR_{50} outcome 66 had an abnormal FBAL (≥1.2 mmol/d) and 28 a normal FBAL. Combination of both ^{75}SeHCAT parameters resulted in a lower number of false-positives (8 / 148 = 5 %) and false-negatives (2 / 47 = 4 %), but also in a higher number of equivocal results, requiring additional chemical bile acid analysis (108 / 195 = 55 %) (table 4).

Only for purposes of evaluation and comparison of the reference values reported in the literature the mean equilibrium time was calculated as 0.7 days (interquartile range 0.1 - 1.2 d) for the total group of 195 patients.

An overview of the calculated ^{75}SeHCAT WBR_{50} reference values using the equation WBR_{50}=ln2.(t - eqt)/(ln100 - ln(ret)) from the reference values reported in the literature is presented in table 5.

Indicated in this table are also the percentage of false-negative and false-positive results of the ^{75}SeHCAT test in predicting the actual chemically measured bile acid loss, when the reference values reported in the literature would have been applied to our patient population (n=195).

According to the equation **ln(ret) = k . (- t)** the reference values for retention after one week could be calculated by extrapolation of the results of the reference values of FL4D and WBR_{50} determinations, when ^{75}SeHCAT excretion is assumed to follow a monoexponential curve within the first seven days, which is the case if excretion is less than 96% of the administered dose (3). This would have resulted in a 7-day retention < 8 % indicating bile acid malabsorption (as reported by Merrick et al. (11) and a 7-day retention > 20 % (as reported by Nyhlin et al. (3) indicating normal bile acid excretion with a 90 % reliability.

Table 5: Evaluation of ^{75}SeHCAT test reference values in the literature in predicting bile acid excretion in the 195 patients in this study

		*calculated		application in the 195 patients in this study:	
		normal	abnormal		
Author	(ref)	WBR_{50}	WBR_{50}	% false-neg	% false-pos
Hess Thaysen	(8)	> 2.85	< 2.48	9	55
Nyhlin	(3)	> 2.64	< 2.06	16	26
Merrick	(11)	> 2.31	< 1.73	23	9
Scheurlen	(12)	> 1.2	< 1.2	72	4
manufacturer		> 2.72	< 2.31	10	51
this study		> 2.8	< 1.7	9	6

*for purposes of comparison and evaluation WBR_{50} was calculated from the reported reference values using the equation WBR_{50} = ln2.(t-eqt)/(ln100-ln(ret)); eqt was assumed 0.7 d, WBR_{50}: whole-body retention half-life (days), pat.no.: number of patients investigated with 75SeHCAT, false-pos: false-positive test results, false-neg: false-negative test results.

Table 5 illustrates that the sensitivity and specificity of the ^{75}SeHCAT test depend on the reference values chosen (12). In general the test does not combine a high sensitivity with a high specificity in predicting bile acid excretion.

DISCUSSION

Our study confirms that the kinetics of ^{75}SeHCAT closely resemble the kinetics of the labelled natural bile acid ^{14}C-CAT (7,23). The widely used ^{75}SeHCAT test protocol with retention measurements on only 2 occasions approximated the accuracy of the protocols with daily retention or excretion measurements during 4 or 5 days.
Incomplete fecal collection, which is sometimes inevitable in case of severe diarrhea or fecal incontinence is mentioned to be a disadvantage of fecal ^{75}SeHCAT excretion measurements. This is avoided with abdominal retention measurements. However incomplete collection is in our experience rare. In this study fecal collection was accurate in at least 92 % of the patients.
An advantage of the fecal collection method is that it only requires 2 visits to the laboratory, regardless of the number of 24 hr stool collections. Counting of the 4 or 5 closed plastic pots with 24 hr stool collections can simply be performed at a moment suitable for the laboratory staff.
Calculation of the ^{75}SeHCAT WBR_{50} based on 4 or 5 24 hr stool collections is more accurate than calculation based on 2 retention measurements. More than 2 retention measurements, as applied by various authors (12,13,24,25) require extra visits to the hospital, making it less attractive to perform the test on an outpatient base. In addition the fecal collection method allows for essential visual inspection (steatorrhea etc.), assessment of 24 hr stool weight and additional analysis of stools, including Na^+ and K^+ measurements, revealing a possible secretory character of the diarrhea, and chemical bile acid determinations.
In order to be able to compare and evaluate the reference values of the ^{75}SeHCAT test reported in the literature and recommended by the manufacturer, a mean equilibrium time of 0.7 days representing the initial mixing phase as well as colonic transit was calculated from the combined ^{75}SeHCAT test data of the 195 patients.
If we had used the manufacturers reference values on the data of the present study, 10 % false-negative and 51 % false-positive ^{75}SeHCAT test results would have been found in predicting fecal 3α-OH bile acid excretion. In addition in 13 % of the patients the test result would have been equivocal. This implies that only 26 % of the patients would have been correctly classified and that an unacceptably high percentage of patients (51 %) would have been inappropriately investigated as to unravel the etiology of their putative bile acid malabsorption.
In our opinion the ^{75}SeHCAT test should be used as a screening test for bile acid malabsorption and therefore accurately reflect FBAL. We accepted an a priori score of 10% false-negative and 10% false-positive results of the ^{75}SeHCAT test parameters, in predicting whether FBAL was increased or normal, defining FBAL $\geq$ 1.2 mmol/day abnormal (1,14 -17). Using these criteria a ^{75}SeHCAT WBR_{50} < 1.7 d or a FL4D > 73 % indicate bile acid

malabsorption and a WBR_{50} > 2.8 d or a FL4D < 58 % indicate normal bile acid excretion.
The WBR_{50}, as a parameter of ^{75}SeHCAT excretion, has the theoretical advantage over fractional loss or retention measurements of being independent of initial mixing of ^{75}SeHCAT with the bile acid pool as well as colonic transit time. Combining of WBR_{50} and FL4D data in this study resulted in a lower percentage of false classifications (5 %), however at the cost of a higher percentage of equivocal results (55 %).
In principle, in case of an equivocal test result FBAL should be determined chemically. When the ^{75}SeHCAT test is performed using the reference values listed above with selective determination of FBAL, a minimum of FBAL measurements (45 %) is combined with a maximum of confidence with regards to the interpretation of the ^{75}SeHCAT test result.

Conclusion and recommendation

The ^{75}SeHCAT test, using the fecal collection method or the abdominal scanning method, is much easier to perform than measurements of ^{14}C-CAT excretion or fecal bile acids. We recommend the fecal collection method controlled by recovery of radio-opaque markers, as it allows inspection and simultaneous analysis of stools including chemical bile acid measurements, which are indicated in 40-50 % of the patients in whom the ^{75}SeHCAT test result is equivocal. The abdominal scanning method, which can be reliably executed with abdominal scanning after ingestion and again after one week, as recommended by the manufacturer, is a good alternative especially in patients unable to collect their stools accurately. Application of the reference values, recommended by the manufacturer (< 15 % retention after 7 days is abnormal, > 20 % is normal) results in an unacceptably high percentage (51 % in this study) of false-positive tests. Based on our data we recommend the following reference values: ^{75}SeHCAT WBR_{50} > 2.8 d: normal bile acid excretion; WBR_{50} < 1.7 d: increased bile acid loss. In the range 1.7-2.8 d the test result is termed 'equivocal' and additional fecal bile acid measurements are indicated.

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CHAPTER 6

Assessment of bile acid malabsorption: aetiology and faecal analysis in 385 patients.

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Submitted for publication

SUMMARY

In order to obtain insight into possible mechanisms of increased intestinal bile acid loss, we reviewed the clinical data and results of the ^{75}Se-homocholic acid taurine (^{75}SeHCAT) test, supplemented with faecal 3α-OH bile acid measurements in 385 patients, who had been referred for chronic diarrhoea or ileal disease. In addition data on faecal weight, oro-anal transit time, Na^+ and K^+ excretion and concentration were analyzed in various diagnostic groups.
Type 1 bile acid malabsorption (ileal disease) was found in 76 patients, type 2 (idiopathic, responding to cholestyramine) in 12 patients, type 3 (associated with other conditions) in 58 patients, including 14 patients with not previously reported associations such as hyperthyroidism (n=2), short bowel syndrome (1), chronic intermittent pseudo-obstruction (1), intestinal lymphoma (1), lambliasis (1) and alcohol induced diarrhoea (8). In 22 patients the cause of bile acid malabsorption remained unknown and a response to cholestyramine was not documented. In 4 patients only colitis was present. The ^{75}SeHCAT test reflected faecal 3α-OH bile acid excretion in most patients, being false-positive in 5 % and false-negative in 13 % of the patients.
A statistically significant positive correlation between faecal bile acid loss and faecal weight, indicating a possible role of bile acids in the pathogenesis of the diarrhoea was only found in ileitis (r=0.69) and alcohol related diarrhoea (r = 0.80).

INTRODUCTION

The distal ileum plays a major role in the enterohepatic circulation of bile acids as it is the only site in the intestine where active absorption of conjugated bile acids takes place (1). Bile acid malabsorption, which often leads to diarrhoea due to the cathartic effect of bile acids on the colon (2), is well documented in ileal disease and after ileal resection (3,4). However abnormal bile acid loss can occur in conditions without demonstrable ileal disease. Three types of bile acid malabsorption (BAM) are now recognized (5-8). Type 1 BAM is due to ileal inflammation or resection. The pathogenesis of type 2 BAM, also called primary or idiopathic bile acid malabsorption, first described by Hess Thaysen in 1973 (9,10), is unknown. This type of BAM is probably less rare than previously thought (7,11-13). Many conditions and drugs have been reported to be associated with type 3 BAM which was first described by Fromm et al. in 1977 (14) (table 1). The precise mechanism of bile acid malabsorption in this type of BAM is unknown, but it is thought that the efficiency of bile acid reabsorption is decreased by altered small intestinal motility or unfavourable intra-luminal conditions. An analysis of all causes of type 3 BAM has not been the subject of any study probably due to the small numbers of patients described.
The introduction of the gamma-labeled synthetic bile acid [23-^{75}Se]-25-homocholic acid taurine (^{75}SeHCAT), replacing the previously used isotope [24-^{14}C]-cholic acid taurine, has facilitated the diagnosis of BAM considerably (22,27).

Table 1: Conditions and drugs reported in association with bile acid malabsorption in the absence of ileal disease

post-cholecystectomy diarrhoea (15)
post-vagotomy diarrhoea (16)
diabetic diarrhoea (17)
medullary thyroid carcinoma (18,19)
sequel of gastroenteritis (19)
cystic fibrosis (20)
pancreatic insufficiency (7,21)
coeliac disease (7)
renal failure (5,14)
'allergic diarrhoea'/ food intolerance (19,22)
secretory diarrhoea caused by an apudoma (22)
collagenous colitis (19)
fat hyperalimentation (19)
bacterial overgrowth (11)
lactose malabsorption (23)
drugs: bile acid binding agents (cholestyramine and $Al(OH)_3$)
various laxatives (19,22)
neomycin and colchicine (24,25)
phenphormin (14,26)
theophylline (19)

The reference values for the ^{75}SeHCAT test reported in the literature were primarily based on clinical data such as the presence or absence of ileal disease (7,22,28). We recently defined the reference values for the ^{75}SeHCAT test in our laboratory based on simultaneous faecal bile acid loss measurements in 195 patients (29). The ^{75}SeHCAT test was introduced in our laboratory in 1981. The test has gained a place in the analysis of diarrhoea and has up to now been performed in more than 600 patients. In case of an equivocal or abnormal ^{75}SeHCAT test result, faecal bile acid loss (FBAL) was also measured chemically. In all patients faecal weight was measured, and when faecal weight or FBAL were increased, faecal Na^+ and K^+ content were determined as well. From 1983 onwards the daily excretion of 25 radio-opaque markers, which were simultaneously administered with the ^{75}SeHCAT dose, was determined as a control on the accuracy of faecal collection. In addition this allowed a calculation of the oro-anal transit time.

