

75Se HCAT test in the detection of bile acid malabsorption in functional diarrhoea and its correlation with small bowel transit

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SUMMARY The purpose of this study was to evaluate whether bile acid malabsorption assessed by the 75SeHCAT test, had a pathogenetic role in functional chronic diarrhoea and to ascertain whether the small bowel transit time (SBTT) could be correlated with the 75SeHCAT test results. The test was based on the counting of the abdominal retention of a 75-selenium labelled homotaurocholic acid. The 75SeHCAT test was carried out in a control group of 23 healthy adults and in 46 patients, 38 of whom were suffering from irritable bowel syndrome (IBS) of diarrhoeic form and eight patients who had undergone cholecystectomy and were suffering from chronic diarrhoea. Faecal bile acid loss was determined in nine patients, and in 14, serum bile acid increase after a standard meal was measured. In 17, SBTT was studied by hydrogen breath test after lactulose administration (21 g in 300 ml water). In 15 patients, choledochocaecal transit time was estimated by Tc99m-HIDA (111 MBq) cholescintigraphy. In 20 of 46 subjects, 75SeHCAT retention was below normal level, and in 19 cholestyramine administration relieved diarrhoea. 75SeHCAT results were related to faecal bile acid loss, while no correlation was found with serum bile acids and SBTT. The data suggest a possible wider use of the 75SeHCAT test in chronic diarrhoea to estimate bile acid malabsorption in irritable bowel syndrome, diarrhoeic form, and provide an effective treatment. In our patients small bowel transit velocity does not seem to be a pathogenetic factor of bile acid malabsorption.

Irritable bowel syndrome (IBS) is quite a common disease in gastroenterology, and diagnosis is based on the exclusion of intestinal or extraintestinal organic pathology. Bile acids may play a pathogenetic role in chronic diarrhoea, but in practice bile acid malabsorption is not easily proved and for this very reason some consider that bile acid diarrhoea is underdiagnosed.¹

Idiopathic bile acid malabsorption² is now being investigated with renewed interest since a new radioisotopic test (75SeHCAT test) was brought into clinical practice.^{3,4} The 75SeHCAT test proved to be useful in the investigation of ileal pathology,^{5,7} and is highly sensitive and specific in bile acid malabsorp-

tion assessment.⁸ Clinical research has recently drawn attention to the finding of abnormally low 75SeHCAT retention in diarrhoeic patients considered to suffer from irritable bowel syndrome.^{1,8,9}

In this study, 75SeHCAT test was carried out in patients suffering from chronic or recurrent diarrhoea which was thought to be functional and to investigate whether bile acid malabsorption had a pathogenetic role in this syndrome. The results of the 75SeHCAT test were correlated with SBTT and other parameters of bile acid absorption.

Methods

PATIENTS

The study was carried out under the Helsinki Declaration. Forty six patients were studied, all of

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whom had given written informed consent. Twenty were male, 26 female, aged between 17 and 73 years (mean 41). In 38 cases diagnosis of irritable bowel syndrome was established, according to Gudmand-Hoyer *et al.*,¹⁰ based on the clinical picture and the absence of organic diseases affecting the alimentary tract. In the other eight, a cholecystectomy had already been done. Chemical and microbiological faecal analysis showed normal results. Radiographic examinations of large and small bowel, carried out using two contrast media were negative. Diabetes, other endocrine diseases, and food allergies were excluded, and all the patients were on a lactose free diet. The clinical picture was characterised by chronic or recurrent diarrhoea with liquid or semiliquid stools. The general nutritional condition appeared good, however, abdominal pain was often present and anticholinergic agents, loperamide and high fibre diet had not relieved the symptoms. In six of eight cholecystectomised patients diarrhoea occurred after surgery, while in the other two it was already present.

Different methods were used to investigate bile acid malabsorption and small bowel transit.

75SeHCAT TEST

The 75SeHCAT test was carried out in all patients using the method we described elsewhere and the control group consisted of the same 23 subjects.⁸ Results are expressed as percentage retention values calculated by the exponential time activity curve on day 3. Measurements of abdominal radioactivity were taken by gamma camera counting on the day of administration of 370 KBq 75Se-homocholytaurine (75SeHCAT, Amersham Radiochemical Centre, England) (time zero) and on days 1–3–5 and 7. An abdominal retention of 34% or more on day 3 is considered normal by our method.⁸ The percentage abdominal retention on day 7, measured directly by gamma camera for both the control and the functional diarrhoea groups was considered. An abdominal retention of less than 8% (the lowest value in a normal subject) is considered pathologic.

