CROHN’S DISEASE WITH SPECIAL REFERENCE TO INTESTINAL MALABSORPTION

A clinical study based on patients from Northern Sweden

Av

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UMEÅ 1984
ABSTRACT

Crohn's disease is a chronic inflammatory bowel disease which may affect any part of the gastrointestinal tract with a preference for the terminal ileum and ileocaecal region. The disease was first described in 1932 and has increased during the last decades. The clinical manifestations could be referred to as inflammation, malabsorption and obstruction.

The annual incidence of Crohn's disease in the county of Västerbotten, North Sweden, was found to be 4.9/10^5 inhabitants. In a study of 87 patients in a medical gastrointestinal unit, 23% of non-operated patients and 66% of resected patients had increased fecal fat excretion. D-xylose test and lactose tolerance test were abnormal in 19% and 24% respectively of the non-operated patients. No clear relation could be found between the outcome of these malabsorption tests and localization, extension or activity of the disease. This suggests the cause of malabsorption in Crohn's disease to be complex and multifactorial.

The morphology of jejunal biopsies from 18 patients with Crohn's disease elsewhere in the gastrointestinal tract demonstrated an abnormal picture in 13 patients when assessed by light microscopy and scanning electron microscopy. A high proportion of these patients had abnormal intestinal absorptive tests.

Skeletal muscle biopsies were performed in 13 patients showing a depletion of muscle potassium content and more infrequently low skeletal muscle magnesium content. This depletion is not reflected by subnormal plasma concentration.

In the initial clinical assessment of a new gamma labelled synthetic bile acid-SeHCAT, 45 patients, 19 of whom had Crohn's disease, were studied. The outcome of the test correlated well with the excretion of fecal bile acids. It was possible to discriminate patients with terminal ileal disease from other patient groups.

In a follow-up study, the SeHCAT test was modified as to make it simpler and to shorten the test period. Nine patients with Crohn's disease were tested, showing a sufficient accuracy of the outcome of the test within 48 hours, using simple equipment available in many hospitals. The elimination of radioactivity was calculated as WBR50; the time for 50% of the administered dose to be excreted. This gives information as to the rate of excretion, reflecting the degree of terminal ileal malfunction.

Key words: Crohn's disease, intestinal absorption tests, muscle electrolytes, intestinal mucosa, terminal ileal function, SeHCAT
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ORIGINAL PAPERS

The present thesis is based on the following papers which are referred to by their Roman numerals:

I Nyhlin H, Danielsson Å, Ek B.
Submitted for publication

II Nyhlin H, Stenling R.
The small intestinal mucosa in patients with Crohn's disease assessed by scanning electron and light microscopy.
Scand J Gastroenterol. In press

III Nyhlin H, Dyckner T, Ek B, Wester PO.
Plasma and skeletal muscle electrolytes in patients with Crohn's disease.
J Amer Coll Nutr. In press

IV Nyhlin H, Merrick MV, Eastwood MA, Brydon WG.
Gastroenterology 1983;84:63-68

V Nyhlin H, Brydon WG, Danielsson Å, Westman S.
Clinical application of a selenium($^{75}$Se)-labelled bile acid for the investigation of the terminal ileal function.
Hepato-Gastroenterol, in press
INTRODUCTION

History
At the annual meeting of the American Medical Association held in New Orleans, 1932, Burril B Crohn and coworkers (1) presented a paper containing 14 cases of terminal ileitis. The term regional enteritis was later suggested as the disease occasionally may occur in the upper small bowel.

This was the start of what today is known as Crohn's disease. However, in retrospect, several cases of possible Crohn's disease could be found in the literature. The first goes back to 1769 when Morgagni (2) described a case with ileal ulceration and enlarged mesenteric lymph glands. Another case with thickening of the terminal ileum was related 1813 by Combe (3). In 1913, the Glasgow surgeon Dalziel described nine cases of whom seven made a perfect recovery after intestinal resection (4). Dalziel concludes: "My friends the pathologists prefer to call it hyperplastic enteritis, and I can only regret that the ethiology of the condition remains in obscurity, but I trust that ere long further considerations will clear up the difficulty". Dalziel's prophetic ability was however poor and still, 70 years later, no definite answer has been given to the cause of Crohn's disease.

Epidemiology
Irrespective of improvement in diagnostic procedures, there has been a general agreement that the incidence of Crohn's disease has increased in several western countries, although, still being low in Africa and Asia (5-10).

Epidemiological studies have been carried out in Sweden (11, 12) showing an average annual incidence of Crohn's disease of 4-5 per 100,000 inhabitants with a tendency of increased incidence rates during later years. Hellers (13), showed that the incidence of Crohn's disease in Stockholm county had increased from approximately 1.0 to 4.5 in the early 70's, reaching a plateau during the 70's. A decrease in incidence has recently been reported from Scotland (14).
Clinical characteristics of Crohn's disease

Shortly after the initial description by Crohn et al (1), it was demonstrated that the disease could affect the jejunum (15) as well as the caecum (16). Later, Crohn's disease was found to affect simultaneously or exclusively the large bowel, and could be distinguished from ulcerative colitis (17, 18). It is now established that Crohn's disease may affect any part of the gastrointestinal tract even including the mouth (19), the esophagus (20), the stomach (21) and the duodenum (22).

There is a preponderance for ileocaecal involvement as the initial site of Crohn's disease. Frequencies of 41-55% with ileocaecal involvement, 29-41% small intestinal, and 14-27% colonic have been described, whereas only 1-3% has a location elsewhere in the gastrointestinal tract (23-25).

