Intestinal bile salts in cystic fibrosis

Studies in the patient and experimental animal

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SUMMARY The quantitative and qualitative distribution of bile salts in the duodenal juice of 13 patients with cystic fibrosis (CF) was studied after a test meal. The effects of triolein (TO), bovine serum albumin (BSA), and ricinoleic acid (RA) on the absorption of taurocholate (TCA) in the distal ileum of the rat *in vivo* was also studied.

The mean (and ranges) of total bile salt concentrations, glycine:taurine conjugate ratios, and percentage of dihydroxy bile salts in the patients with CF and pancreatic insufficiency were 3.5 (1.3-6.6) mmol/l, 8.6 (>10-3.1), and 37 (10-60) compared with control values of 7.4 (3.0-16.0) mmol/l, 3.0 (1.3-4.5), and 61 (52-70) respectively. The differences between the control and CF values were statistically significant ($P < 0.01$ - $P < 0.001$). Three of the 13 CF patients had total bile salt concentrations <2 mmol/l, 8 had much higher glycine:taurine ratios, and 8 had a reduced percentage of dihydroxy bile salts. In 2 patients with normal pancreatic enzyme activities, duodenal bile salts were both quantitatively and qualitatively normal. TO (10 and 30 mmol/l), BSA (3%), and RA (5 mmol/l) had no inhibitory effect on the ileal absorption of TCA.

These results show pronounced abnormalities of duodenal juice bile salts in CF with pancreatic insufficiency consistent with a broken enterohepatic circulation (EHC); such abnormalities may contribute to defective lipid absorption in CF. The data in the experimental animal do not support the suggestion that unhydrolysed dietary substrates play a role in the pathophysiology of the broken EHC.

Recent studies have shown that children with cystic fibrosis (CF) excrete similar amounts of faecal bile salts as patients with ileal resections (Weber *et al.*, 1973, 1976; Goodchild *et al.*, 1975), and on the basis of kinetic studies, this increased faecal loss appears to be secondary to a broken enterohepatic circulation (EHC) of bile salts (Watkins *et al.*, 1974, 1977). The pathogenesis of the broken EHC has not been defined. Weber *et al.* (1973, 1976) reported a significant correlation between faecal fat and bile salt excretion, and found that pancreatic extracts and/or a low fat diet supplemented with medium-chain triglycerides markedly reduced the faecal excretion of bile salts. From these findings they suggested that

unhydrolysed triglycerides might impair the ileal absorption of bile salts.

We investigated the total and individual bile salt patterns in the proximal small intestine of children with CF after a test meal, and studied some of the pathophysiological mechanisms that may be responsible for the broken EHC in the rat ileum.

Subjects and methods

Thirteen (6 boys and 7 girls) patients with CF were investigated, their ages varied from 1 to 182 months with a mean of 52.3 months. The diagnosis of CF was established by the finding of sweat Na and Cl concentrations of >70 mmol/l on at least two occasions in volumes of sweat >100 mg. All the patients had typical clinical features, and all except 2 had markedly reduced activities of pancreatic lipase, trypsin, amylase, and esterase compared with control values (McCollum *et al.*, 1977). Pancreatic extracts and antibiotics were discontinued 48 hours before administering the test meal. The control group (6

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boys and 4 girls) consisted of children with suspected malabsorption who were proved to be normal after extensive investigation; their ages ranged from 16 to 192 months with a mean of 40 months. None of the subjects had clinical or biochemical (serum proteins, bilirubin, and transaminases) evidence of liver disease.

Details of the test meal and duodenal intubation procedure have been described elsewhere (McCollum *et al.*, 1977). The meal, which was a modification of the Lundh meal, contained carbohydrate (4%) in the form of glucose, protein (4%) as comminuted chicken, and fat (4%) as corn oil; it was administered via a nasogastric tube after an overnight fast in the older children or after a 4-hour fast in young infants. Two-hour postprandial collections of juice were then obtained by siphonage on ice and aliquots of this postprandial pool were analysed.

Total bile salt concentrations in the duodenal aspirates were determined by the enzymatic procedure of Iwata and Yamasaki (1964) using 3- α -hydroxy steroid dehydrogenase (Cambrian Chemicals Ltd) after their extraction on Amberlite XAD-2 resin (Makino and Sjoval, 1972) supplied by British Drug Houses Chemicals Ltd. Individual bile salt concentrations were determined by the same method after separation on thin-layer chromatography using the solvent system of Hofmann (1964); amyl acetate/propionic acid/n-propanol/water (4:3:2:1 by vol). Both procedures gave consistent recoveries of >85%.