CHOLESTYRAMINE TEST

Cholestyramine was given to all patients twice daily to give a total dose of 2–8 g/day for a period of at least 10 days. When cholestyramine was not effective in relieving symptoms, therapy was discontinued. In those patients where the drug was effective, it was stopped for seven days, and started again if diarrhoea recurred. The test was considered positive when diarrhoea stopped with cholestyramine administration, and recurred without it. In all patients stool frequency which was taken as the average number of bowel actions per day, over a period of one week,

was recorded for the week preceding and after administration.

POSTPRANDIAL SERUM BILE ACID RESPONSE

In 14 patients, conjugated bile acids (cholic, chenodeoxycholic and deoxycholic acid) were tested in serum by a radioimmunologic method (Farmos Diagnostica, Finland). Accuracy of the method is between 90% and 110% and sensitivity is 0.2 µmol/l.^{11,12} Blood samples were taken at –15 min, just before the ingestion of a standard meal (900 cal), and 60, 90, 120, 150, 180 min after eating. Meal ingestion started at midday, according to Aldini *et al.*¹³ for a better sensitivity of the test. The maximum increase (Δ) after meal of each bile acid concentration was used for correlation with the 75SeHCAT test results.

FAECAL BILE ACID DETERMINATION

Faecal bile acid loss was measured in nine patients after three day stool collection. For measuring faecal bile acid, 1 g faeces taken from each sample was dissolved in 10 ml iso-propyl alcohol.¹⁴ After centrifugation at 2500 g for 15 min, 2–5 ml supernatant was put into test tubes and dried under nitrogen stream. The dry residue was mixed with 5 ml NaOH 0.1 M and the solution was passed through Bond-Elut C18 columns which were consecutively activated with 3 ml methanol and 6 ml H₂O and finally eluted with 4 ml methanol. The sample was again dried under nitrogen stream, after which it was ready for bile acid assay. Total bile acid determination was carried out by an enzymatic fluorimetric method (3 α HSD). A faecal bile acid amount less than 250 mg/day was considered normal.^{14,15}

SMALL BOWEL TRANSIT TIME

Small bowel transit time (SBTT) was assessed in 17 patients by hydrogen breath test (H₂-BT) according to the technique of Metz *et al.*¹⁶ after oral administration of an isotonic solution of lactulose (21 g lactulose in 300 ml water). Alveolar air samples for hydrogen content measurement were collected at five minute intervals for two hours or until a marked increase of H₂ concentration was observed. An increase of H₂ concentration of at least 20 ppm above the basal value means that lactulose has reached the caecum and defines SBTT.^{17,18} Two patients were H₂-non-producers, therefore the SBTT could only be ascertained in 15 patients.

RADIOISOTOPIC DETERMINATION OF CHOLEDOCHOCAECAL TRANSIT TIME

Because 75SeHCAT travels in the bowel mixed with bile, choleldochocaecal transit time was measured by Tc99m-HIDA choleldochocecal scintigraphy in 15 patients. For

this purpose the patient received 111 MBq Tc99m-HIDA intravenously 15 minutes after oral ingestion of a fat meal (200 ml cholecystokinetic standard meal) to empty the gall bladder. In this way, bile flows into the duodenum through the choledochus, with minor influence from gall bladder filling and moreover cholecystectomised subjects can be studied using the same method. Images of the abdominal radioactivity were collected by a large field of view gamma camera and fed into a computer, at a rate of 1 frame per minute in 128×128 bite matrix. Data acquisition started at the moment of the injection and lasted until the radiocompound reached the caecum and the ascending colon. On the computer stored images, regions of interest which were the choledochus, the caecum and the ascending colon were outlined, and the corresponding time activity histograms generated.¹⁸ Choledochocaecal transit time was the time that elapsed between the visualisation of the choledochus and the appearance of the radioactivity in the caecum.