The most common clinical symptoms include diarrhea and abdominal pain. The abdominal pain is often postprandial and localized to the right hypochondrium. The major systemic manifestations of Crohn's disease include weight loss, fever, anemia, anorexia and weakness. Fever reflects the underlying inflammatory process, sometimes indicating the presence of an inflammatory mass with localized perforation and multiple abscess formation. Occasionally, generalized septicemia occurs. Sometimes, the initial clinical sign of Crohn's disease may even be fever of unknown origin, without diarrhoea or abdominal pain (26, 27).

Diagnostic considerations

The diagnosis of Crohn's disease is often based on a combination of clinical data, radiological and endoscopical findings as well as on histology. The anatomical manifestation of Crohn's disease is often discontinuous, e.g. scattered ulcers with intervening normal-looking mucosa and asymmetrical involvement of a bowel segment. The bowel wall is considerably thickened and the inflammation is obviously transmural. However, there are no specific diagnostic features of Crohn's disease and the pathologist has to rely on the recognition of a characteristic histological pattern. Three principal macroscopic patterns may distinguish Crohn's disease (28).
1. The ulcerations of the mucous membrane are serpinginous and discontinuous and begin as small aphtoid ulcers. They vary in size from tiny, pinpoint hemorrhagic lesions to small, clearly defined shallow ulcers with a white base.

2. The strictures may be short or long, single or multiple. The classical type is the single "hose pipe" stricture of the terminal ileum, but similar narrowing can affect any part of the gastrointestinal tract.

3. Classic "cobblestone" appearance of the mucous membrane are formed as the result of intercommunicating crevices of fissures surrounding islands of mucous membrane, which are elevated by the underlying inflammation and edema.

Microscopically, the appearance of granulomas composed of epitheloid cells and giant cells of Langhans' type are present in some 60% of the affected tissues and the regional lymph glands. Fissures, although not a specific feature of Crohn's disease, are often found passing deep into the bowel wall (28).

Even in the absence of granulomas and fissures the histological picture of Crohn's disease may be indicative. There is a transmural inflammation with widening of the submucosa by edema. Focal collections of lymphocytes are scattered across all layers of the bowel wall, as well as in the serosa and peri-intestinal fat. Vascular changes may be present. The inflammatory infiltrate is predominantly lymphocytic with some plasma cells, without polymorphs except in crypt abscesses. As a consequence of the diagnostic inaccuracy several suggestions of diagnostic criteria have been made, basically relying on histological and radiological features (7, 29-31).

**Disease activity**

The clinical consequences of Crohn's disease are inflammation, malabsorption and obstruction, with abdominal pain as a major symptom. There is an obvious problem in assessing a disease with such varying
manifestations. Furthermore, the disease has a course with periods of remittance followed by relapses. The complexity of the disease has led to the development of numerical indices supposed to reflect the disease activity. The first index proposed by Best et al (32), was used by the National Cooperative Crohn's Disease Study Group in the USA (33). Being cumbersome in practice it's usefulness has been questioned (34, 35). It has been suggested to have no advantage over a simple index suggested by Harvey and Bradshaw (36).

Another index was suggested by van Hees et al, 1980 (37) consisting of nine variables; albumin, ESR, Quatellet index, abdominal mass, sex, temperature, stool consistency, resection and extraintestinal lesions. The different disease activity indices recognize inflammation, malabsorption and obstruction differently, which is important to be aware of, when applied in clinical studies.

Digestion and intestinal absorption
A short summary of certain basic physiological processes involved in the digestion and intestinal absorption are reviewed.

The human diet is composed of a mixture of large molecules, which are mechanically treated by chewing and gastric motility. Acid hydrolysis takes place in the stomach. The food constituents are subjected to enzymatic degradation at different levels in the gastrointestinal tract. Enzymatic systems in the saliva, gastric, duodenal, jejunal and pancreatic juice degrade the lipid, protein and carbohydrate macromolecules into smaller components. The final enzymatic degradation takes place in the intestinal epithelium and free fatty acids, amino acids and monosaccarides are absorbed. The absorption of these substances is considered to be nearly complete when the bolus reaches the ileum. In the ileum a specific absorption of bile acid and vitamin B₁₂ takes place. In the first part of the colon a number of active processes take place, such as conservation of water and electrolytes, and it also serves as a fermenting chamber (38).

Lipids. Dietary fat is mainly in the form of long chain fatty acid esters of glycerol (triglycerides), which are insoluble in water. The first step of fat digestion takes place in the stomach where lingual
lipase action may promote emulsification of dietary fat. In the upper part of the small intestine lingual lipase also enhance pancreatic lipase action which hydrolyses the emulsion (39). Bile salts inhibit pancreatic lipase, but the inhibition is relieved by the aid of the cofactor colipase. Bile acids are mainly in micellar solution in the upper small intestine but also in monomolecular forms. Micelles are molecular aggregates arranged so that the polar groups are oriented towards the external surface promoting water solubility while the lipophilic groups face internally. When free fatty acids and/or monoglycerides are formed and released, they can be incorporated into mixed micelles with bile acids. The end result is the formation of micellar fat which is water soluble (40). Recently it has been proposed that there are also other phases present in the fat digestion (41). After passing the unstirred layer of water, the micellar fat reaches the cell membrane and easily penetrates by diffusion due to high lipid solubility. Upon entrance into the cells the fatty acids or monoglycerides are re-esterified to triglycerides and the bile acids are left behind in the lumen of the small bowel. The absorption of fat is generally completed in the proximal jejunum (42).

Carbohydrates: The diet contains carbohydrates in the form of starch, sucrose, lactose and fructose. The digestion of starch starts in the oral cavity by salivary amylase, but the principal enzymatic digestion of starch into maltose and dextrose is mediated by pancreatic amylase. The final stage of degradation of the disaccharides is completed by enzymes in the surface membrane of the intestinal mucosa cells covering the villi. The enzymes lactase, glucoamylase and α-dextrinase are located within the brushborder of the enterocyte (43). The monosaccharides are actively transported into the surface epithelial cells. The efficiency of the carbohydrate uptake therefore depends on the integrity of the enterocyte surface.