Animal experiments. The animal experiments were performed using an *in vivo* closed-loop model as previously described (Harries and Sladen, 1972; Sladen and Harries, 1972). A 20 cm segment of terminal ileum was used in male Wistar rats weighing 250–300 g after an overnight fast, water being allowed *ad libitum*.

All solutions were prepared on the day of the experiment. The solutions were mixed by ultrasound and gassed with 95% O₂ and 5% CO₂ to pH 7.1 immediately before instilling approximately 2 ml into the loop. The control solutions contained 10 mmol sodium taurocholate (TCA) (Koch Light)/l, 1 mmol oleate/l and 0.5 mmol monoolein/l (Sigma Chemical Company Ltd), 4 mmol KCl/l, 25 mmol NaHCO₃/l, and were made up to 280 mosmol/l with NaCl. ¹⁴C labelled taurocholate (Radiochemical Centre, Amersham) was added to give an activity of 1 μ Ci/ml. The test solutions were of a similar composition to the control solutions but, in addition, contained 10 or 30 mmol triolein (Sigma Chemical Company Ltd)/l, 3% bovine serum albumin (fraction V from Armour Chemical Company), or 5 mmol sodium ricinoleate (Eastman

Chemical Company)/l. The stock lipid solutions were made up in organic solvents, and appropriate aliquots were evaporated to dryness under nitrogen before mixing with the bile salt-buffer solution.

After the absorptive period a 100 μ l aliquot of the fluid remaining in the loop was counted in 10 ml of a dioxan-based scintillant: naphthalene 70 g, PPO 7 g, POPOP 0.0526 g/l dioxan (Hopkin and Williams Ltd). The solution was counted in a LKB Wallac beta counter with external standardisation.

Statistical analysis. The significance of differences between mean values was assessed by the Student's *t* test.

Results

Duodenal bile salts. Figs 1, 2, and 3 show the values for total bile salt concentrations, the molar ratios of glycine to taurine conjugates, and the percentage of dihydroxy bile salts in the duodenal juice of patients with CF and pancreatic insufficiency compared with controls. Unconjugated bile salts were not detected.

In the controls, the mean (and ranges) of total bile salt concentrations, glycine:taurine conjugate ratios, and percentage dihydroxy bile salts were

7.4 (3.0–16.0) mmol/l, 3.0 (1.3–4.5), and 61 (52–70) respectively. Pronounced differences were found in the CF patients with pancreatic insufficiency, corresponding values being 3.5 (1.3–6.6) mmol/l, 8.6 (>10–3.1), and 37 (10–60); P values were <0.01, <0.001, and <0.001 respectively. Six of these 11 patients with CF had total bile salt concentrations that were below the control range, and in 3 of these the concentrations were less than the critical micellar concentration (i.e. 2 mmol/l). Eight of the 9 patients had markedly increased glycine to taurine conjugate ratios. In six the ratio was 10 or greater, taurine conjugates being undetectable or only present in trace amounts. In 8 of the 9 patients the percentage of dihydroxy bile salts was reduced, and 6 of these patients were less than one year old. There was no apparent relationship between total bile salt concentrations or glycine: taurine ratios and age.

In the 2 patients with normal pancreatic enzyme activities, duodenal bile salts were both quantitatively and qualitatively normal. The total bile salt concentrations were 16.1 and 25.5 mmol/l, the glycine: taurine ratios 4 and 3.5, and the percentage dihydroxy bile salts 56 and 70 respectively.

Animal studies. Preliminary experiments were performed in order to establish the relationship between TCA absorption and the absorptive period, and the results are shown in Fig. 4. Luminal disappearance of TCA was very rapid during the first 30 min. By 30 min the mean percentage absorption of TCA was 89% compared with 77% for the 20-min absorptive period. When expressed as $\mu\text{mol/g}$ wet weight of ileum corresponding mean values for the 1-, 5-, 10-, 20-, 30-, and 40-min absorptive periods were 0.86, 5.77, 9.30, 10.41, 13.74, and 11.20

Values denote means and ranges; figures in parentheses indicate number of animals studied.

Fig. 4 Relationship between percentage disappearance of taurocholate from rat ileum and time.

respectively. For all subsequent experiments a 20-min absorptive period was used unless otherwise stated. In other preliminary experiments the whole loop of ileum was removed, opened, washed with isotonic saline, saponified and counted for radioactivity as described by Dawson and Webb (1973). A constant but small percentage (<5%) of label was present in the tissue, confirming that luminal disappearance reflected absorption of TCA from the loop.