Results

The ⁷⁵SeHCAT retention values shown on the curve on day 3 and from the gamma camera counting on day 7 are shown in Figure 1. From the curve, 20

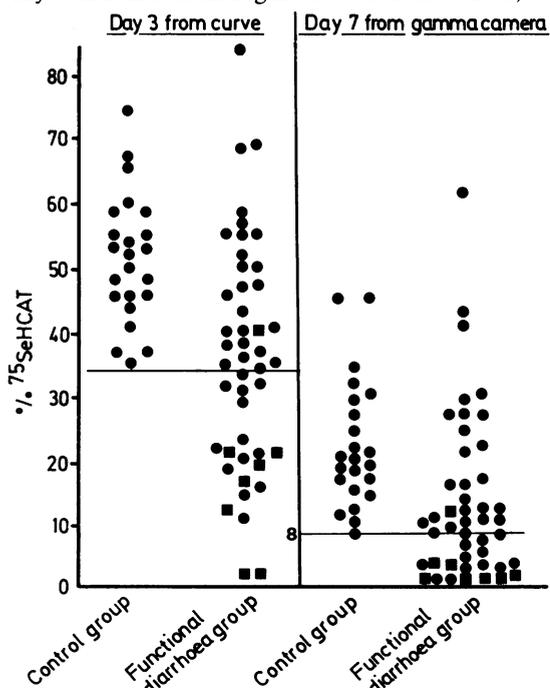


Fig. 1 ⁷⁵SeHCAT values of the 46 patients studied and of the 23 normal controls. Horizontal lines represent, in all figures, the lower normal limit of ⁷⁵SeHCAT retention. Squares mark cholecystectomised patients, in all figures.

patients transpired to be within abnormal range on day 3, while from the gamma camera 18 appeared on day 7 and the other two cases had a retention of 8% which is the lower limit of normal level. Only one of 8 cholecystectomised patients (marked by squares) had a normal ⁷⁵SeHCAT retention. The effects of cholestyramine administration on stool frequency, both in normal and in low ⁷⁵SeHCAT retention patients, are shown in Figure 2. In 19 of 20 patients with low ⁷⁵SeHCAT, cholestyramine was effective in relieving diarrhoea, while 25 of 26 cases with normal ⁷⁵SeHCAT retention had no positive response. In Figure 3 the results of the ⁷⁵SeHCAT test and the maximum increase of serum cholic acid, which is almost completely reabsorbed by the distal ileum, are correlated. No correlation was found between the two tests nor between the ⁷⁵SeHCAT values and the other two serum bile acids. Total faecal bile acids results, with the corresponding ⁷⁵SeHCAT values, are shown in Figure 4. The four cases with normal ⁷⁵SeHCAT retention had normal faecal bile acid loss, while the five patients with low ⁷⁵SeHCAT retention had increased faecal bile acid loss. In Figure 5 SBTT, measured by H₂-BT and the corresponding values of the ⁷⁵SeHCAT test are shown. There is no correlation between the two examinations. Figure 6 represents choledochocaecal transit time measured by choleoscintigraphy and the corresponding results of ⁷⁵SeHCAT test and no correlation was observed.

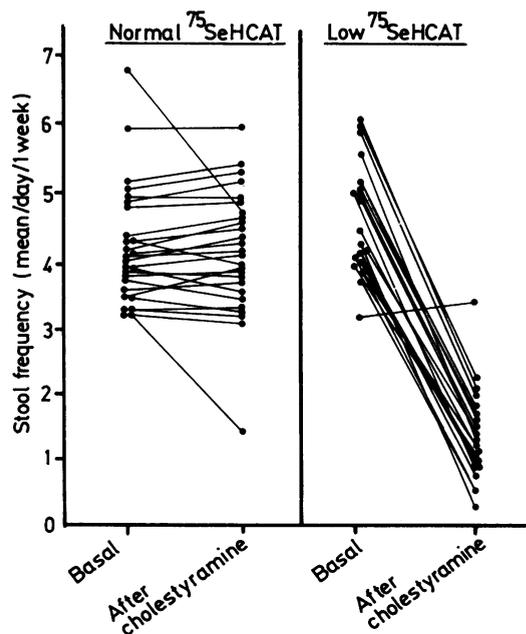


Fig. 2 Stool frequency changes after cholestyramine in patient with normal and low ⁷⁵SeHCAT retention.

Discussion

The 75SeHCAT test, recently introduced in clinical practice, has made the detection of bile acid malabsorption more easily detectable. In Crohn's ileitis and ileal resections, the 75SeHCAT test showed a high diagnostic accuracy both when the test was done

using a whole body counter⁴ and when abdominal retention was measured by gamma camera.^{5,6} As the test is easy to do and causes the patient no discomfort, it can be widely used to investigate irritable bowel syndrome, diarrhoeic form, which is a common pathological condition. Diagnosis of irritable bowel syndrome relies on the exclusion of any demonstrable disease, but bile acid malabsorption which was at one time difficult to detect, seems to play an important pathogenetic role in chronic and recurrent diarrhoea. Some investigators are of the opinion that bile acid diarrhoea is currently underdiagnosed, and many cases where diarrhoea is caused by bile acid malabsorption are erroneously attributed to the diarrheic form of irritable bowel syndrome.^{1,8,9}