The aim of this study was to review the clinical conditions found in all patients in whom a ^{75}SeHCAT test had been performed. This might provide a better insight in the causes of bile acid malabsorption especially in the absence of ileal dysfunction. In addition comparison of the ^{75}SeHCAT test and determination of 3α-OH faecal bile acids in all 3 types of bile acid malabsorption would confirm the alleged higher sensitivity and specificity of ^{75}SeHCAT in detecting distal ileal pathology. As in a large number of patients data on faecal weight, bile acid excretion parameters, Na^+ and K^+ excretion and marker excretion pattern were available, one of the aims of the study was a characterization of the diarrhoea in the various diagnostic groups. This might provide insight into the mechanism of the diarrhoea. Another goal of the study

was to investigate whether these measured parameters and derived para-meters such as Na+- and K+-concentration, approximations of stool osmolality and bile acid pool size could predict the diagnosis in an individual patient.

METHODS

Patients

The clinical data of 385 patients out of a total of more than 600 patients referred to us for bile acid excretion studies between 1981 and 1988 were retrieved and analyzed. Insufficient clinical data were available in the remaining patients, the majority of whom had been referred to us solely for the ^{75}SeHCAT test from surrounding hospitals.

Measurement of bile acid excretion:

Chemical bile acid determination

Total 3α-hydroxy bile acids were enzymatically determined in duplo in 4-5 pooled 24 hr. faecal collections (30). Abnormal bile acid loss was defined as total faecal bile acid loss $\geq$ 1.2 mmol/d (30-32).

^{75}SeHCAT test

Execution

The patients ingested a ^{75}SeHCAT capsule (370 kBq, specific activity 481 GBq/mmol) with a standard Lundh meal after an overnight fast. Stools were then collected in 24 hr. portions for 5 days. Gamma emitting activity of the stool collections was measured with a collimated 5 cm NaI crystal in a selected standard geometry (29).

Calculation and interpretation

The fractional ^{75}SeHCAT loss after 4 days (FL4D) was measured in all patients as a parameter of ^{75}SeHCAT excretion.

In the majority of the patients the ^{75}SeHCAT whole-body retention half-life (WBR_{50}) was calculated as a second parameter. ^{75}SeHCAT WBR_{50} was derived from the fractional turnover, which was calculated using the least squares method.

Based on the combined ^{75}SeHCAT test and FBAL results in 195 patients in our laboratory results of the ^{75}SeHCAT test are placed in one of the following 3 categories (29):

-normal ^{75}SeHCAT excretion: WBR_{50} > 2.8 d or/and FL4D < 58%
-abnormal ^{75}SeHCAT excretion: WBR_{50} < 1.7 d or/and FL4D > 73%
-equivocal ^{75}SeHCAT excretion: WBR_{50} 1.7-2.8 d or/and FL4D 58-73%
or discrepancy between WBR_{50} and FL4D results in an individual patient

Bile acid pool size calculation

The size of the bile acid pool was approximated with the following formula:

pool = daily faecal bile acid loss / fractional clearance
pool = FBAL * (ln2 / WBR_{50})

Radio-opaque marker excretion
The completeness of stool collection was in most patients checked (from 1983 onwards) by the recovery of 25 radio-opaque markers which were administered simultaneously with the ^{75}SeHCAT dose. For this purpose an X-ray was made of the 5 stool collections.
Collection was considered to be accurate when at least 23 markers were present in the collected stools.

Oro-anal transit time
The excretion data of the radio-opaque markers allowed calculation of the mean oro-anal marker transit time according to the following formula:

$$\text{transit time} = ((n_1*1) + (n_2*2) + (n_3*3) + (n_4*4) + (n_5*5))/n$$

In this formula n_1, n_2, n_3, n_4, n_5 and n represent the number of markers on each day and the total number of markers counted (33).

Faecal weight
The weight of the faeces collected during 5 days was measured and faecal weight was expressed in grams/d. Diarrhoea was defined as faecal weight exceeding 200 grams/d.

Faecal Na^+ and K^+ excretion
Na^+- and K^+-concentration were measured in faecal water using a flame-photometer and expressed in mmol/kg. Daily Na^+ and K^+ excretion were calculated using the daily faecal weight and expressed in mmol/d.

Calculation of faecal osmolality
Faecal osmolality was approximated using the following formula:
osmolality = 2•(Na^+-concentration + K^+-concentration). The actual faecal osmolality was not systematically measured during the ^{75}SeHCAT test and therefore not used in this study.

Statistics
Calculation of correlation coefficients, analysis of variance and regression analysis were performed with the statistical program Stata 2.0. Discriminant analysis was performed with the BMDP statistical software package.

RESULTS
BAM defined as 3α-OH bile acid excretion ≥ 1.2 mmol/d was present in 172 patients. Table 2 shows the clinical conditions encountered in these patients.

Briefly the diagnoses encountered were based on the following criteria:
Ileitis: small bowel X-rays and/or endoscopy and biopsy.
Ileal resection: patients history and/or operation reports.
Radiation enteritis: radiotherapy to intra-abdominal organs and small bowel X-rays.
Laxative abuse: demonstration of laxatives in urine or faeces.
Post-cholecystectomy and post-vagotomy diarrhoea: diarrhoea as a new symptom after these operations.

Lactase deficiency: diarrhoea related to ingestion of milk products and an abnormal lactose H_2 breath test.
Pancreas insufficiency: abnormal pancreas function such as steatorrhoea and abnormal pancreas morphology.
Coeliac disease: villous atrophy in jejunal biopsies and recovery of histology and symptoms on a gluten free diet.
Bacterial overgrowth: abnormal $^{14}CO_2$ rise within 4 hours at a ^{14}C-glycocholate breath test and response to antibiotics.
Lambliasis: jejunal biopsy or microscopy of faeces.
Neuropathic diarrhoea: diarrhoea coinciding with autonomic neuropathy.
Medullary thyroid carcinoma: histology, diarrhoea parallelling the onset and the course of the disease.
Short bowel syndrome: malabsorption and operation reports.
Chronic intermittent pseudo-obstruction: recurrent symptoms and signs of intestinal stasis and exclusion of obstruction.
Alcohol related diarrhoea: otherwise unexplained diarrhoea coinciding with alcohol abuse.
Hyperthyroidism: laboratory results and diarrhoea.
Small bowel lymphoma: small bowel X-rays and histology.
Renal insufficiency: laboratory results, unexplained diarrhoea.
Large bowel pathology - colitis (colitis Crohn, ulcerative colitis, theophylline induced colitis, colitis as a manifestation of systemic vasculitis), idiopathic proctitis and solitary rectal ulcer: endoscopy and biopsy.
Carcinoid syndrome:laboratory results and liver ultrasound.
Candida diarrhoea: faecal culture.
With the exception of candida diarrhoea and carcinoid syndrome all these diagnoses were, at least in some patients, associated with BAM.

Table 2: conditions found in 380 patients investigated with ^{75}SeHCAT for diarrhoea or as an ileal function test with their respective ^{75}SeHCAT test and 3α-OH bile acid excretion data

	FBAL ≥ 1.2 ^{75}SeHCAT*				FBAL < 1.2 ^{75}SeHCAT*				FBAL ? ^{75}SeHCAT*			
	abn	eq	nl	?	abn	eq	nl	?	abn	eq	nl	total
ileitis	16	6	2			1	1		1		5	32
ileitis/colitis	6	4	4			1	3			1	9	28
ileal resection	25	1		1		1	1	1				30
ileal resection/colitis	5		2								1	8
radiation enteritis	3	1				1						5
total type 1 BAM	55	12	8	1		4	5	1	1	1	15	103
laxative abuse		1								1		2
post-chol diarrhoea	4	5			1	4	1			1	3	19
post-vag diarrhoea	2	2				4	2				8	18
lactase deficiency	1	1	1			2	2			1	10	18
pancreas insufficiency		4	1								1	6
coeliac disease	1		2				1				3	7
bacterial overgrowth	2	1	1								6	10
neuropathic diarrhoea	1	1									1	3
uraemia		1										1
medullary thyroid carc	2											2
hyperthyroidism	1	1										2
short bowel syndrome	1											1
chron int pseudo-obs		1										1
alcohol rel diarrhoea	3	3	2		1		3				4	16
intestinal lymphoma			1									1
lambliasis	1											1
more than 1 diagnosis	4	5	1		1	3				1	10	25
total type 3 BAM	23	26	9		3	13	9			4	46	133
primary (type 2) BAM	7	3	2									12
no diagnosis:												
BAM +	5	14	3									22
BAM — / diarrhoea +						3	3				11	17
BAM — / diarrhoea —						7	4			5	42	58
colitis		4					11				13	28
proctitis						1					2	3
solitary rectal ulcer						1						1
carcinoid syndrome							2					2
candida diarrhoea											1	1
total	90	59	22	1	3	29	34	1	1	10	130	380

FBAL: faecal bile acid loss in mmol/d, * ^{75}SeHCAT test result: abn: abnormal, eq: equivocal, nl: normal, ?: result not available or unreliable, BAM: bile acid malabsorption (≥ 1.2 mmol/d), diarrhoea +/—: faecal weight >/< 200 g/d, post-chol: post-cholecystectomy, post-vag: post-vagotomy, carc: carcinoma, chron int pseudo-obs: chronic intermittent pseudo-obstruction, rel: related

As might be expected, the most frequent causes of bile acid malabsorption were ileitis or ileal resection. Type 1 BAM was present in 44% of the bile acid losers. Type 3 BAM was found in 58 patients (34%), including several conditions not previously described to be associated with BAM such as hyperthyroidism, short bowel syndrome, chronic intermittent pseudo-obstruction, alcohol related diarrhoea, intestinal lymphoma and lambliasis. In 34 patients (20%) no cause of the BAM was found, 12 of these patients fulfilling Hess Thaysens criteria for idiopathic bile acid malabsorption (type 2 BAM) as a positive response to cholestyramine was documented. In only 4 patients with BAM (2%) only large bowel pathology (colitis) was documented.
In general there was a good agreement between results of the ^{75}SeHCAT test and chemically measured bile acid loss.
In 22 (13 %) of the 172 patients with BAM the ^{75}SeHCAT test was normal. In 3 of the 66 (5 %) patients with normal FBAL ($\geq$ 1.2 mmol/d) the ^{75}SeHCAT test was abnormal. In 130 patients FBAL measurement was not performed as the ^{75}SeHCAT test was normal. In 11 patients with an equivocal or abnormal ^{75}SeHCAT test FBAL measurement was omitted for unknown reasons. In 5 patients both ^{75}SeHCAT excretion as well as FBAL were not measured as faecal collection was inaccurate.