The effect of adding triolein (10 and 30 mmol/l), bovine serum albumin (3%) and ricinoleic acid (5 mmol/l) on TCA absorption is shown in the Table. None of these agents had a statistically significant effect on TCA absorption during the 20-min absorptive period. The effect of triolein (10 mmol/l) was studied at different absorptive periods from 1 to 40 min and had no effect on TCA absorption at any of these times.

Discussion

This study has shown pronounced quantitative and qualitative abnormalities in the distribution of duodenal bile salts in children with CF and pancreatic insufficiency after a test meal compared with control subjects.

The mean ratio of glycine:taurine conjugates (3.0) and the percentage of dihydroxy species (61%) in our control subjects are almost identical with those reported for normal adult bile (Heaton, 1972). The mean age of our control group was 40 months, an age when duodenal bile salts have previously been shown to be similar to those found in adults (Poley *et al.*, 1964). We have previously shown, using the same intubation procedure, that the concentration of total bile salts in duodenal juice varies during a 2-hour postprandial collection (McCollum *et al.*, 1977). In this study determinations were performed on an aliquot of the mixed postprandial collection, thus representing a mean of the postprandial concentration fluctuations. By definition this mean value will overestimate the lowest bile salt concentration during the postprandial period.

Table Effect of triolein (TO), bovine serum albumin (BSA), and ricinoleic acid (RA) on the ileal absorption of taurocholate (TCA)

	No. of animals	Absorption of TCA ($\mu\text{mol/g}$ wet weight ileum per 20 min)
Controls	7	10.41 ± 1.20
10 mmol TO/l	8	12.85 ± 0.92
30 mmol TO/l	4	10.29 ± 0.96
5 mmol RA/l	3	14.4 ± 0.78
3% BSA	9	17.6 ± 0.83

Absorptive values expressed as mean $\pm$ 1 SE.

Under the conditions of the present study 6 of the 11 CF patients with pancreatic insufficiency had total bile salt concentrations below our normal range, and in 3 the concentrations were below the critical micellar concentration (CMC), if this value is taken as 2 mmol/l. There was a marked increase in the ratio of glycine:taurine conjugates (mean 8.6), and a concomitant reduction in the percentage of dihydroxy bile salts (mean 37%); although these two qualitative abnormalities may be present in non-CF subjects during early infancy (Heaton, 1972), this cannot be the case in our older patients. The CMC is higher for trihydroxy compared with dihydroxy bile salts (Heaton, 1972), and the pKa of glycine conjugates (approximately 4.0) higher than that for taurine conjugates (approximately 2.0). These observations, coupled with the relatively low pH of the duodenal juice in some CF patients, would be anticipated to result in defective micellar solubilisation of lipid in CF, and contribute to the malabsorption of fat. This would be in keeping with the well established fact that pancreatic supplements only partially reduce the faecal losses of fat in most CF patients. Goodchild *et al.* (1975) using a different test meal after hormonal stimulation found mean total bile salt concentrations in duodenal juice to be no different in 5 CF patients compared with 2 adult controls. After stimulation with CCK-PZ and then secretin in the same patients the same authors reported mean bile salt concentrations (mmol/l/per 10 min) as 22.3 and 36.2 respectively. After stimulation with CCK-PZ the mean ratios of total trihydroxy:dihydroxy, and glycine to taurine conjugated bile salts were 1.9 and 5.2 respectively; these ratios show a similar trend to those which we observed although the glycine to taurine ratio in our patients was more pronounced. The marked discrepancies in total bile salt concentrations between the study of Goodchild *et al.* (1975) and ours may be related to technical differences. For example, intravenously administered CCK-PZ provides information on the maximal capacity of the pancreas to secrete its proteolytic juice and on maximal gallbladder contraction; in addition Goodchild *et al.* (1975) reported a reduced volume of duodenal fluid in response to hormonal stimulation, which might be anticipated to result in 'apparently' normal total bile salt concentrations. In the study of Goodchild *et al.* (1975) the test meal was administered soon after IV CCK-PZ and secretin, and bile salt data were reported for periods after both the hormonal stimuli and the test meal. The data after their test meal may not therefore accurately reflect the physiological events which are associated with entry of gastric contents into the proximal small intestinal lumen in CF, but rather the net

response to maximal hormonal stimulation in addition to the test meal.

Marked increases in faecal output of bile salts have been reported in young children with CF (Weber *et al.*, 1973, 1976; Goodchild *et al.*, 1975), with reduced output (often within the normal range) in older children and young adults (Goodchild *et al.*, 1975; Roller and Kern, 1977). These observations together with our data are entirely consistent with a broken EHC secondary to defective absorption of bile salts by the distal ileum in young children with CF.