Bile acids. Bile acids, the detergent factor in the bile, are formed in the liver from cholesterol by a complex metabolic process, which is partly under hormonal control. In man, the bile acid pool size is 3 to 5 g and circulates 6-10 times per day. A 200-600 mg daily loss via the feces is compensated for by de novo synthesis from cholesterol. The
primary bile acids are chenodeoxycholic acid (3α, 7α-dihydroxycholanic acid) and cholic acid (3α, 7α, 12α-trihydroxycholanic acid). The detergent effect of bile acids is produced by the α-orientated hydroxyl groups protruding from the molecule, which give rise to a water soluble facet. Conjugation with taurine or glycine further increases the water solubility.

The gallbladder stores and concentrates the bile. Upon meal stimulation gastrointestinal hormones are released from the duodenal and jejunal mucosa by acid and breakdown products of protein and fat. Cholecystokinin (CCK-PZ) causes contraction of the gallbladder and bile acids are discharged into the duodenum. This effect is augmented by the simultaneous release of secretin. In the jejunum, bile acids combine with phospholipids and monoglycerides to form mixed micelles. After the fat has been absorbed, a bulk of bile acids are reabsorbed in the terminal ileum. Some bile acids are also passively taken up in jejunum, ileum and proximal colon. The absorbed bile acids are transported to the liver via the portal vein.

Deconjugation and 7α-dehydroxylation of bile acids are the main metabolic effects caused by intestinal bacteria. The secondary bile acids, deoxycholic acid and lithocholic acid, are produced from the primary bile acids, cholic and chenodeoxycholic acid, respectively. The secondary bile acids which constitute the major part of fecal bile acids, may selectively be absorbed from the colon to appear in small amounts in the bile.

Abnormal bacterial colonization of the gut leads to increased deconjugation of bile acids. Hoffman (44) has suggested that when the enterohepatic circulation is broken by resection of the ileum, an excess of bile acids passes into the colon and watery diarrhea may result. The diarrhea from these patients contains primary bile acids, which have not undergone dehydroxylation either because there is inhibition of 7α-dehydroxylation by high concentrations of bile acids or because there is, too rapid a transit of feces through the colon. Chenodeoxycholic acids appears to be the bile acid with the most pronounced cathartic properties (45).
BACKGROUND TO THE STUDY

In the assessment of small bowel function, many complex biochemical processes have to be investigated before a final conclusion could be reached about impairment in the uptake of different nutrients. No techniques for direct measurements exist in practical clinical use. The measurement of small intestinal function is therefore indirect. Abnormalities in digestion and a subsequent malabsorption of dietary constituents can occur due to impairment at various stages involving the digestive enzymes: the luminal environment, the integrity of either the structural or metabolic properties of the mucosal cell; the transport system or the necessary physical contact between the mucosal cell and the products of digestion (46).

The initial symptoms of Crohn's disease may be nonspecific and the time period from the onset of symptoms until the correct diagnosis is established is often delayed for up to four or five years (47, 48). Weight loss is a common symptom which either can be attributed to the patient's anorexia and decreased food intake caused by postprandial pain or to the catabolic state induced by the active inflammatory process, and corticosteroid therapy. Malabsorption of fat, carbohydrates and proteins may also contribute to the weight loss. Malabsorption may occur in Crohn's disease for a variety of reasons including loss of absorptive surface as a consequence of the disease process, intestinal stasis and/or bacterial overgrowth associated with narrowing or stricture formation in the bowel as well as previous segmental resection of bowel (49).

Defining the initial anatomical localization of the disease has been suggested to be of value in the prediction of subsequent complications, clinical features and indications for surgery (50). Attempts have been made to localize diseased bowel by specific malabsorption tests (51-62). The results are conflicting in that patients with radiologically defined disease in the ileocaecal region may present biochemical tests indicating jejunal malabsorption. In a group of 52 patients with Crohn's disease of which only five had jejunal involvement, Sladen and Kumar found 19 patients with abnormal D-xylose test
In 61 patients, Beeken found no relation between steatorrhea and disease localization (62). Thus, Crohn's disease may diffusely involve the gastrointestinal tract, as has been suggested in several studies (63-66). Previous studies on malabsorption in Crohn's disease have shown steatorrhea to be present in 15-69% (52, 60-62), abnormal D-xylose test in 17-39% (51, 52, 60-62) and abnormal lactose tolerance test in 9-22% (60, 62). However, these studies comprise selected patient groups and some studies lack distinct diagnostic criteria or make no clear distinction between resected and non-resected patients. As abnormal malabsorption tests are not always related to the radiological localization of the disease, it is thought to be of value to assess the morphology of the jejunal mucosa in relation to the malabsorption tests.

In the clinical setting of Crohn's disease, electrolyte deficiencies has been described and suggested to be of significant importance (67-69). Magnesium deficiency has been demonstrated to cause neurological dysfunction, weakness and mental confusion in patients with Crohn's disease (68). However, as both potassium and magnesium are located predominantly intracellularly plasma estimation may be of little value. In evaluating potassium and magnesium deficiencies as in malabsorptive states of Crohn's disease the intracellular content has to be estimated.