Of all 103 patients with conditions falling in the type 1 BAM category (ileal pathology) the ^{75}SeHCAT test was abnormal in 56 and equivocal in 17 patients. In 28 patients (27%) with ileal disease the ^{75}SeHCAT test was normal. More than half (16) of these patients suffered from both ileitis and colitis. FBAL was measured in 86 patients with ileal disease and normal in 10 of these patients (12 %).
In the patients with ileal pathology and BAM ($\geq$ 1.2 mmol/d) the ^{75}SeHCAT test was abnormal in 72 %, equivocal in 16 % and normal in 11 % of the 76 patients. In type 3 BAM the ^{75}SeHCAT test was abnormal in 40 %, equivocal in 45 % and normal in 16 % of the 58 patients. In the type 2 BAM group the number of patients was to low to make a useful calculation. This suggests that the ^{75}SeHCAT test is more likely to be abnormal in patients with BAM due to ileal pathology than in type 3 BAM. However, when the ileal resection group is left out of the type 1 BAM group, the diagnostic yield of the ^{75}SeHCAT test becomes lower, being abnormal in 60 %, equivocal in 26 % and normal in 14 % of the remaining 42 patients.
In tables 3, 4 and 5 the data on faecal weight, FBAL, ^{75}SeHCAT FL4D and WBR_{50}, bile acid pool size, marker transit time, Na^+ and K^+ content and concentration and calculated osmolality are presented for all diagnostic groups in case of proved accurate collection. Diarrhoea (defined as a mean 24-hr stool weight > 200 g) was present in only 51 % of the patients, although it was a main symptom and indication for the ^{75}SeHCAT test in most patients. In a minority of the patients the ^{75}SeHCAT test was performed because of suspected or established ileal pathology.
Our results show that a faecal weight below 200 g did not preclude BAM. Of the 114 patients with BAM ($\geq$ 1.2 mmol/d) the mean faecal weight was below 200 g/d in 31 (27 %), below 150 g/d in 12 (11 %) and below 100 g/d in 1 (1 %). An abnormal ^{75}SeHCAT test (FL4D > 73 %) was present in 66 patients. The mean faecal weight was below 200 g/d in 17 (26 %), below 150 g/d in 7 (11 %) and below 100 g/d in 1 (1 %) of these 66 patients.

The relevance of BAM in patients without obvious diarrhoea remains to be established. If one accepts a priori that 5 % of the cases of BAM (defined as faecal 3α-OH bile acid excretion ≥ 1.2 mmol/d) will be missed, this would imply that in cases with a faecal weight below 140 g/d bile acid measurements would not be indicated (25 % of the patients in whom a ^{75}SeHCAT test was performed).

Table 3: faecal weight, fractional ^{75}SeHCAT loss after 4 days and oro-anal marker transit time of the patients with proven accurate faecal collection per diagnosis (mean (range) indicated)

	n	FW	FL4D	MTT
ileitis	20	258 (128-437)	72(16-99)	1.8(1.0-3.4)
ileitis/colitis	20	449 (57-1413)	58(23-100)	1.9(1.0-4.2)
ileal resection	13	551 (96-1691)	89(73-100)	1.5(1.0-4.0)
ileal res/colitis	4	352 (94-543)	78(47-93)	1.5(1.0-3.1)
radiation enteritis	3	150(112-189)	82(63-95)	2.4(1.5-4.3)
post-chol diarrhoea	13	153 (64-263)	56(16-79)	2.0(1.0-3.5)
post-vag diarrhoea	13	208 (86-604)	53(16-91)	1.8(1.0-2.5)
lactase deficiency	14	202 (46-705)	46(13-87)	2.1(1.0-4.2)
pancreas insuffiency	4	353(169-723)	53(26-67)	1.5(1.3-1.7)
coeliac disease	4	461(235-1065)	40(14-77)	1.8(1.1-2.8)
bacterial overgrowth	6	326(121-504)	57(28-98)	1.8(1.3-2.9)
neuropathic diarrhoea	1	521	77	1.5
uraemia	1	221	65	1.8
medullary thyroid carc	1	428	80	1.0
hyperthyroidism	2	498(141-855)	76(69-82)	1.2
alcohol rel diarrhoea	11	180 (62-340)	60(41-89)	1.8(1.1-3.5)
intestinal lymphoma	1	217	53	1.8
more than 1 diagnosis	20	235 (75-960)	58(11-96)	2.0(1.0-3.8)
primary BAM	8	304(142-612)	70(50-85)	1.3(1.0-1.7)
no diagnosis:				
BAM +	20	238(133-385)	68(39-96)	1.6(1.0-3.3)
BAM — / diarrh +	15	378(201-821)	50(30-71)	1.5(1.0-2.1)
BAM — / diarrh —	47	133 (49-197)	39 (5-72)	2.0(1.0-4.3)
colitis	17	327(104-755)	40 (6-77)	1.9(1.0-3.7)
proctitis	2	198(160-237)	49(40-58)	1.5(1.4-1.6)
candida diarrhoea	1	327	46	1.2

n: number of patients, FW: faecal weight (g/d), FL4D: fractional ^{75}SeHCAT loss in 4 days (%), MTT: oro-anal marker transit time (d), chol: cholecystectomy, vag: vagotomy, carc: carcinoma, rel: related, BAM: bile acid malabsorption (≥ 1.2 mmol/d), diarrhoea +/—: faecal weight >/< 200 g/d.

Table 4: faecal bile acid loss, ^{75}SeHCAT whole-body retention half-life and bile acid pool size of the patients with proven accurate faecal collection per diagnosis (mean (range) indicated)

	n	FBAL	WBR_{50}	pool
ileitis	14	2.5(1.2-7.0)	2.0(0.6-9.9)	4.0(1.1-6.8)
ileitis/colitis	10	2.2(0.4-5.0)	3.1(0.2-6.7)	4.9(1.3-8.5)
ileal resection	7	6.1(1.0-12.0)	0.8(0.3-1.9)	4.3(2.2-8.7)
ileal resection/colitis	2	3.6(0.5-5.1)	0.9(0.5-1.3)	5.0(3.7-6.4)
radiation enteritis	3	2.4(1.6-3.7)	1.3(0.6-1.8)	4.8(1.6-9.6)
post-chol diarrhoea	7	1.2(0.9-1.8)	2.4(0.8-5.3)	3.3(1.0-4.2)
post-vag diarrhoea	6	1.4(1.0-2.2)	3.1(2.0-5.5)	5.3(3.2-7.6)
lactase deficiency	6	1.4(0.6-3.3)	3.8(1.1-6.6)	4.3(1.6-5.9)
pancreas insufficienty	3	1.9(1.6-2.2)	2.5(2.0-2.7)	6.6(4.9-8.6)
coeliac disease	2	1.8(1.6-1.9)	7.4(1.5-13.0)	5.7(3.5-7.9)
bacterial overgrowth	2	2.7(2.0-3.3)	1.8(0.6-3.2)	3.9(2.9-4.9)
neuropathic diarrhoea	1	1.40	1.7	3.4
uraemia	1	1.30	2.7	5.1
medullary thyroid carc	1	2.10	1.2	3.6
hyperthyroidism	2	2.4(1.4-3.3)	1.6(1.2-1.9)	5.7(2.4-9.0)
alcohol rel diarrhoea	7	3.1(0.7-6.0)	3.1(1.0-5.1)	7.7(3.8-11.4)
intestinal lymphoma	1	1.20		
more than 1 diagnosis	11	1.9(0.5-4.5)	2.4(0.9-6.3)	4.9(1.2-11.0)
primary BAM	8	2.5(1.4-3.7)	2.1(1.1-4.0)	7.0(3.5-17.3)
no diagnosis:				
BAM +	19	2.2(1.2-6.0)	2.3(0.5-5.2)	7.5(1.2-34.6)
BAM — / diarrh +	6	0.9(0.6-1.1)	3.7(2.1-5.6)	4.1(3.1-5.0)
BAM — / diarrh —	8	1.0(0.8-1.1)	4.3(1.8-10.0)	3.7(2.2-5.4)
colitis	7	1.2(0.2-2.2)	6.8(1.8-36.0)	5.7(3.4-8.3)
proctitis	1	0.90	2.9	3.8
candida diarrhoea	0			

n: number of patients, FBAL: faecal bile acid loss (mmol/d), WBR_{50}: ^{75}SeHCAT whole-body retention half-life (d), pool: bile acid pool (mmol/d), chol: cholecystectomy, vag: vagotomy, carc: carcinoma, rel: related, BAM: bile acid malabsorption ($\geq$ 1.2 mmol/d), diarrhoea +/— : feacal weight >/< 200 g/d.

Table 5: Na^+ and K^+ excretion and calculated osmol data of the patients with proven accurate faecal collection per diagnosis (mean (range) indicated)

	n	Na^+	K^+	Na^+c	K^+c	osmc
ileitis	15	10 (2-35)	20(13-32)	34(10-102)	83(41-133)	235(163-192)
ileitis/colitis	17	34 (1-126)	22 (9-40)	55 (4-114)	56(21-101)	223(167-280)
ileal resection	13	31 (2-100)	28 (7-45)	51(24-80)	62(27-101)	227(172-319)
ileal res/colitis	3	28(19-35)	19(13-27)	64(53-74)	46(28-60)	219(204-246)
radiat enteritis	2	5 (4-6)	10(10-11)	32(20-43)	63(52-74)	189(145-233)
post-chol diarrh	8	7 (1-12)	19 (5-32)	39 (7-84)	102(42-165)	282(224-343)
post-vag diarrh	7	17 (3-56)	24(20-29)	54(18-93)	98(41-126)	304(255-432)
lactase defic	7	14 (2-40)	26(12-49)	36 (8-79)	99(44-160)	270(218-336)
pancreas insuff	4	15 (3-36)	31 (8-74)	36(18-50)	77(47-102)	227(132-305)
coeliac disease	3	38 (8-95)	35(16-55)	55(30-89)	78(52-126)	266(176-340)
bacterial overgr	3	40(35-45)	22(15-30)	88(77-94)	48(40-62)	272(238-312)
neuropathic diarrh	1	33	40	63	77	280
uraemia	1	8	27	36	122	316
medullary thyr ca	1	43	23	101	54	309
hyperthyroidism	2	52 (8-97)	11(11-11)	84(55-114)	45(13-78)	259(253-265)
alcohol rel diarrh	8	8 (2-17)	17(7-28)	36(23-70)	81(45-124)	234(190-293)
intest lymphoma	1	5	23	21	106	255
more than 1 diagn	14	9 (2-24)	21(10-35)	34(13-58)	87(23-145)	241 (71-337)
primary BAM	8	14 (3-41)	22(11-36)	41(21-79)	77(41-113)	236(134-268)
no diagnosis:						
BAM +	18	10 (1-25)	24 (9-49)	37 (5-74)	97(45-159)	269(159-349)
BAM — / diarrh +	11	18 (2-51)	33(18-61)	40 (7-99)	87(35-160)	253(105-366)
BAM — / diarrh —	8	5 (1-7)	19 (9-30)	26(11-39)	110(76-176)	273(173-390)
colitis	12	21 (3-81)	23(10-44)	47(22-107)	66(21-100)	225(148-262)
proctitis	1	6	23	25	97	245
candida diarrhoea	1	7	41	22	125	295

n: number of patients, FW: faecal weight (g/d), Na^+: Na^+ excretion in mmol/d, K^+: K^+ excretion in mmol/d, Na^+c: Na^+concentration in mmol/kg, K^+c: K^+ concentration in mmol/kg, osmc: calculated osmol ($2 \cdot (Na^+ + K^+)$), radiat: radiation, chol: cholecystectomy, vag: vagotomy, diarrh: diarrhoea (+ > 200 g/d, — < 200 g/d), defic: deficiency, insuff: insufficiency, overgr: overgrowth, thyr ca: thyroid carcinoma, rel: related, intest: intestinal, diagn: diagnosis, BAM: bile acid malabsorption (≥ 1.2 mmol/d).