The pathophysiological mechanisms responsible for the broken EHC in CF have not been defined and raise intriguing and fundamental questions concerning bile salt metabolism in general. The ileal absorption of bile salts occurs by an active, carrier-mediated, sodium-dependent transport system which represents a highly efficient biological process for the conservation of the total bile salt pool of the body, and is basic to the maintenance of a normally operating EHC (Dowling, 1972). It has been suggested that the broken EHC in CF results from unhydrolysed triglycerides or other unhydrolysed dietary constituents interfering with the ileal absorption of bile salts (Weber *et al.*, 1973, 1976; Goodchild *et al.*, 1975; Watkins *et al.*, 1977). The finding of increased faecal bile salt outputs in other causes of pancreatic insufficiency lends support to this hypothesis (Goodchild *et al.*, 1975), as does the observation of Weber *et al.* (1973) who reported a good correlation between faecal bile salts and fat. This however, was not confirmed by Goodchild *et al.* (1975). In this context it is of interest that in the 2 patients with normal pancreatic enzymes in our study, duodenal bile salts were quantitatively and qualitatively normal. The relatively high concentrations of total bile salts in these 2 patients may be related to the reduced volume output of pancreatic secretions which occurs in CF, even in the absence of pancreatic insufficiency. The above observations prompted our studies on the possible effects of unhydrolysed fat and protein, as well as an hydroxy fatty acid, on the absorption of a bile salt in the terminal ileum of the rat *in vivo*.

High intraluminal concentrations of triolein, bovine serum albumin, and ricinoleic acid did not influence absorption rates of TCA in the rat ileum (see Table). Although caution should be exerted in extrapolating data from the experimental animal to man, our results do not appear to support the hypothesis that impaired pancreatic hydrolysis of dietary fat and protein (or the production of hydroxy fatty acids) plays a pathophysiological role in the broken EHC in CF.

There are a number of other explanations which

can be considered. For example, there may be a defect in the mucosal phase of bile salt absorption which is unrelated to the nature of the luminal contents. This possibility rests on reports which have described increased faecal outputs of amino-acids (Gibbons *et al.*, 1969), impaired *in vitro* uptake of phenylalanine by small intestinal mucosa (Morin *et al.*, 1976), impaired vitamin B12 absorption (Deren *et al.*, 1973), and reduced mucosal lactase activity (Antonowicz *et al.*, 1968) in CF patients. It has also recently been shown using a steady state perfusion technique that the jejunal absorption of phenylalanine, but not lysine, is impaired in CF patients (P. Milla, personal communication). These two amino-acids are probably absorbed by separate transport systems (Matthews and Laster, 1965) which suggests that phenylalanine malabsorption may reflect a selective and primary defect of mucosal function in CF. These abnormalities are not likely to be related to gross mucosal damage as the only described alterations of mucosal morphology in CF are dilated mucosal glands (Thomaidis and Arey, 1963) and thickening of surface mucus (Freye *et al.*, 1964). The possibility of a primary mucosal defect is supported by the finding that faecal bile salt losses in children with active coeliac disease are not significantly different from controls (Weber *et al.*, 1973), as the mucosal lesion in coeliac disease is predominantly a proximal one. This is not supported by a recent report in which marked reductions in faecal losses of bile salts were shown in older children and young adults with CF (Roller and Kern, 1977). The reason for the difference in bile salt excretion in older children and young adults compared with younger patients is not at present clear. A progressive reduction in the bile salt pool secondary to inadequate compensatory synthesis of bile salts by the liver is one possibility which requires further study; such a possibility is supported by the increased incidence of gallstones which occurs in older children with CF (Roy *et al.*, 1977). Alternatively more proximal regions of the small intestine may develop an increased capacity to absorb bile salts.

There is evidence that a component of pancreatic juice facilitates the absorption of vitamin B12 in the distal ileum (Toskes *et al.*, 1973), and a similar process could participate in bile salt absorption resulting in excessive faecal losses of bile salts in CF. It is difficult, however, to reconcile this possibility with the observed reduction in faecal bile salt losses which is associated with low fat or medium chain triglyceride diets in CF patients (Weber *et al.*, 1976).

The marked increases in faecal bile salt excretion in CF patients may have major clinical implications

to the patient, and the pathophysiological mechanisms responsible for the broken EHC require further elucidation. Our animal studies, however, do not support the hypothesis that defective pancreatic hydrolysis of dietary substrates is responsible for the increased excretion of bile salts in this condition.

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