Recognition of a diseased terminal ileum is of importance in the investigation of Crohn's disease. Bile acid malabsorption and choleretic diarrhea is a clinical feature of patients with Crohn's disease of the terminal ileum (70). Various methods are available including barium follow through, colonic endoscopy and function tests such as the Schilling test, $^{14}$C-cholyglycine breath test and the estimation of fecal bile acids. All of these tests have shortcomings. The radiology have technical and subjective limitations. The Schilling test is a test of vitamin $B_{12}$ absorption and is influenced by a number of factors independent of the state of the ileum. The others require specialized laboratory facilities. It would be of great value if more specific and simple tests for the investigation of ileal function could be developed.
AIMS OF THE STUDY

In patients with Crohn's disease:

1. To characterize the patient population with Crohn's disease at the Department of Medicine, Umeå University Hospital, with respect to some malabsorption tests and to study the incidence of the disease in the county of Västerbotten 1974-1981.

2. To describe the jejunal mucosa as assessed by light and scanning electron microscopy in patients with distal localization of the disease.

3. To study skeletal muscle contents of potassium and magnesium.

4. To evaluate SeHCAT - a new isotope test for bile acid absorption and to make an application for practical clinical use.
PATIENTS

Diagnostic criteria
The diagnosis of Crohn's disease was validated using the criteria proposed by Garland et al (7). A definite diagnosis is confirmed by characteristic histology from an operative specimen. A probable diagnosis is suggested by either: a laparotomy report of characteristic naked-eye appearance of the small bowel or an equivocal histological report from an operative specimen with typical macroscopic features or a colonoscopic report compatible with Crohn's disease and biopsy with features strongly suggestive of Crohn's disease or a radiologic examination strongly suggestive of intestinal or colonic inflammatory disease with obstructive or fistulose features. Finally a possible diagnosis of Crohn's disease is defined as a medical history and a discharge diagnosis of Crohn's disease, regional enteritis or granulomatous colitis; no clinical or radiological findings inconsistent with the diagnosis.

Incidence study population (I)
During 1974-1981, 96 new cases of Crohn's disease were recorded in the county of Västerbotten (245,000 inhabitants). The information was derived from the medical notes of patients with a diagnosis of Crohn's disease discharged from the departments of internal medicine, surgery and pediatrics at the three hospitals of the county. The diagnoses were retrospectively validated using the criteria described by Garland et al (7) and included were only patients with a definite or a probable diagnosis.

Clinical study population (I)
The North Health Region of Sweden included three counties with, in all, 645,000 inhabitants. The University Hospital of Umeå serves, with its specialized units, as a Region Hospital and is situated in the county of Västerbotten with 245,000 inhabitants.

During 1974-1981, 103 patients with a diagnosis of Crohn's disease were investigated at the Department of Medicine, Umeå University Hospital. Eighty-seven of these patients fulfilled the criteria of a de-
finite or probable diagnosis of Crohn's disease and were included in the study. Of these patients, 51 were females with a mean age of 31 years (18-66) and 36 males with a mean age of 37 years (16-74). Sixty-one of the patients had not been subjected to intestinal resection. The medical records of all 87 patients were retrospectively reviewed. Fecal fat excretion had been examined in 53 (87%) of the non-resected patients and in 18 (69%) of the operated patients. D-xylose test had been carried out in 53 (87%) of the non-resected patients and in 11 (42%) of the resected patients whereas lactose tolerance test could be validated in 41 (67%) and 9 (35%) respectively. Diagnostic criteria and localization of the disease are presented in Table I.

Table I. Patients with Crohn's disease included in the different investigations

<table>
<thead>
<tr>
<th>Study n</th>
<th>Definite</th>
<th>Probable</th>
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<th>Localization</th>
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<tr>
<td>I</td>
<td>87</td>
<td>28</td>
<td>59</td>
<td>32 15 14 26</td>
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<td>II</td>
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*not done

Small intestinal morphology (II)

Eighteen patients with a diagnosis of Crohn's disease were studied. Eleven of the patients were males, mean age 42 years (18-73) and seven females, mean age 26 years (19-36). Six healthy volunteers served as controls, mean age 36 (29-46) years. The diagnostic criteria and disease localization are presented in Table I.

In order to appraise the accuracy of the evaluation of the X-ray findings an experienced radiologist accomplished a blind assessment of the radiograms of the patients in Study II. The findings were categorized
in falling order of certainty of Crohn's disease, 1 being the most certain and 3 the most uncertain finding. The radiologists assessment in relation to the diagnostic criteria by Garland et al (7) is presented in Table II.

Table II. Radiologist assessment

<table>
<thead>
<tr>
<th>Diagnostic criteria by Garland et al (7)</th>
<th>1</th>
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<tbody>
<tr>
<td>Def.</td>
<td>6</td>
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<tr>
<td>Prob.</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Poss.</td>
<td>1</td>
<td>1</td>
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</table>

Of the six patients with a definite diagnosis all were considered to have certain diagnoses on the radiograms. In the nine patients with a diagnosis based solely or partly on typical radiological findings (probable), six had certain and two fairly certain radiological diagnoses (in these two colonoscopic reports were contributing to our diagnosis). In one patient the radiograms could not be found.

Of the three patients with a possible diagnosis one had a certain, one a fairly certain and one an uncertain radiological diagnosis of Crohn's disease. This underlines the validity in our rating of diagnostic criteria and the standard of the X-ray reports of the patients.

Skeletal muscle electrolyte content (III)

Sixteen patients with the diagnosis of Crohn's disease and 10 healthy controls matched for age and sex, were studied. Five of the patients were females, 23-44 years old (mean 33 years) and 11 were males, 22-55 years old (mean 34 years). The controls were four females, 23-39 years old (mean 31 years) and six males, 25-51 years old (mean 38 years). Diagnostic criteria and localization of the disease are presented in Table I.

Bile acid absorption

Two different populations of patients with Crohn's disease with matched controls were investigated. The initial study was carried out at
the Gastrointestinal Unit, Department of Medicine and Department of Nuclear Medicine, Western General Hospital, Edinburgh, Scotland (IV).