Using discriminant analysis it was not possible to make a reliable diagnostic classification in individual patients based on their bile acid excretion data in combination with all other measured parameters. This was due to the large number of diagnostic groups in relation to the small number of independent variables and due to the considerable number of incomplete observations with respect to Na^+ and K^+ excretion.

A mutual comparison of the various parameters in the various diagnostic groups large enough to allow evaluation is presented in table 6, using analysis of variance. In this respect the group with normal faecal weight and FBAL and without diagnosis served as a reference group, as a proper control group was

not present due to the retrospective nature of the study. ^{75}SeHCAT and 3α-OH bile acid excretion as well as faecal weight were high in the groups with ileal pathology. Faecal bile acid excretion was also remarkably high in the alcohol related diarrhoea group. In colitis ^{75}SeHCAT excretion was lower than in the reference group. In general Na^+ and K^+ excretion were higher in case of a higher faecal weight and bile acid malabsorption and Na^+ concentration tended to be higher at the cost of a lower K^+ concentration in these patients, possibly reflecting a secretory effect of bile acids on the colonic mucosa.

Table 6: Analysis of variance of faecal weight, bile acid excretion data, marker transit time, faecal Na^+ and K^+ excretion data and calculated stool osmolality of the patients with proven accurate faecal collection per diagnostic group in relation to the reference group

	FW	FBAL	FL4D	WBR_{50}	pool	MTT	Na^+	K^+	Na^+c	K^+c	osmc
ileitis		⇑									
ileitis/colitis	⇑⇑⇑						⇑⇑⇑		⇑⇑	⇓⇓⇓	
ileal resection	⇑⇑⇑	⇑⇑⇑	⇑⇑⇑				⇑⇑	⇑	⇑	⇓⇓	
ileal res/colitis		⇑⇑							⇑	⇓⇓	
post-chol diarrh											
post-vag diarrh									⇑		⇑
lactase defic											
pancreas insuff								⇑			
coeliac disease	⇑			⇑			⇑	⇑			
bacterial overgr							⇑		⇑⇑⇑	⇓⇓	
alcohol rel diarrh		⇑⇑			⇑						
primary BAM		⇑									
no diagnosis:											
BAM +		⇑			⇑						
BAM — / diarrh +	⇑										
colitis			⇓	⇑⇑						⇓	

FW: faecal weight, FBAL: faecal bile acid loss, FL4D: fractional ^{75}SeHCAT loss after 4 days, WBR_{50}: ^{75}SeHCAT whole-body retention half-life, pool: bile acid pool size, MTT: marker transit time, Na^+: Na^+ excretion, K^+: K^+ excretion, Na^+c: Na^+ concentration, K^+c: K^+ concentration, osmc: calculated osmol ($2*(Na^+ + K^+)$), ⇑ or ⇓ : $p < 0.05$, ⇑⇑ or ⇓⇓ : $p < 0.01$, ⇑⇑⇑ or ⇓⇓⇓ : $p < 0.001$, res: resection, chol: cholecystectomy, vag: vagotomy, diarrh: diarrhoea (+ < 200 g/d, — > 200 g/d), defic: deficiency, insuff: insufficiency, overgr: overgrowth, rel: related, BAM: bile acid malabsorption (≥ 1.2 mmol/d).

Table 7 shows the correlation between the various parameters for the total group of patients, in whom all these parameters were measured. The best correlation was found between faecal weight and faecal Na^+ excretion (r = 0.88). The coefficient of correlation between FBAL and faecal weight was only 0.39. As the mechanisms of diarrhoea might be different in the various diagnostic groups, analysis within each diagnostic group might result in a better correlation between particular bile acid or faecal parameters in certain groups.

Table 7: correlation* between bile acid excretion data, bile acid pool size, oro-anal marker transit time, faecal weight and electrolytes in 127 patients.

	FBAL	FL4D	WBR_{50}	pool	MTT	FW	Na^+	K^+	Na^+c	K^+c	osmc
FBAL	1										
FL4D	.55	1									
WBR_{50}	-.28	-.61	1								
pool	.31	-.31	.24	1							
MTT		-.23	.18		1						
FW	.39	.21				1					
Na^+	.33	.19	.19			.88	1				
K^+	.24					.53	.36	1			
Na^+c	.23	.26			-.26	.50	.76		1		
K+c	-.31	-.34			.26	-.54	-.58	.23	-.63	1	
osmc						-.18		.37	.20	.63	1

*only statistically significant ($p < 0.05$) correlations listed

FBAL: faecal bile acid loss, FL4D: fractional ^{75}SeHCAT loss in 4 days, WBR_{50}: ^{75}SeHCAT whole-body retention half-life, pool: bile acid pool size, MTT: oro-anal marker transit time, FW: faecal weight, Na^+: Na^+ excretion, K^+: K^+ excretion, Na^+c: Na^+ concentration, K^+c: K^+ concentration, osmc: calculated osmol ($2*(Na^+ + K^+)$).

Table 8 represents the correlation between the various parameters and faecal weight. A significant relation between one of the parameters and faecal weight could imply a causal role for this particular parameter in the aetiology of the diarrhoea, while an absent or low correlation would exclude this possibility.

Table 8: Correlation* between the 24 hr faecal weight and other parameters (^{75}SeHCAT and 3α-OH bile acid excretion, bile acid pool size, marker transit time, faecal Na^+ and K^+ content and concentration and calculated stool osmolality)

	FBAL	FL4D	WBR_{50}	pool	MTT	Na^+	K^+	Na^+c	K^+c	osmc
ileitis	0.69					0.73	0.65	0.58	-0.62	
ileitis/colitis						0.97	0.59	0.76	-0.70	
ileal resection		0.72				0.96	0.66		-0.75	
il res/colitis										
post-chol diarrh					-0.63		0.72			
post-vag diarrh		0.61			-0.65	0.95			-0.94	
lactase defic						0.93				
pancreas insuff		-0.99				0.99	0.99			
coeliac disease						0.99				
bacterial overgr										
alc rel diarrh	0.80	0.74	-0.75			0.82				
primary BAM			0.77	0.97		0.92	0.75			
no diagnosis:										
BAM +		0.46				0.63	0.67			
BAM — /diarrh +										
BAM — /diarrh —		0.47	-0.44		-0.32	0.83		0.75		
colitis			0.78			0.94		0.88	-0.70	

*only statistically significant ($p < 0.05$) correlations listed

FW: faecal weight, FBAL: faecal bile acid loss, FL4D: fractional ^{75}SeHCAT loss after 4 days, WBR_{50}: ^{75}SeHCAT whole-body retention half-life, pool: bile acid pool size, MTT: marker transit time, Na^+: Na^+ excretion, K^+: K^+ excretion, Na^+c: Na^+ concentration, K^+c: K^+ concentration, osmc: calculated osmol ($2*(Na^+ + K^+)$), res: resection, chol: cholecystectomy, vag:vagotomy, diarrh: diarrhoea (+ > 200 g/d, — < 200 g/d), defic: deficiency, insuff: insufficiency, overgr: overgrowth, alc rel: alcohol related, BAM: bile acid malabsorption (≥1.2 mmol/d).

Table 9 represents the correlation between 3α-OH bile acid excretion and the other parameters. This might provide insight in the role of the other parameters in the pathophysiology of bile acid malabsorption.

Table 9: Correlation* between faecal 3α-OH bile acid excretion and other parameters (^{75}SeHCAT excretion data, bile acid pool size, marker transit time, faecal Na^+ and K^+ excretion and concentration, calculated stool osmolality)

	FL4D	WBR_{50}	pool	MTT	Na^+	K^+	Na^+c	K^+c	osmc
ileitis						0.61			
ileitis/colitis	0.77	-0.76							
ileal resection	0.57		0.89	-0.61		0.73			
il res/colitis						0.99			
post-chol diarrh									
post-vag diarrh			0.87						
lactase defic	0.96								
pancreas insuff									
coeliac disease									
bacterial overgr									
alc rel diarrh	0.82	-0.85			0.95		0.90		
primary BAM								-0.80	
no diagnosis:									
BAM +			0.71		0.57				
BAM — /diarrh +									
BAM — /diarrh —									
colitis	0.84					0.75			

*only statistically significant ($p < 0.05$) correlations listed

FW: faecal weight, FBAL: faecal bile acid loss, FL4D: fractional ^{75}SeHCAT loss after 4 days, WBR_{50}: ^{75}SeHCAT whole-body retention half-life, pool: bile acid pool size, MTT: marker transit time, Na^+: Na^+ excretion, K^+: K^+ excretion, Na^+c: Na^+ concentration, K^+c : K^+ concentration, osmc: calculated osmol ($2 \cdot (Na^+ + K^+)$), res: resection, chol: cholecystectomy, vag: vagotomy, diarrh: diarrhoea (+ > 200 g/d, — < 200 g/d), defic: deficiency, insuff: insufficiency, overgr: overgrowth, alc rel: alcohol related, BAM: bile acid malabsorption (≥ 1.2 mmol/d).

DISCUSSION

This retrospective study describes the conditions found in 385 patients, in whom a ^{75}SeHCAT test with additional faecal analysis was performed in the work-up of chronic diarrhoea or as a test of ileal function. BAM defined as FBAL ≥ 1.2 mmol/d was present in 172 patients.
BAM was classified as type 1 (ileal pathology) in 44 %, as type 2 (idiopathic, symptomatic response to cholestyramine) in 7 % and as type 3 (associated with other conditions) in 34 % of the patients. BAM was associated with colitis in 2 % and BAM could not be classified in 12 % of the patients, many of these patients having been labelled as suffering from the irritable bowel syndrome. It is possible that some of these patients could have been suffering from idiopathic bile acid malabsorption, but a positive response of symptoms to cholestyramine was not documented.
Several conditions not previously described to be associated with BAM were encountered such as hyperthyroidism, short bowel syndrome, chronic intermittent pseudo-obstruction, alcohol related diarrhoea, intestinal lymphoma and lambliasis. We propose that these conditions are added to the list of conditions representing the type 3 BAM category (table 1) as in all these conditions nutrient absorption by the small bowel or small bowel motility are altered in some way, often leading to diarrhoea. A considerable number of patients were classified as alcohol related diarrhoea. Mean faecal bile acid loss was high in this group. The mechanism of the diarrhoea is unknown. Folate and amino acid uptake have been reported to be decreased in these patients (34,35). Amino acid as well as bile acid transport depend on the Na^+,K^+,-ATPase and in animal experiments alcohol has been shown to inhibit this enzyme (36) in various tissues. In concordance with this theory is the observation that the correlation between FBAL and faecal weight was the best of all diagnostic groups ($r = 0.80$) suggesting a role of the bile acids in the pathogenesis of the diarrhoea in these patients.

Using our previously defined reference values the ^{75}SeHCAT test was generally in accordance with FBAL, being 'falsely' negative or positive in 11 % (25 / 237) of the patients. An equivocal ^{75}SeHCAT test result was present in 98 of the 378 patients (26%), which implies that in a lower number of patients FBAL measurements were indicated to detect BAM than reported in a previous study in our laboratory (29).