In patients affected by chronic or recurrent diarrhoea we carried out the 75SeHCAT test which showed evidence of an increased bile acid loss in more than 40% of our cases. In those cases where total faecal bile acids were tested, the 75SeHCAT results were also consistent, thus confirming that an abnormally low retention value of 75SeHCAT corresponds to an increased bile acid loss. In 19 of 20 patients with a pathologic 75SeHCAT test, a positive clinical response to cholestyramine confirmed that bile acid malabsorption played an important role in causing diarrhoea. In one patient whose 75SeHCAT value was 32%, cholestyramine was not able to

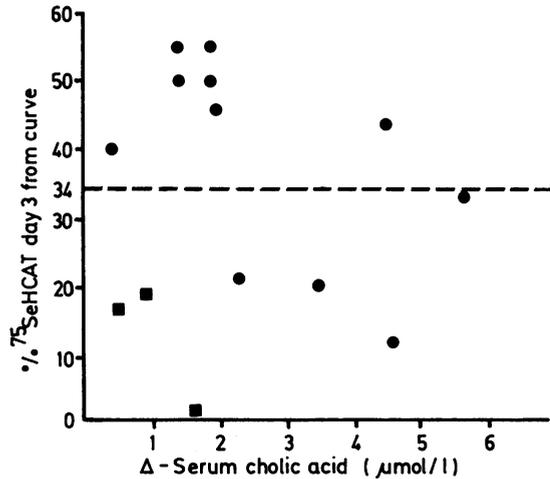


Fig. 3 Maximum postprandial increase of conjugated cholic acid and 75SeHCAT values: no correlation is present.

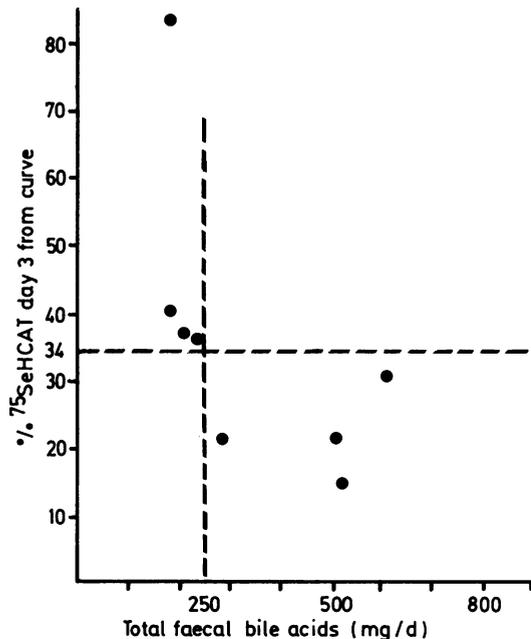


Fig. 4 75SeHCAT values and total faecal bile acids of nine patients. Vertical dotted line marks the lower normal limit for total faecal bile acids (250 mg/day).

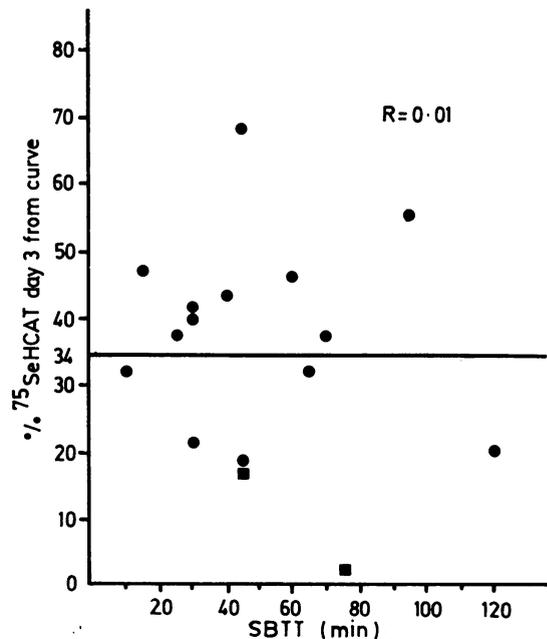


Fig. 5 Small bowel transit time, measured by hydrogen breath test with lactulose, and 75SeHCAT values of 15 patients: no correlation found.