Forty-five patients were investigated of which 19 patients were diagnosed as suffering from Crohn's disease. The controls were 17 healthy volunteers. Twenty-nine of the patients were women and 16 men with ages ranging from 14-72 years (mean 41 years). The diagnoses of Crohn's disease in the 19 patients were confirmed by at least one positive biopsy or by typical radiological appearances. The localization of Crohn's disease in these patients is presented in Table I.

In the second study nine patients with the diagnosis of Crohn's disease and seven healthy volunteers were studied at the Department of Medicine, Umeå University Hospital (V). Four of the patients were females, 22-46 years old (mean 33 years) and five were males, 26-44 years old (mean 32 years). Controls were two females, 23 and 36 years old and five males, 25-41 years old (mean 34 years). The diagnostic criteria and disease localization is presented in Table I.
METHODS

Disease activity index
In an attempt to obtain an objective and quantitative standard of disease activity a modification of the index proposed by van Hees et al (37) was used in the studies I, II, III and V. The van Hees index embraces serum albumin, ESR, body mass (Quatelet) index, abdominal mass, sex, the mean of afternoon body temperature for seven days, stool consistency, resection and extraintestinal lesions. The index applied was slightly modified due to different hospital routines. Thus, the afternoon body temperature was replaced by the morning body temperature. Serum albumin was assessed by another laboratory method (immunoassay) instead of cellulose acetate electrophoresis and the size of an abdominal mass had not been recorded regularly and was therefore underestimated in the calculations.

Fecal fat
Fecal fat was measured according to the method by van de Kamer (71) being the routine method at the laboratory of the hospital. Three days prior to and during the three days collecting period an additional 40 g fat was given to the patient. A fecal fat excretion exceeding 6 g/24 h (7 mmol/24 h) was regarded as abnormal.

D-Xylose absorption test
D-Xylose is a pentose sugar, which is absorbed exclusively in the upper jejunum (72). To the fasting patient 25 g D-xylose in 500 ml water was given orally. The urine was collected for the following five hours and the excreted D-xylose was measured according to Roe and Rice (73). To ascertain a normal renal function and a complete urine collection endogenous creatine clearance was calculated. Patients with impaired renal function and urine collection less than 250 ml were excluded. Values below 4.5 g/5 h urin was considered abnormal.

Lactose tolerance test
Lactose is a disaccaride and is the major part of the carbohydrates in milk which contains approximately 50 g/l of lactose. Lactose is degraded into the monosaccarides glucos and galactose in the brush bor-
der of the jejunal mucosa under the influence of lactase. To the fasting patient 50 g lactose in 500 ml water was administered orally. Blood tests were taken on two occasions prior to the intake to give the fasting values and every 15 minutes for two hours after the intake. Blood glucose levels were measured according to the standard method of the hospital laboratory. The lactose tolerance test was considered abnormal when the rise in blood glucose was less than or equal to 2.2 mmol/l, provided that an oral glucose tolerance test was normal.

Procedure for biopsy of the upper small intestine
One intestinal biopsy specimen was taken at the region of the ligament of Treitz by means of a Watson capsule attached to an endoscope (Olympus GIF-Q). The Watson capsule contains a rotating knife, which is released by aspiration. After intubation the tip of the endoscope was positioned in the ascending part of the duodenum and the capsule was guided down a further 20-30 cm before aspiration was applied. The specimen was divided into two halves, one for light microscopy and one for scanning microscopy. The specimen for light microscopy was fixed in 10% Neutral Formaline and that for scanning electron microscopy was immediately (within 60 seconds) fixed at room temperature in phosphate buffered 6.5% glutaraldehyde, pH 7.2-7.4 (74).

Morphological methods
For light microscopy the specimens were orientated, embedded in paraffin, serially cut parallel to villi and crypts, stained with hematoxylin-eosin and according to the periodic-acid-schiff (PAS) procedure. For scanning electron microscopy (SEM) the specimens were fixed for at least 24 hours. They were then carefully rinsed in cold physiological saline, dehydrated in increasing concentrations of ethanol and isopropanol-acetate and dried with liquid carbon dioxide in a critical-point-drying apparatus (Polaron E-3000). The specimens were coated with approximately 20 nm gold in a modified vacuum coating unit during automatic tilting and rotation and finally examined in a scanning electron microscope (Cambridge Stereoscan S-4).
Each SEM-specimen was studied at three different magnifications during the same sequence: At low power magnification (x 100-200) the general architecture of the mucosa was assessed. At medium power magnification (x 1.000-2.000) the cell structure was studied and at high power magnification (x 10.000) the surface ultrastructure was analysed.

Muscle biopsy
Muscle biopsy was obtained by the method elaborated by Bergström (75) i.e. by a percutaneous needle biopsy from the lateral position of the quadriceps femoris muscle, 15-20 cm proximal to the knee. The muscle biopsies, 40 to 80 mg wet weight, were rapidly dissected free from all visible connective tissue and fat and were rolled on a piece of quartz glass to remove all traces of blood. The muscle tissue was then attached to a preweighed platinum hook and repeatedly weighed on a Cahn 4700 electrobalance. The original wet weight was obtained by extrapolation to zero time. The platinum hook with adhering muscle tissue was then placed in an oven at 110°C until constant weight, to obtain the dry weight and the water content of the specimen.

Fat was extracted with redistilled petroleum ether. After a new drying period the fat-free dry solid weight (FFDS) was obtained. The muscle tissue was then wet ashed in 1 N nitric acid and the electrolytes were determined by atomic absorption spectrophotometry in the solution left, according to methods of Bergström et al (76). Plasma electrolytes were determined by a conventional autoanalyzer technique (Na, K) atomic absorption spectrophotometry (Mg), and by precipitation with silver nitrate (Cl).