A statistically significant positive correlation between faecal weight and FBAL was only found in ileitis and in alcohol related diarrhoea suggesting that bile acids did not play a major role in most other types of diarrhoea. In post-cholecystectomy diarrhoea a negative correlation between faecal weight and FBAL (not reaching statistical significance) was present. This is in concordance with a study by Fromm et al. (37), who could not demonstrate secretory levels of dihydroxy bile acids or a response to cholestyramine in patients with this condition.
In the primary BAM group the coefficient of correlation between faecal weight and FBAL was only 0.35 (not statistically significant), making an important role for bile acids in the pathogenesis of the diarrhoea less likely. It is remarkable that in this condition the marker transit time was the lowest of all groups, being

significantly different from most other groups including the control group. In a previous study we demonstrated that primary or idiopathic BAM is not due to an absent active bile acid transport in the distal ileum (38). Our findings support the hypothesis that rapid ileal transit, not allowing enough time for an efficient absorption, might be implicated in this condition. Another remarkable feature of primary BAM was the correlation ($r = 0.97$) between faecal weight and bile acid pool size. The mean bile acid pool size was also higher than the pool size of most other groups. It is therefore tempting to speculate that the BAM in these patients might be related to a large bile acid pool with a normal ileal function resulting in a net higher colonic overspill.
What can be concluded from the observation that motor, osmotic, secretory and combined types of diarrhoea are all represented in type 3 BAM ? Active absorption of bile acids in the distal ileum must play a key role in the enterohepatic circulation of bile acids as BAM occurs after relatively small ileal resections (6,39). Another argument is found by the close parallel in all categories of BAM between excretion of 3α-OH bile acids and ^{75}SeHCAT, claimed to be specifically actively absorbed in the distal ileum. Apparently a small decrease in the efficiency of intestinal bile acid absorption can lead to BAM, the cycling frequency of the enterohepatic circulation augmenting a small difference in absorption efficiency per cycle to a considerable extent (7).
In contrast to bile acid malabsorption type 1, in which there is a distinct defect in distal ileal bile acid absorbing **capacity**, in bile acid malabsorption type 3 the **availability** of bile acids to the ileal mucosa is somehow altered in an unfavourable way by a more rapid transit, lower conjugated bile acid concentration, altered pH or other mechanisms.
In a few patients with BAM only large bowel pathology - colitis -was documented. The pathophysiology of BAM in these patients is not clear and it seems not appropriate to classify them as type 3 bile acid malabsorbers.
As can be concluded from table 2 the ^{75}SeHCAT test is far from specific for the detection of ileal disease. However a comparison of the ^{75}SeHCAT test results in type 1 and type 3 BAM suggests a somewhat higher specificity for the detection of ileal disease, although this is accentuated by the group of patients with an ileal resection, in which BAM is more severe than in all other groups.

Discriminant analysis could not reliably classify patients in a particular diagnostic group due to the large number of diagnostic groups and the relatively small number of independent variables available.

In conclusion the spectrum of gastro-intestinal conditions associated with BAM has been extended. Alcohol is an important cause of diarrhoea and BAM, one of the mechanisms implicated being its effect on Na^+,K^+,- ATPase. The enigma of primary bile acid bile acid malabsorption remains to be solved, although this study indicates that a rapid intestinal transit as well as a large bile acid pool might be implicated.
This study illustrates that the faecal collection method has several advantages over the abdominal scanning method as a way of performing the ^{75}SeHCAT test. It allows inspection of faeces and establishment whether diarrhoea is really present (51 % of the patients in this study). Faecal bile acid analysis can selectively and relatively simply be carried out in the same 24 hr stool collections in case the ^{75}SeHCAT test result falls in the equivocal range. The

combination of ^{75}SeHCAT WBR_{50} and FBAL allows for an approximation of the bile acid pool size, which can be useful for clinical as well as research purposes. The radio-opaque markers not only serve as a control of the accuracy of the faecal collection, but also allow calculation of the oro-anal transit time, which can be useful in selected patients. Faecal electrolytes can be selectively measured reflecting a possible secretory character of the diarrhoea.

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CHAPTER 7

The ^{75}Se-homocholic acid taurine (^{75}SeHCAT) test and faecal 3α-OH bile acid excretion in ileal disease. An analysis in 57 patients.

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Submitted for publication

SUMMARY

We compared the ability of ^{75}Se-homocholic acid taurine (^{75}SeHCAT) and 3α-OH faecal bile acid excretion to detect ileal disease (ileitis and ileal resection) in 57 patients and to predict the extent of ileal involvement in a subgroup of 18 patients. ^{75}SeHCAT whole-body retention half-life (WBR_{50}) and fractional ^{75}SeHCAT loss after 4 days (FL4D) were calculated from 5 consecutive 24 hr stool collections and the mean daily 3α-OH bile acid excretion was measured in 3 consecutive 24 hr stool collections.
The ^{75}SeHCAT test was false-negative in 15 of a subgroup of 40 (38%) patients with ileitis. Faecal (3α-OH) bile acid loss (FBAL), measured in 46 patients with ileal disease was superior to ^{75}SeHCAT excretion in detecting ileal disease. FBAL was higher after ileal resection than in ileitis. The lowest bile acid excretion was found in ileitis with concurrent colitis, 60 % of the ^{75}SeHCAT tests in this group falling in the normal range. It is concluded that the ^{75}SeHCAT test cannot be used to predict the presence or absence of ileitis. Although ^{75}SeHCAT as well as FBAL were related to the extent of ileal malfunction, individual results of the ^{75}SeHCAT test or FBAL measurements were not useful in predicting the extent of ileal disease. Whether repeated bile acid excretion measurements can be used to follow the course of the disease in individual patients remains to be established.

INTRODUCTION

Conjugated bile acids are largely reabsorbed in the distal part of the ileum by an active Na^+-dependent transport mechanism (1-4). Resection or disease of the distal ileum can result in an increased spill-over of bile acids to the colon, where dihydroxy bile acids may interfere with water and electrolyte absorption (5-9) and may alter motility (10), frequently leading to diarrhoea. Increased faecal bile acid loss (FBAL) can be detected by the gamma emitting bile acid marker [23-^{75}Se]-25-homocholic acid taurine (^{75}SeHCAT) (11-13). The rate of ^{75}SeHCAT excretion can be calculated by simple measurement of gamma activity in collected stools or by abdominal retention measurements. Bile acid malabsorption is well documented in ileal disease (5-9, 14,15). ^{75}SeHCAT was developed because it was thought to be resistant to bacterial deconju- gation, thus preventing passive absorption in the proximal small bowel or colon. However, a recent study in our laboratory showed that significant deconjugation does occur in vivo but passive absorption is negligible due to the polarity of the deconjugate ^{75}Se-homocholic acid (16). As ^{75}SeHCAT is only actively absorbed it has been claimed to be a more specific marker of ileal dysfunction than total bile acid excretion (13). Whether ileal disease can reliably be detected using ^{75}SeHCAT (17,18) and whether ^{75}SeHCAT can provide additional information about the length of non-functioning ileum is still a matter of dispute. Schroth, Balzer and Sciaretta found a correlation between the ^{75}SeHCAT test results and the length of diseased or resected ileum (19-21), while Fagan and Merrick did not (22,23). Comparison of their studies is however difficult as they did not use similar ^{75}SeHCAT test parameters. Balzer, Fagan and Merrick measured ^{75}SeHCAT retention after one week,

while Schroth and Sciaretta calculated the ^{75}SeHCAT whole-body retention half-life (WBR_{50}) based on more frequent retention measurements. A comparative study to establish whether the ^{75}SeHCAT test is indeed superior to FBAL measurements in detecting ileal disease and predicting the extent of ileal involvement has never been performed. In addition there are little data as to whether concurrent colonic disease influences bile acid excretion in these patients (22).

We have analyzed the results of ^{75}SeHCAT tests performed in a group of patients with ileal disease, using the faecal collection method. The accuracy of stool collection was controlled by the recovery of simultaneously administered radio-opaque markers. In addition in most patients FBAL was determined enzymatically in the same stool collections.

In a subgroup of the patients data on the length of non-functioning ileum based on small bowel x-ray reports and operation reports were available, allowing an analysis of the relation between the length of ileal impairment and bile acid excretion. A group of 12 patients, not suffering from ileal disease or other conditions affecting bile acid kinetics was used as a reference. Results were also compared with bile acid excretion data of a heterogenous group of patients investigated because of diarrhoea, not suffering from ileal disease, and without a history of abdominal irradiation, gastric surgery or cholecystectomy.

METHODS

Patients

The data of 98 patients with ileal disease, who were investigated with ^{75}SeHCAT, were analyzed. Ileitis was established by radiologic and endoscopic findings and biopsy, ileal resection was based on operation reports. Most patients with ileitis suffered from Crohn's disease, with the exception of one patient with transient unexplained ileitis and one patient with ileitis as a manifestation of Reiter's syndrome. The most frequent indication for ileal resection was Crohn's disease. Other indications were intestinal ischaemia (2x), a volvulus, a coecal perforation, a peri-appendicular infiltrate, an unexplained ileal stenosis, chronic non-granulomatous jejuno-ileitis, a large retro-peritoneal fibroma and a complicated abdominal hysterectomy.

Thirty two patients had not received radio-opaque markers. In 9 patients faecal collection was possibly inaccurate as judged by a marker-recovery below 90 % (less than 23 of the 25 administered markers). The number of recovered markers in these 9 patients were 22 (2x),21,19,16,15,14,12(2x) respectively. In 57 patients faecal collection was proved to be accurate by a marker-recovery > 90 % ($\geq$ 23). In 18 of these 57 patients data on the length of abnormal or resected ileum were available based on small-bowel X-rays, colonoscopy and operation reports. As a precise length of ileal involvement is often difficult to determine, patients were placed in three groups: limited impairment (< 20 cm), moderate impairment (20-40 cm) and extensive impairment (> 40 cm). The presence or absence of concurrent colitis was based on endoscopy and colonic X-rays. The ^{75}SeHCAT test results of 12 patients without ileal disease or other diseases known to affect bile acid kinetics, with normal FBAL (< 1.2 mmol/d (24-27)) and adequate faecal collection, as judged by marker recovery, were used as a control.

The results of ^{75}SeHCAT tests of a group of 151 patients referred for diarrhoea, not suffering from ileal disease and without a history of abdominal irradiation, gastric surgery or cholecystectomy were also used for evaluation. This group was labeled the non-ileitis group.

^{75}SeHCAT test

The ^{75}SeHCAT test was performed with 370 kBq ^{75}SeHCAT (Amersham) administered with a standard meal, consisting of one slice of bread together with 25 radio-opaque markers, after an overnight fast. Five consecutive 24 hr faecal collections were examined for gamma activity by a gamma counter in a standard geometry. The completeness of stool collection was controlled by counting the number of radio-opaque markers on an X-ray made of the 5 stool collections. Mean 24 hr stool weight was measured.

Although the elimination of ^{75}SeHCAT follows a biexponential curve (14,28), the first component comprises 96 % of the administered radioactivity and only in patients with substantially increased bile acid loss will the second part of the curve be reached within one week (29). As the first component follows first order kinetics (11,19,21), the rate of ^{75}SeHCAT excretion can be expressed as its whole-body retention half-life (WBR_{50}) i.e. the time necessary for excretion of 50 % of the administered dose. ^{75}SeHCAT WBR_{50} was calculated in the following way:

$WBR_{50}=\ln 2/k$ (k = fractional turn over rate (11,30))

The fractional turnover rate was calculated by the least squares method.

In addition the fractional ^{75}SeHCAT loss after 4 days (FL4D) was always measured and used as a simple, always available second ^{75}SeHCAT test parameter.

Establishment of reference values of the ^{75}SeHCAT test in our laboratory

Reference values for the ^{75}SeHCAT test in predicting 3α-OH bile acid excretion were established based on results of 195 patients investigated in our laboratory because of diarrhoea (31).