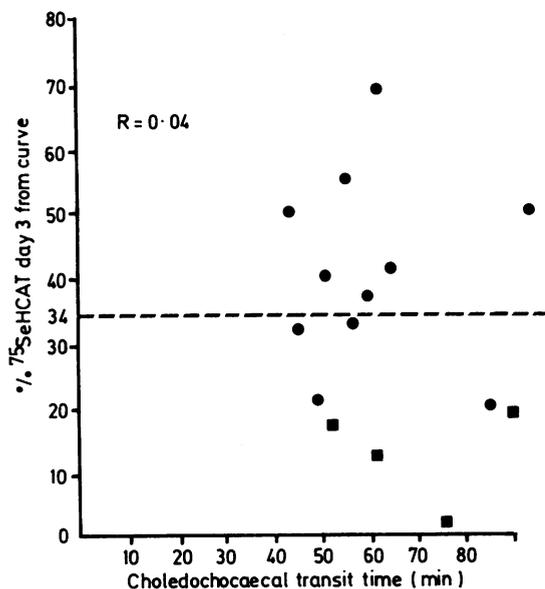


Fig. 6 Cholelochocaecal transit time, studied by cholescintigraphy with Tc99m-HIDA, and $^{75}\text{SeHCAT}$ results in 15 patients: no correlation observed.

control the symptoms. The low doses of cholestyramine sufficient enough to control diarrhoea (2–8 g/day) are justified by the moderate bile acid malabsorption observed in these patients who were diarrhoeic patients, but not to the same extent as some Crohn's ileitis or ileum resected patients where the $^{75}\text{SeHCAT}$ retention is very low and often zero on the third day.⁸ Determination of serum bile acids is not a sensitive test when undertaken to detect bile acid malabsorption in patients with chronic or recurrent functional diarrhoea, where bile acid malabsorption is not severe. Moreover bile acid concentrations in serum do not depend on intestinal absorption alone, but many other factors are involved including principally liver function. Diarrhoea caused by idiopathic bile acid malabsorption is a well known physiopathological entity, but it is currently considered to be uncommon. We estimate moderate bile acid malabsorption to be much more frequent in patients suffering from functional diarrhoea. More than 40% of our patients had a pathologic low $^{75}\text{SeHCAT}$ retention. Our results are consistent with the opinion of Merrick *et al*¹ that bile acid malabsorption as a cause of diarrhoea is underestimated. In the population they examined, however, bile acid malabsorption was present in a lower percentage of patients: 12%. To explain this difference three factors may be taken into account: (1) none of the patients studied by Merrick *et al* was cholecystectomised: eight of our patients were, and in seven the $^{75}\text{SeHCAT}$ test was abnormal; in these

subjects, an increased frequency of enterohepatic cycling and a greater postprandial bile load into the ileum may have been involved. (2) In the valuation of the $^{75}\text{SeHCAT}$ test, Merrick *et al* considered a 'range of values of uncertain attribution . . .', but a different approach was used here, and results were considered to be either normal or abnormal. (3) Nutritional and racial differences may also play a role.

As far as the mechanism of bile acid malabsorption is concerned, three situations may be considered in patients with intact gall bladder function: (1) the efficiency of distal ileum mucosa in reabsorbing bile salts is reduced; (2) an accelerated transit of intestinal content in the distal ileum reduces the time for bile salts reabsorption; (3) the enterohepatic cycling frequency may be increased as in cholecystectomised subjects. In this study, small bowel transit time was investigated by means of hydrogen breath test after lactulose ingestion and cholescintigraphy. Lactulose solution, used to measure small bowel transit time,^{17,18} may not be considered a physiological meal and it passes through the small bowel more quickly than a solid meal. Thus a difference between those patients with and without bile acid malabsorption may be concealed, and further studies should be carried out using more physiological meals. In patients with a pathologic $^{75}\text{SeHCAT}$ test, in which bile acid malabsorption is present, these two methods did not show a decreased SBTT or cholelochocaecal transit time, and no correlation was found between these two results and the $^{75}\text{SeHCAT}$ test result. No correlation was found, either between SBTT and cholelochocaecal transit time which were both done in 11 patients. This may be because of both the wide variability of the results and of the different mechanisms of the two tests.

On the basis of these data, we conclude that $^{75}\text{SeHCAT}$ test, systematically carried out in patients suffering from chronic or recurrent diarrhoea, is able to detect those cases where bile acid malabsorption is present and a diagnosis of irritable bowel syndrome would not be correct. Moreover, although the processes involved are unknown, bile acid malabsorption seems to be unrelated to small bowel transit velocity in our patients.

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