Fecal bile acids
The stools for the first three days of the test week were collected. The collections were pooled, mixed and an aliquot was freeze dried. Bile acids were measured using the method of Evrard and Janssen (77) and methyl ketone derivatives were separated by gas-liquid chromatography using OV17 on 100/120 Gas Chrom Q.
Whole body retention of SeHCAT

23-Selen-25-homotaurocholate is a synthetic bile acid labelled with a γ-ray-emitting radioisotope $^{75}$Se. It is readily measured using external detectors (78). Forty kBq (1μCi) SeHCAT was administered orally as an aqueous solution (IV). Whole body retention of selenium radioactivity was measured on three occasions, 0, 4 and 7 days, using a shadow-shield whole-body counter equipment with four 100 mm thick x 150 mm diameter NaI (Tl) detectors. Each measurement took 10 minutes on each occasion (IV).

150 kBq (4μCi) SeHCAT was administered intravenously (V). Measurements were carried out 20 minutes after injection and after 6, 24, 48, 72, 96 and 168 hours. The measurements were performed in a low background room with the patient in chair geometry approximately 25 cm from a scintillation spectrophotometer with a single 12.7 cm x 12.7 NaI (Tl) detector, anteriorly attached to an one-channel pulse height analyser. Each measurement took 20 minutes on each occasion.
RESULTS AND DISCUSSION

Incidence of Crohn's disease

During 1974-1981, 96 patients had a definite or a probable diagnosis of Crohn's disease (diagnostic criteria according to Garland et al) (7)) in the county of Västerbotten (245,000 inhabitants). The annual incidence ranged from 2.9 to 8.2 per $10^5$ inhabitants (mean value 4.9) (I). The demonstrated incidence of Crohn's disease is in accordance with previous Swedish studies (11-13). Patients with minor clinical manifestations of the disease might not be referred to hospital or may be treated as outpatients only and have not been included in our study. The present annual incidence may thus be slightly underestimated.

Crohn's disease has been suggested to be more common in urban areas (79, 80). Although the county of Västerbotten is a rural area, the same incidence was demonstrated as that of the biggest cities in Sweden (12, 13). Moreover, the mean annual incidence of the district of Umeå (the largest city of the county) was even higher, 6.5 per $10^5$ inhabitants (I). Taken together, these data may indicate that the overall incidence of Crohn's disease is higher in the northern part of Sweden.

The onset of the disease occurred mainly in young adults. This is in agreement with earlier studies (11-13, 80). A second peak of incidence in older age groups has been described (11, 83), which, however, has been questioned (13). In the present study, there was a gradual decline in incidence with age (I).

Clinical characteristics

The clinical investigation was performed by reviewing the notes of all patients with a diagnosis of Crohn's disease discharged from the Department of Medicine, Umeå University Hospital, between 1974 and 1981.

During these years, 87 patients with a definite or probable diagnosis of Crohn's disease were treated. Sixty-one patients had not been subjected to intestinal resection. The 26 patients with intestinal resec-
tion had a slightly shorter diagnostic delay from the onset of symp-
toms until the diagnosis was confirmed than had the non-resected, 3.3
and 3.7 years respectively.

The patient population studied, consisted of patients with gastroin-
testinal symptoms referred for diagnostic evaluation as well as pa-
tients with an established diagnosis treated at the department for re-
lapses, complications, or treatment failures. One group of patients
living in the Umeå district was referred for diagnostic investigation
and was separately analysed. However, they did not differ from the
rest of the non-resected patients with respect to age, sex, disease
duration or localization. Nor was there any difference with respect to
the outcome of the laboratory tests studied. Therefore, the results
from the whole group of 61 non-operated patients were presented with
the presumption that the results would be representative for patients
with Crohn's disease attending a medical clinic.

The localization of Crohn's disease showed a predominance for ileal
disease, whereas ileocaecal involvement was comparatively rare. During
the time period investigated, 33 patients were directly referred to
the surgical department of the hospital and 24 were operated upon with
intestinal resection whereas nine were subjected to laparotomy without
resection. In the surgical group of patients, there was a predominance
for ileocaecal disease as diagnosed by direct inspection or by histol-
ogy. These findings suggest that patients with ileocaecal Crohn's dis-
ease may be more prone to present with symptoms of intestinal obstruc-
tion and thus treated surgically. Thus, the patients with Crohn's dis-
ease recruited from the Umeå district showed a disease localization of
40% ileal, 40% ileocaecal and 20% colonic involvement, which is con-
sistent with previous reports (24).

Malabsorption
The presence of malabsorption of fat and carbohydrates in Crohn's dis-
ease has previously been recognized (52, 54-57, 60, 62). In the pre-
sent investigation (I) 23% of the non-resected patients had increased
fecal fat excretion, whereas steatorrhea was significantly more common
in the group of patients with intestinal resection (66%) (p < 0.01).
Fat malabsorption after intestinal resection has been demonstrated to correlate with the length of the resection (57, 61). In the present study (I), there was a tendency for steatorrhea to be more common in patients with ileal disease, than in patients with ileocaecal or colonic localization. The difference was, however, not statistically significant.

Increased fecal fat excretion may reflect an impairment of any stage of the digestive and absorptive processes. Disturbances of the enterohepatic circulation of bile salts influence the fat absorption and fecal fat is consequently increased in conditions such as obstructive jaundice, terminal ileal disease, or intestinal resection. An increased loss of fecal bile acids in patients with Crohn's disease localized to the ileum has directly been demonstrated by means of a -emitting bile acid (SeHCAT) (IV, V).

Damage to the enterocyte function decreases fat absorption and also leads to increased fecal fat as can be seen in coeliac disease.