A 3α-OH faecal bile acid excretion ≥ 1.2 mmol/d was considered abnormal (24-27). This resulted in the following reference values for the ^{75}SeHCAT test:

-normal bile acid loss: WBR_{50} >2.8 days or FL4D < 58 %
-abnormal bile acid loss: WBR_{50} <1.7 days or FL4D > 73 %
-equivocal bile acid loss: WBR_{50} 1.7-2.8 days or FL4D 58-73 %
or discrepancy between WBR_{50} and FL4D results in an individual patient.

Faecal bile acid analysis

Mean daily 3α-OH bile acid loss was enzymatically determined in duplo in the 5 pooled 24 hr stool collections (24), which were also used for ^{75}SeHCAT excretion measurements (normal value in our laboratory < 1.2 mmol/d (24-27)).

Calculation of the bile acid pool size

Assuming that the behaviour of ^{75}SeHCAT resembles the behaviour of other natural bile acids in the enterohepatic cycle an approximation of the pool size can be made using the following equation:

$$pool = FBAL * 1 / k \qquad (k = \text{fractional turnover})$$
$$pool = FBAL * (^{75}SeHCAT\ WBR_{50} / \ln 2)$$

Statistics

Results of ^{75}SeHCAT test results and FBAL determinations were compared using the unpaired Wilcoxon test.

RESULTS

The 57 patients with ileal disease and proven accurate faecal collection were placed in 4 diagnostic categories: ileitis (n=20), ileitis with concurrent colitis (20), ileal resection (13) and ileal resection with concurrent colitis (4).
Table 1 shows the results of the ^{75}SeHCAT test, FBAL measurements and bile acid pool calculations in the 4 ileal disease subgroups, the non-ileitis group and the control group. There was a wide range of bile acid excretion in all groups. It can be seen that after ileal resection bile acid excretion tends to be higher than in ileitis. The calculated bile acid pool size also showed a wide variation.

Table 1: Bile acid excretion parameters in the clinical groups (mean values and range indicated)

category	n	^{75}SeHCAT FL4D	^{75}SeHCAT WBR_{50}	FBAL	pool
ileitis	16/20	72 (16-99)	2.0 (0.6-9.9)	2.5 (1.2-7.0)	4.0 (1.1-6.8)
ils/cls	13/20	58 (23-100)	3.1 (0.2-6.7)	2.3 (0.4-5.0)	4.9 (1.3-8.5)
il res	13	89 (73-100)	0.8 (0.2-6.7)	6.2 (1.0-12.0)	4.3 (2.2-8.7)
il res/cls	3/4	78 (47-93)	0.9 (0.5-1.3)	3.7 (2.5-5.1)	5.0 (3.7-6.4)
non-ils	73/78	63 (6-98)	1.4 (0.5-10)	1.8 (0.2-6.0)	6.0 (1.2-34.4)
controls	12	54 (11-71)	3.0 (1.8-4.1)	0.8 (0.6-1.1)	3.4 (1.9-4.8)

n: number of patients investigated with ^{75}SeHCAT; the first figure represents the number of patients in whom FBAL and pool data were available in addition to the ^{75}SeHCAT data, FL4D: fractional loss in 4 days (%), WBR_{50}: whole-body retention half-life (d), FBAL: faecal bile acid loss, pool: calculated bile acid pool size (mmol), ils: ileitis, cls: colitis, il res: ileal resection, non-ils: non-ileitis.

Table 2 shows that bile acid excretion as expressed by the ^{75}SeHCAT test and FBAL excretion was significantly higher in the ileal resection category than in all other categories of ileal disease. Both in ileitis and after ileal resection the presence of concurrent colitis seemed to result in a trend towards lower bile acid excretion (table 1), but this did not reach statistical significance (table 2). The category with ileal resection and concurrent colitis was to small (n=4) to allow statistical analysis. Only in the ileitis/colitis category was the ^{75}SeHCAT test not significantly different from the control group. The control group differed from all other categories with regards to FBAL. The bile acid pool size was similar in all groups.

Table 2: Significance of differences in ^{75}SeHCAT and 3α-OH bile acid excretion results between the 3 groups with ileal disease, the non-ileitis group and the control group

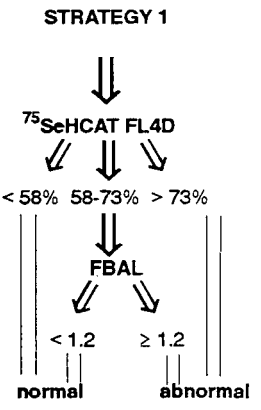
category	FL4D p	WBR_{50} p	FBAL p	pool p
ileitis vs. ils/cls	NS	NS	NS	NS
ileitis vs. il res	< 0.01	< 0.05	< 0.01	NS
ils/cls vs. il res	< 0.01	< 0.05	< 0.01	NS
control vs. ils	< 0.01	< 0.01	< 0.01	NS
control vs. ils/cls	NS	NS	< 0.01	NS
control vs. il res	< 0.01	< 0.01	< 0.01	NS
control vs. non-ils	NS	NS	< 0.01	NS

FL4D: fractional ^{75}SeHCAT loss after 4 days (%), WBR_{50}: ^{75}SeHCAT whole-body retention half-life, FBAL: faecal bile acid loss (mmol/d), pool: calculated bile acid pool size (mmol), ils/cls: ileitis with colitis, il res: ileal resection, non-ils: non-ileitis, NS: not significant (Wilcoxon-test).

Table 3a illustrates the results when bile acid excretion data were interpreted according to the proposed strategy of performing a ^{75}SeHCAT test and in addition only measuring FBAL in case of an equivocal ^{75}SeHCAT test result (31). Table 3a shows that in 16 of the 40 patients with ileitis (40%) this strategy for the ^{75}SeHCAT test led to a normal result. In the subgroup of patients with ileitis and concurrent colitis 12 of the 20 (60%) had a normal ^{75}SeHCAT test. The ^{75}SeHCAT test was normal in only 1 of the 17 patients with an ileal resection (6%). Directly measuring FBAL without measuring ^{75}SeHCAT resulted in a higher percentage of abnormal tests. As FBAL had in practice sometimes not been measured in case of a normal ^{75}SeHCAT result, the percentage of abnormal FBAL measurements is presented with the minimal and maximal score of abnormal results when FBAL would have been measured in all patients.

In table 3b, the proposed strategy is applied to the data of 42 patients with proven diarrhoea (defined as a mean 24 hr stool weight > 200 g). The results are listed in a similar fashion as in table 3a.

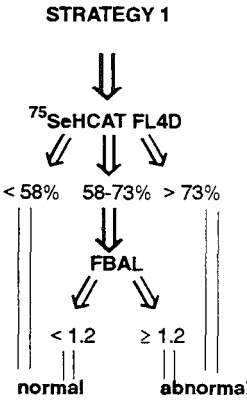
Table 3a: distribution of patients of various diagnostic groups according to 2 strategies for classification of bile acid excretion : strategy 1: ^{75}SeHCAT excretion supplemented with faecal 3α-OH bile excretion in case of equivocal ^{75}SeHCAT excretion, strategy 2: faecal 3α-OH bile acid excretion



category	n			
ileitis	20	4 + 0	4 + 12 = 16 (80%)	16-20 (80-100%)
ileitis/colitis	20	11 + 1	4 + 4 = 8 (40%)	11-17 (55-85%)
ileal resection	13	0 + 1	0 + 12 = 12 (92%)	12 (92%)
il res/colitis	4	1 + 0	0 + 3 = 3 (75%)	3-4 (75-100%)
non-ileitis	151	99 + 9	23 + 20 = 43 (28%)	53-126 (35-83%)

FBAL: faecal (3α-OH) bile acid loss (mmol/d), *FBAL: percentual range of abnormal FBAL results (N < 1.2), abn: abnormal, eq: equivocal, nl: normal, il res: ileal resection.
* presented as a range (minimal and maximal score in case FBAL would have been measured in all patients)

Table 3b: distribution of patients of various diagnostic groups with proven diarrhoea (≥ 200 g/d) according to 2 strategies for classification of bile acid excretion: strategy 1: ^{75}SeHCAT excretion supplemented with faecal 3α-OH bile excretion in case of equivocal ^{75}SeHCAT excretion, strategy 2: faecal 3α-OH bile acid excretion



category	n			
ileitis	13	3 + 0	2 + 8 = 10 (77%)	10-13 (77-100%)
ileitis/colitis	15	8 + 1	4 + 2 = 6 (40%)	9-13 (60-87%)
ileal resection	11	0 + 0	0 + 11 = 11 (100%)	11 (100%)
il res/colitis	3	0 + 0	0 + 3 = 3 (100%)	3 (100%)
non-ileitis	69	34 + 4	13 + 18 = 31 (45%)	38-50 (55-72%)

FBAL: faecal (3α-OH) bile acid loss (mmol/d), *FBAL: percentual range of abnormal FBAL results (N < 1.2), abn: abnormal, eq: equivocal, nl: normal, il res: ileal resection.
* presented as a range (minimal and maximal score in case FBAL would have been measured in all patients)

Of the 18 patients with ileal disease with a known length of ileal impairment, 11 had Crohn's ileitis (extent 10-120 cm) and 7 had undergone an ileal resection (30-80 cm).
The mean results of both ^{75}SeHCAT test parameters and chemical measured FBAL in relation to the extent of ileal impairment are presented in table 4. A wide range of bile acid excretion was found in all 3 groups with ileal malfunction with considerable overlap between the various groups. FBAL and ^{75}SeHCAT excretion tended to be higher, when ileal malfunction was more extensive. All patients with extensive ileal malfunction had severe bile acid malabsorption and this was the only group in which all 3 bile acid excretion parameters showed no overlap with the control group.

Table 4: Bile acid excretion for the 3 groups with various degrees of ileal malfunction in relation to a group of 12 control patients (mean and range indicated)

length	n	(ils/res)	^{75}SeHCAT FL4D	^{75}SeHCAT WBR_{50}	FBAL
< 20 cm	5	(5/0)	73(56-89)*	2.1(1.0-3.4)	3.1(0.7-7.0)*
20-40 cm	8	(4/4)	86(68-95)**	1.2(0.6-2.1)**	3.8(1.3-8.2)**
> 40 cm	5	(2/3)	90(84-97)**	0.7(0.4-1.2)**	4.8(4.1-5.6)**
control	12		54(11-71)	3.0(1.8-4.1)	0.8(0.6-1.1)

length: extent of ileal malfunction in cms as defined in text, n: number of patients, (ils/res): number of patients with ileitis/number of patients with ileal resection, ^{75}SeHCAT WBR_{50} is expressed in days and FL4D in %. FBAL: faecal 3α-OH bile acid loss in mmol/day. p-value obtained with the Wilcoxon sign rank test. NS: not significant, ** $p < 0.01$, * $p < 0.05$.

^{75}SeHCAT excretion and FBAL in all 3 groups with ileal disease were significantly different from the control group, with the exception of ^{75}SeHCAT WBR_{50} in the limited ileal disease group.
Table 5 shows the correlation between the length of ileal disease or resection and bile acid excretion. In general ^{75}SeHCAT excretion showed a better correlation with the extent of ileal involvement than FBAL. Separate calculation for the ileitis and ileal resection groups did not markedly affect the correlation coefficients.

Table 5: Coefficients of correlation between ^{75}SeHCAT excretion, faecal bile acid excretion and extent of ileal involvement.

		all	ils	il res
length	- FBAL	0.67	0.63	0.79
length	- ^{75}SeHCAT WBR_{50}	-0.79	-0.75	-0.82
length	- ^{75}SeHCAT FL4D	0.72	0.67	0.74

all: ileitis and ileal resection, ils: ileitis, il res: ileal resection, length: extent of ileal involvement, FBAL: faecal 3α-OH bile acid loss, WBR_{50}: whole-body retention half-life, FL4D: fractional loss after 4 days.

DISCUSSION

This study confirms that ileal disease leads to malbsorption of endogenous bile acids. The excretion of the gamma-labeled bile acid analogue ^{75}SeHCAT can also be increased in ileal disease, and sometimes also in other diarrhoeal disorders without demonstrable ileal pathology (table 1). 3α-OH bile acid and ^{75}SeHCAT malabsorption were higher after ileal resection than in ileitis (table 2). This is not surprising as less than 20 cm of ileum is rarely resected, often together with the coecum and the ileo-coecal valve, and as an inflamed ileum is probably capable of some residual bile acid uptake.

The role of ^{75}SeHCAT in the detection of ileal disease is disputed. Some studies have reported high sensitivity rates (20-22), but it should be born in mind that one of the factors determining the sensitivity of a test is the choice of cut-off points (17). The specificity of the ^{75}SeHCAT test as a test for ileal pathology is limited as many conditions other than ileal disease can give rise to bile acid malabsorption (32) as well as increased ^{75}SeHCAT excretion. In addition, as shown in table 2, in patients with both ileitis and colitis ^{75}SeHCAT excretion was not significantly different from the control group.

The ^{75}SeHCAT test was introduced as a relatively simple screening test for bile acid malabsorption. Reference values for the test have been based on clinical grounds (13) and not on chemically measured FBAL. Recently we have proposed new reference values based on simultaneous ^{75}SeHCAT and 3α-OH bile acid excretion measurements in a large number of patients with and without bile acid malabsorption (31). Using these reference values the ^{75}SeHCAT test result can be classified as normal, abnormal or equivocal. When faecal 3α-OH bile acid loss is only measured in case of an equivocal ^{75}SeHCAT test result all patients investigated with ^{75}SeHCAT can be classified as normal or as bile acid losers with a minimum of additional chemical FBAL measurements. One of the objectives of this study was to apply this strategy for the ^{75}SeHCAT test with selective FBAL measurements to the patients with known ileal disease.

It is not surprising that this strategy correctly identified virtually all patients with an ileal resection (table 3a) as abnormal. However, for diagnostic purposes the group of non-operated patients with ileitis is more interesting. The described strategy for the ^{75}SeHCAT test would have missed 35% of all patients with ileitis and a remarkably high proportion of 60% of the patients in the subgroup of patients with combined colitis and ileitis. These results do not support the opinion that the ^{75}SeHCAT test can replace small bowel X-rays as a first investigation in patients, who are thought to suffer from ileal disease (33).

Measurement of FBAL without preselecting patients with the ^{75}SeHCAT test would have improved the detection rate for the group of patients with ileitis with or without colitis to some extent (table 3a). The low sensitivity of the proposed ^{75}SeHCAT test strategy with selective FBAL measurements in detecting ileitis did not markedly improve when only patients with proven diarrhoea (> 200 g/d) were selected for the test (table 3b). The number of false-positives in the non-ileitis group even increased from 28% to 40%. Why the presence of colitis reduces bile acid excretion remains speculative. Small bowel transit might be slowed, allowing more efficient bile acid absorption. Alterations in the colonic bacterial flora and pH might result in colonic retention of ^{75}SeHCAT and/or increased passive bile acid absorption.

Some authors have proposed that ^{75}SeHCAT retention can be used as an indicator of the extent of ileal disease, but this has not been confirmed by all studies (19-23).
In a subgroup of 18 patients with a known length of ileitis or ileal resection we found a correlation between the extent of ileal disease and the severity of bile acid malabsorption. However there was a considerable overlap between the control group and the groups with various extents of ileal malfunction which limits the role for the ^{75}SeHCAT test in predicting the length of ileal involvement in individual patients. No overlap in chemically measured FBAL was found between the patients with moderate or extensive ileal disease (> 20 cm) and the control group. No overlap in the ^{75}SeHCAT test was only found between the group with extensive ileal disease (> 40 cm) and the control group.

In conclusion our results indicate that the ^{75}SeHCAT test is inferior to the FBAL determination as a screening test for ileal disease. Especially in combined ileitis and colitis the ^{75}SeHCAT test is often normal. Bile acid (and ^{75}SeHCAT) excretion is virtually always abnormal after ileal resection. Although bile acid excretion shows a correlation with the extent of ileal disease, the results of the ^{75}SeHCAT test and/or FBAL measurements cannot be used to predict the extent of ileal disease in individual patients. At present it is still not known whether the ^{75}SeHCAT test can be used to follow the evolution of ileal disease in patients with Crohn's disease.

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CHAPTER 8

Summary/samenvatting

Summary

Bile acid malabsorption is frequently found in the analysis of chronic diarrhoea. Bile acids are involved in the absorption of fat from the small intestine. The distal ileum plays a key role in the enterohepatic circulation of bile acids as it is the only intestinal site where bile acids are actively reabsorbed via a Na^+-dependent, carrier mediated transport mechanism.

In **chapter 1** the enterohepatic circulation of bile acids is described with special emphasis on intestinal bile acid absorption. Clinical syndromes characterized by a somehow altered bile acid metabolism, especially conditions associated with bile acid malabsorption, are discussed. Diagnostic approaches which provide insight into various aspects of the enterohepatic circulation of bile acids are listed, with specific attention to methods of diagnosing bile acid malabsorption.

Chapter 2 outlines the scope of this thesis.

Chapter 3 describes the results of measurement of active ileal bile acid transport in various patient groups with intestinal disorders known to affect bile acid metabolism. A micro-assay of Na^+-dependent ^3H-taurocholate uptake into brush border membrane vesicles prepared from terminal ileal biopsies, taken at retrograde ileocolonoscopy was used. The results indicate a decreased transport in ileal disease and surprisingly also in chronic constipation. An increased transport was found in diarrhoea with concomitant bile acid malabsorption which was not due to ileal disease. These findings suggest that intestinal bile acid transport is somehow regulated by the availability of bile acids to the ileal enterocytes.

In **chapter 4**, ten patients fulfilling the criteria of idiopathic bile acid malabsorption - chronic diarrhoea with unexplained bile acid loss responding to cholestyramine - are described. It had been postulated that idiopathic bile acid malabsorption was due to a defect of active ileal bile acid transport. In vitro Na^+-dependent bile acid transport as quantified by the micro-assay applied in chapter 3 showed no evidence of a carrier defect in any of these 10 patients: in contrast there was an increased bile acid transport compared to a control group of 132 patients. Our findings imply that the mechanism of idiopathic bile acid malabsorption is different from the previously assumed mechanism. The actual pathophysiology remains obscure, although it is tempting to postulate motor disturbances of the ileum which result in a reduced contact time and inhibit optimal bile acid absorption.

In **chapter 5** an evaluation is presented of the utility of the gamma emitting bile acid analogue [23-^{75}Se]-25-homocholic acid taurine (^{75}SeHCAT) in predicting chemically measured (3α-OH) faecal bile acid excretion. An initial evaluation in the laboratory confirmed that the ^{75}SeHCAT test can equally well be executed by scanning of daily collected faeces as by abdominal retention measurements. We advocate the faecal collection method as it allows inspection of stools and additional chemical analysis when the ^{75}SeHCAT test is not conclusive. Our results in 195 patients indicate that the reference values for the ^{75}SeHCAT test reported in the literature and recommended by its manufacturer do not accurately predict faecal bile acid excretion. The reference values recommended by the manufacturer resulted in a large percentage (51 %) of false-positive tests, when applied to the patients in our study. New reference values were proposed with a maximal rate of 10 %

false-negative and false-positive results. As a consequence in 40-50 % of the ^{75}SeHCAT tests the outcome was equivocal and additional faecal bile acid measurements were indicated.

In **chapter 6** an evaluation of the conditions associated with bile acid malabsorption is made in a large number of patients investigated with the ^{75}SeHCAT test. Results of chemical bile acid measurements and ^{75}SeHCAT excretion are compared in various types of bile acid malabsorption. Conditions not previously reported to be accompanied by bile acid malabsorption were encountered, including diarrhoea related to alcohol abuse, hyperthyroidism, short bowel syndrome, chronic intermittent pseudo-obstruction, intestinal lymphoma and lambliasis (type 3 bile acid malabsorption). The pathophysiology of bile acid malabsorption in these conditions is unknown. It is concluded that bile acid malabsorption can occur in a wide variety of conditions characterized by abnormal small intestinal morphology or physiology. In general bile acid malabsorption was not a feature of large bowel pathology.

Analysis of the available variables (^{75}SeHCAT excretion data, 3α-OH bile acid excretion, bile acid pool size, faecal weight, oro-anal marker transit time, faecal Na^+ and K^+ content and concentration) revealed that the faecal weight only showed a significant positive correlation with faecal bile acid excretion in ileitis and alcohol related diarrhoea. The oro-anal transit time of radio-opaque markers was shorter in the group with primary bile malabsorption than in any other group with diarrhoea and/or bile acid malabsorption. Only in primary bile acid malabsorption a significant positive correlation was found between faecal weight and pool size, implicating that in this condition a large pool size might play a role in the diarrhoea. In addition analysis of a correlation between faecal weight, bile acid excretion parameters, bile acid pool size, oro-anal transit time, Na^+ and K^+ excretion data revealed a good relation between faecal weight and Na^+ excretion. Using discriminant analysis on all variables a reliable classification of the patients was not possible due to the wide variety of diagnostic groups in relation to the small number of variables.

Chapter 7 handles about the value of two ^{75}SeHCAT test parameters - whole-body retention half-life and fractional loss after 4 days - and faecal bile acid measurements in detecting ileal disease and predicting the length of non-functioning ileum. In our series of 57 patients the ^{75}SeHCAT test was often normal in ileal disease, especially in ileitis with concurrent colitis. Bile acid excretion as well as both ^{75}SeHCAT test parameters showed a correlation with the extent of resected or inflamed ileum in a subgroup of 18 patients, without being able to predict the length of ileal involvement in individual patients accurately. Whether the ^{75}SeHCAT test can be used to follow the course of the disease remains uncertain.

In conclusion, the ^{75}SeHCAT test is neither a very sensitive nor a specific test for the detection of ileal disease, but it facilitates the diagnosis of bile acid malabsorption.

Samenvatting

Bij onderzoek naar de oorzaak van diarrhee kan malabsorptie van galzuren vaak worden vastgesteld. Galzuren spelen een rol bij de opname van vet uit de dunne darm. Het distale ileum speelt een belangrijke rol bij de enterohepatische kringloop van galzuren omdat het de enige plaats is waar galzuren door middel van een Na^+-afhankelijke opname via een transport-eiwit vanuit de darm worden opgenomen.

In **hoofdstuk 1** wordt de enterohepatische galzuurkringloop beschreven met speciale aandacht voor de opname van galzuren vanuit de darm. Ziektebeelden, waarbij het galzuurmetabolisme veranderd is en met name waarbij malabsorptie van galzuren optreedt, worden besproken. Onderzoeksmethodes die inzicht geven in de enterohepatische galzuurkringloop worden genoemd met speciale aandacht voor de diagnostiek van galzuur- malabsorptie.

In **hoofdstuk 2** wordt het bestek van dit proefschrift geschetst.