Abnormal D-xylose absorption tests were present in 16% of the non-operated patients (I). D-xylose is absorbed in the upper small intestine and is believed to reflect the area of intestinal absorptive surface. However, no relation between the outcome of this test and the localization of the disease was found. A higher proportion of abnormal lactose tolerance tests (24%) was found among the patients, which exceeds the figure expected for Northern Sweden (approximately 5%) (I).

Jejunal enzymatic abnormalities as well as subtle morphometric changes have been encountered in patients with distal localization of the disease as assessed by radiology (66). The radiological assessment of disease localization does not seem to meet with the sensitivity of biochemical tests. Ultrastructural changes in intestinal absorptive cells may be one reason for impaired absorption of fat and carbohydrates, and consequently the disease may be more widely spread than indicated by radiology. In fact, the jejunal mucosa as investigated by scanning electron microscopy displays abnormalities in a high proportion of patients with the disease radiologically localized to the distal part of the intestine (II).
The cause of the absorptive dysfunction seems to be multifactorial. The localization and extension of the disease assessed at the microscopic level appear to be important, and so may biochemical disturbances as well as intestinal bacterial overgrowth (62).

Morphology
The function of the small bowel can be evaluated by clinical absorption tests, and the jejunal mucosal biopsy enables a direct histological diagnosis. Jejunal biopsies from 18 patients with Crohn's disease were assessed by light (LM) and scanning electron microscopy (SEM) (II). A high frequency (13 out of 18) of abnormalities of the upper small intestinal mucosa in patients with a disease radiologically localized to the distal intestine was encountered. Five patients had normal jejunal biopsy specimens when assessed by means of LM and SEM. On the other hand, six patients had jejunal biopsy specimens with LM abnormalities compatible with partial villous atrophy, and the mucosal biopsies were also abnormal when assessed by SEM. Seven patients had normal LM appearance but displayed ultrastructural abnormalities by means of SEM.

Thirteen of the patients had convoluted villi in the jejunal mucosa, a morphological condition which is seen stereomicroscopically in malabsorptive states and corresponds to partial villous atrophy in the LM assessment (81, 82). However, convoluted villi with normal LM appearance as shown in seven of the biopsies has not been described previously in Crohn's disease. Enterocyte irregularity and surface ultrastructural distortion was also found, which indicates a pathologic state of the enterocytes. Such morphological deviations have been encountered in coeliac disease (83, 84). In contrast to coeliac disease in which the mucosal lesions are believed to be generalized (85), the distribution in Crohn's disease appear to be patchy (II). Abnormalities of the jejunal intestinal mucosa were demonstrated in 13 out of 18 patients with Crohn's disease by means of LM and/or SEM. However, in five of these 13 patients the malabsorption tests were normal.

The results indicate that in patients with Crohn's disease, morphological abnormalities in jejunal mucosa specimens may be present without
radiological evidence of disease. The use of SEM in this group of patients might contribute to the explanation of malabsorption. The results also add further weight to the concept of an involvement along the entire gastrointestinal tract in Crohn's disease.

**Muscle electrolytes**

Reports have underlined the clinical importance of magnesium depletion in Crohn's disease (67-69, 86), but little is known of skeletal muscle content of magnesium and potassium. Skeletal muscle biopsies were performed in 13 patients with Crohn's disease and in 10 healthy controls (111). Patients with Crohn's disease demonstrated significantly lower muscle potassium values than the controls (p<0.01). Patients with involvement of both the ileum and the colon tended to have lower muscle potassium content than patients with isolated ileal disease. Muscle potassium content showed no correlation to the occurrence of malabsorption as judged by the absorption tests.

Although the plasma potassium values did not differ significantly between the patients and the controls, the muscle potassium content correlated to plasma potassium concentration in the patient group (r_s = 0.56, p<0.05). The depletion of potassium although often present in patients with Crohn's disease is not reflected by subnormal plasma potassium values.

As a whole, the patient population did not differ significantly from the controls with regard to skeletal muscle magnesium content. The range, however, was greater. Two patients had values below the range of the controls and two above.

A great fecal loss of potassium in Crohn's disease has been described (87). Also, a volume depletion may occur in patients with inflammatory bowel disease caused by a high fecal water discharge. These events could in turn enhance aldosterone secretion leading to an increased urinary potassium loss.

Magnesium is believed to mainly be absorbed from the small intestine. However, it has been shown that magnesium could be absorbed from the colon as well (88, 89). An enhanced colonic absorption may thus compensate for the impaired small intestinal function. This is supported in this study since patients with involvement of the ileum and the
colon had lower muscle magnesium values than did the patients with ileal disease only.

An enhanced magnesium absorption in patients with Crohn's disease put on a low fat diet has previously been reported (90). In this study, more than half of the patients were recommended such a diet which may explain the small proportion of magnesium depleted patients.

**Bile acid malabsorption**

Conjugated bile acids and vitamin B$_{12}$ are actively absorbed in the terminal ileum. Bile acid malabsorption in disease or resection of the terminal ileum is well documented (91-95). It is generally recognized that existing methods of detecting functional disturbances of the terminal ileum are imperfect. Investigations such as the Schilling test are neither sensitive nor specific (95), and excretion of $^{14}$C-conjugated bile acids, although both sensitive and accurate as an index of distal ileal function (94, 96), is not easy to measure under routine clinical conditions.

The selenium-labelled bile acid SeHCAT (tauro-23-$^{75}$Se-Selena-25-homocholic acid) is a synthetic conjugated trihydroxy bile acid, which is resistant to deconjugation and dehydroxylation. The γ-ray emission is readily measured externally by whole-body counting. SeHCAT is absorbed and excreted at the same rate as $^{14}$C-cholic acid (97, 98), and it has been shown that the main route of excretion is biliary. The radioactivity is almost entirely confined to the lumen of the biliary tree, the gut and the liver (97, 99). The biological half-life of 2.6 days does not differ from that previously reported for $^{14}$C-cholic acid (100, 101).

A clinical assessment of SeHCAT in the evaluation of ileal function was carried out (IV). Forty-five patients were investigated, 19 of these had been diagnosed as suffering from Crohn's disease, 11 had ulcerative colitis, 8 had diarrhea of mixed origin, 5 had ileostomy after pancolectomy due to ulcerative colitis and 2 patients had ileal resection due to volvulus. Seventeen healthy volunteers served as controls.
SeHCAT was excreted in a biexponential fashion with the first phase of excretion being rapid. The median whole body retention (WBR) for the controls was 62% of administered dose (32-90%) at 4 days and 42% (19-81%) at 7 days. Essentially similar results were obtained in most of the patients with ulcerative colitis or Crohn's disease confined only to the colon.

At 7 days all patients with ileal disease or resection retained less than 19%. Similar results have been reported using $^{14}$C-taurocholate (99). All patients with ileostomy except one, had less than 19% retention at 7 days. This low retention was unexpected. The WBR for patients with ileal disease, resection or ileostomy was significantly lower than in the control, colonic and diarrhea groups at 4 and 7 days ($p<0.01$).

There was a correlation between SeHCAT retention at 4 as well as 7 days and total fecal bile acid excretion ($p<0.001$), particularly primary bile acids, a finding which is consistent with previous findings (101).

The initial SeHCAT-study was undertaken at a hospital with advanced equipment using a whole body counter not available in most hospitals. In order to make the method more widely applicable, SeHCAT radioactivity was measured by means of a scintillation spectrophotometer with a single detector located in a low background room (V). The SeHCAT was administered intravenously and the results were expressed as WBR$_{50}$, denoting the time for 50% of SeHCAT radioactivity to be excreted. WBR$_{50}$ could accurately be calculated using measurements obtained within 48 hours. The data were obtained from 9 patients with Crohn's disease and 7 healthy subjects. The results obtained with this experimental design corresponded well with those obtained after oral administration (IV). In patients and healthy controls the SeHCAT retention as calculated by WBR$_{50}$ was 63 h (15-163) and 120 h (99-141) respectively.

By calculating the individual time for elimination of 50% of the SeHCAT, information may be obtained that reflects the degree of terminal ileal malfunction. In line with the results of the previous stu-
dy (IV), there was a significant correlation between WBR$_{50}$ and the excretion of total fecal bile acids ($p<0.05$).

As intestinal transit time may influence the retention of bile acids the transit time was measured using radio opaque pellets in the patients and the controls during the SeHCAT-test. There was a significant correlation between total intestinal transit and WBR$_{50}$ in the patient group ($p<0.05$), but not in the control group. Within the control group the transit times varied considerably, although the WBR$_{50}$ only differed to a minor extent. The results suggest that the WBR$_{50}$ is not a direct function of the transit time, but rather that the impairment of the terminal ileum gives rise to both a decreased transit time as well as to an increased fecal elimination of the labelled bile acid.

The SeHCAT technique seems to be a simple and accurate method for studying the function of the terminal ileum (IV, V; 102, 103). The current methods of diagnosing Crohn's disease confined to the small intestine rely on radiology and function tests. The SeHCAT method may be clinically useful in conditions where the ileum is compromised, especially in Crohn's disease.

GENERAL SUMMARY AND CONCLUSIONS

1. During 1974-1981 the mean annual incidence of Crohn's disease was 4.9/10$^5$ inhabitants in the county of Västerbotten (245.000 inhabitants). This is in accordance with the incidence in the rest of Sweden, and even may be somewhat higher.

2. In a department of medicine there was an underrepresentation of patients with ileocaecal disease, a group of patients mainly presenting with obstructive symptoms and treated surgically. Abnormal asorption test results were frequently encountered. Steatorrhea was present in 23% of non-operated patients and in 66% of resected patients. The results of D-xylose and lactose tests were abnormal in 19% and 24% respectively of non-operated patients. There was no relation between the outcome of malabsorption tests
and localization, extension or activity of the disease although patients with ileal disease tended to have more steatorrhea and abnormal D-xylose tests than those with ileocaecal and colonic disease. The results suggest that the cause of malabsorption in Crohn's disease is complex and multifactorial.

3. Jejunal biopsies from patients with radiologically localized disease elsewhere in the gastrointestinal tract demonstrated morphological changes in routine light microscopical assessment. Scanning electron microscopy revealed ultrastructural changes with a pattern previously not described. These changes may be one reason for intestinal malabsorption and add further weight to the concept of an involvement along the entire gastrointestinal tract in Crohn's disease.

4. Patients with Crohn's disease often had reduced muscle potassium content, which did not correlate to tests of intestinal absorption. Involvement of a combination of both ileum and colon tends to be a contributable factor. Low muscle potassium content is not reflected in subnormal plasma concentration. Reduced muscle magnesium content is infrequently encountered in patients with Crohn's disease. The results suggest a need for awareness of the risk of potassium and probably magnesium depletion in patients with Crohn's disease.

5. With the introduction of SeHCAT, a γ-labelled synthetic bile acid, the evaluation of bile acid absorption in the terminal ileum is facilitated. The test may be helpful in discriminating patients with diarrheal diseases due to terminal ileal malfunction from other diarrheal states. The SeHCAT test results correlate well with the excretion of fecal bile acids, particularly primary bile acids. By calculating the time for elimination of 50% SeHCAT, information may be obtained as to the rate of excretion, reflecting the degree of terminal ileal malfunction. The SeHCAT test is a simple, safe and rapid test of bile acid absorption.
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