In **hoofdstuk 3** worden de resultaten beschreven van metingen van het actief galzuurtransport in het ileum bij verschillende darmziekten, die gepaard gaan met een veranderd galzuurmetabolisme. Voor dit doel wordt een micro-bepaling gehanteerd die de Na^+-afhankelijke opname meet van ^{3}H-taurocholzuur in borstelzoom-membraan blaasjes, vervaardigd uit terminale ileum biopten verkregen bij retrograde ileocolonoscopie. Het transport bleek verlaagd bij ziekten van het terminale ileum, maar ook bij chronische obstipatie. Het transport was verhoogd bij diarrhee met verhoogd galzuurverlies, als dit niet veroorzaakt werd door ziekte van het ileum. Deze bevindingen suggereren dat het galzuurtransport in het ileum beinvloed wordt door het lokale aanbod van galzuren aan de darmepitheelcellen.

In **hoofdstuk 4** worden 10 patienten beschreven met idiopathische galzuurdiarrhee, dat is diarrhee met galzuurmalabsorptie door onbekende oorzaak, reagerend op behandeling met cholestyramine. Verondersteld werd dat bij deze aandoening de galzuurmalabsorptie het gevolg was van een stoornis van het aktieve transport in het ileum. In vitro Na^+-afhankelijk galzuur transport gemeten met behulp van de in hoofdstuk 3 beschreven microbepaling toonde bij geen van deze 10 patienten aanwijzingen voor een afwijking van het transport-eiwit. Het transport bleek zelfs significant verhoogd ten opzichte van een groep van 132 controle patienten. Dit betekent dat de pathogenese van idiopathische galzuurdiarrhee anders is dan werd verondersteld. De pathogenese blijft onduidelijk hoewel het voor de hand ligt om te veronderstellen dat er sprake is van motoriek veranderingen in het ileum, die resulteren in een korter contact tussen galzuren en het darmslijmvlies en een optimale galzuuropname belemmeren.

In **hoofdstuk 5** wordt het gamma stralende galzuur isotoop [23-^{75}Se]-25-homotaurocholzuur (^{75}SeHCAT) geevalueerd in het voorspellen van de chemisch gemeten (3α-OH) faecale galzuurexcretie. Een eerste evaluatie wees uit dat meting van retentie in het abdomen even betrouwbaar is als meting van excretie in de faeces. Wij prefereren de faeces verzamel methode omdat hiermee ook inspectie van de faeces en verdere chemische bepalingen mogelijk zijn, met name als het resultaat van de ^{75}SeHCAT test niet eenduidig is. Onze bevindingen in 195 patienten tonen aan dat de normaalwaarden opgegeven door de fabrikant de faecale galzuur excretie niet

betrouwbaar voorspellen. Toepassing van deze normaalwaarden op onze patienten populatie resulteerde in 51 % fout-positieve uitslagen. Wij stellen dan ook nieuwe referentie waarden voor gebaseerd op een maximum van 10% fout-positieve en fout-negatieve uitslagen. Het gevolg van deze voorgestelde referentiewaarden is dat in 40-50 % van de onderzoeken de uitslag van de ^{75}SeHCAT test niet in het abnormale of normale gebied valt en aanvullende chemische galzuurbepalingen noodzakelijk zijn.

In **hoofdstuk 6** wordt de klinische achtergrond geevalueerd van een groot aantal patienten met galzuurmalabsorptie die met behulp van de ^{75}SeHCAT test onderzocht zijn. De resultaten van de ^{75}SeHCAT test en chemische galzuur excretie bepaling worden vergeleken bij patienten groepen met diverse typen galzuurmalabsorptie. Aandoeningen, waarvan niet eerder beschreven was dat ze gepaard konden gaan met galzuurmalabsorptie worden beschreven, zoals diarrhee bij alcohol misbruik, hyperthyreoidie, het korte darm syndroom, chronisch intermitterende pseudo-obstructie, dunne darm lymphoom en lambliasis (type 3 galzuur malabsorptie). Het mechanisme van het galzuurverlies bij deze aandoeningen is onbekend. Geconcludeerd wordt dat galzuurmalabsorptie kan optreden bij aandoeningen waarbij er morfologische of functionele afwijkingen van de dunne darm optreden.
In het algemeen treedt bij dikke darm pathologie geen galzuurmalabsorptie op.
Analyse van de beschikbare variabelen (^{75}SeHCAT test gegevens, 3α-OH galzuur excretie, grootte van de galzuurpool, faecesgewicht, oro-anale markerpassagetijd, faecale Na^+ en K^+ gehalte en concentratie) toonde dat het faeces gewicht alleen een significante positieve correlatie vertoonde met de faecale galzuur excretie bij patienten met ileitis of alcohol gerelateerde diarrhee. De oro-anale passagetijd van radio-opaque markers was korter in de idiopathische galzuur diarrhee groep dan in alle andere groepen. Alleen in de idiopathische galzuurdiarrhee groep werd een significante positieve correlatie gevonden tussen de galzuur pool grootte en het faecesgewicht, duidend op een mogelijke rol van een grote pool bij het mechanisme van de diarrhee bij deze aandoening.
Analyse van een verband tussen faecesgewicht, galzuurexcretie parameters, galzuurpoolgrootte, oro-anale passagetijd, Na^+ en K^+ excretie gegevens toonde een goede relatie tussen faeces gewicht en Na^+ excretie. Het was niet mogelijk om met discriminant analyse patienten te classificeren op grond van bovengenoemde parameters door het te kleine aantal parameters in verhouding tot het grote aantal diagnostische groepen.
In **hoofdstuk 7** worden twee ^{75}SeHCAT test parameters - de halfwaardetijd in het lichaam en de fractionele excretie na 4 dagen - en faecale galzuurexcretie vergeleken in hun vermogen ileumpathologie te detecteren en de lengte van niet-functionerend ileum te voorspellen. De ^{75}SeHCAT test was vaak normaal in onze groep van 57 patienten met ileum-ziekten, met name in geval van ileitis en gelijktijdige colitis. In een sub-groep van 18 patienten bleken zowel de galzuurexcretie als beide ^{75}SeHCAT test parameters weliswaar te correleren met de lengte van het zieke of verwijderde ileum segment, maar konden zij de lengte van het bewuste segment in individuele gevallen niet betrouwbaar voorspellen. Het blijft onzeker of de ^{75}SeHCAT test bruikbaar is om het beloop van ziekten van het ileum te vervolgen.

Samenvattend is de ^{75}SeHCAT test noch zeer gevoelig noch specifiek voor de aanwezigheid van ileumpathologie, maar vergemakkelijkt de test het vaststellen van galzuurmalabsorptie.

Nawoord

Dit proefschrift is bewerkt op de afdeling inwendige geneeskunde 2 van het Academisch Ziekenhuis Dijkzigt te Rotterdam.
Vele mensen zijn betrokken geweest bij de tot standkoming ervan. Enkelen wil ik met name noemen.
dr. F.W.M. de Rooij, Felix, het heeft jou zeer veel tijd en energie gekost. Jouw volhardendheid en originele ideeën waren onmisbaar. Jij hebt de transport-assay ontwikkeld, die de basis vormt van enkele artikelen. Je streven naar perfectie resulteerde in gemiddeld minstens 25 versie's van een artikel, maar had wel tot gevolg dat ingezonden stukken meestal ook voetstoots werden geaccepteerd.
prof. J.H.P. Wilson, Paul, ik vind het bewonderenswaardig, dat iemand die zoveel dingen aan zijn hoofd heeft, toch altijd gelegenheid heeft om snel artikelen door te lezen en te bespreken zonder daarbij zijn goede humeur te verliezen. Het was steeds zeer verfrissend om met jou over de grote lijnen in het onderzoek te discussiëren.
prof. dr.M. Frenkel, die als opleider mij de grondbeginselen van de Inwendige geneeskunde in zijn geheel eigen stijl heeft bijgebracht.
drs. M. van Blankenstein, Mark, jij hebt mijn interesse gewekt voor galzuren en galzuur-diarrhee en daarmee de aanzet gegeven tot dit proefschrift. Jouw creatieve geest en bekende cynische humor wil ik op deze plaats graag noemen.
dr. J.W.O. van den Berg, Wim jij bent van oudsher bij het galzuuronderzoek en ook de ^{75}SeHCAT-test betrokken geweest en hebt de artikelen kritisch bestudeerd en van nuttige suggestie's voorzien.
Elly Bosman-Jacobs, Gardi Voortman en Jeanette Touw-Blommesteijn wil ik bedanken voor hun toegewijdheid bij de galzuurtransport metingen. Ook de overige medewerkers van het lab interne 2 wil ik bedanken voor hun aandeel in de ^{75}SeHCAT testen en galzuurbepalingen.
prof.dr. S.G.M. Meuwissen, Stephan, zonder jouw permissie voor mijn vrije dinsdagmiddag, die ik steevast in Rotterdam op het lab doorbracht, was het niet gelukt. Het heeft langer geduurd dan we beide gehoopt en verwacht hadden. In die kleine 3 jaar ben je mijn opleider in de Gastroenterologie geweest, een belangrijke periode voor mij, waar ik met plezier op terug kijk. Ik ben je zeer erkentelijk voor het plaatsnemen in de beoordelings-commissie.
prof.dr. E.P.Krenning en dr. H.R. de Jonge wil ik bedanken voor het beoordelen van het proefschrift.
Mijn ouders, die mij altijd gestimuleerd hebben en daadwerkelijk hebben bijgedragen door middel van de schenking van een PC.
Mijn schoonvader heeft diverse waardevolle software programma's ontwikkeld. Helaas heeft hij de afronding van het proefschrift niet meer mogen meemaken.
Dorothé, het heeft heel veel van je gevergd, maar gelukkig heb je altijd het belang ervan ingezien en mij gesteund. Na 5 jaar nu voor het eerst de PC niet meer mee op vakantie!

Curriculum vitae

De schrijver van dit proefschrift werd geboren op 14 september 1956 te Rotterdam. Na het behalen van het gymnasium-ß diploma aan het Rotterdams Montessori Lyceum in 1974, werd de studie geneeskunde aangevangen aan de Erasmus Universiteit Rotterdam, alwaar in 1981 het arts-examen werd afgelegd.
Aansluitend werd gestart met de opleiding tot internist op de afdeling Inwendige Geneeskunde II (afdelingshoofd prof.dr.M.Frenkel en na diens emeritaat prof.J.H.P.Wilson) van het Academisch Ziekenhuis Dijkzigt Rotterdam. Eén jaar van de opleiding (1 november 1981 tot 1 november 1982) werd in het kader van een uitwisselingsprogramma doorgebracht in het St. Bartholomews Hospital in Londen onder leiding van prof.C.J.Dickinson. In dit jaar werd met succes het Amerikaanse Visa Qualifying Examination afgelegd.
Tijdens de opleiding werd gestart met onderzoek hetgeen uiteindelijk resulteerde in dit proefschrift. Na de inschrijving als internist in het Specialisten Register op 1 augustus 1986 was hij als zodanig tot 1 februari 1988 werkzaam op de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Dijkzigt.
Op 1 februari 1988 werd de opleiding tot gastroenteroloog aangevangen in het Academisch Ziekenhuis der Vrije Universiteit te Amsterdam onder leiding van prof.dr.S.G.M. Meuwissen. Vanaf 1 augustus 1990 was hij als gastroenteroloog registreerbaar en tot 1 januari bleef hij aldaar werkzaam.
Vanaf 1 januari 1991 is hij werkzaam als internist in het Sint Franciscus Gasthuis te Rotterdam.