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Intraluminal Precipitation of Bile Acids in Stagnant Loop Syndrome

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Summary

The postprandial concentrations of free and conjugated bile acids were measured in total content and micellar phase of jejunal aspirates from nine patients with steatorrhoea due to the stagnant loop syndrome and from 11 normal controls. Aspirates from the stagnant loop syndrome patients, but not from the normal controls, had a high concentration of free (unconjugated) bile acids. There was a reciprocal decrease in the concentration of conjugated bile acids, but total bile acid concentration in the whole aspirate remained normal. Total bile acid concentration in the micellar phase of intestinal content was reduced, indicating precipitation of bile acids. These findings suggest that precipitation of unconjugated bile acids, rather than passive absorption, leads to a reduced postprandial concentration of bile acids in the micellar phase of jejunal content, and are consistent with the hypothesis that fat malabsorption in the stagnant loop syndrome results from decreased micellar dispersion of lipolytic products.

Introduction

Current evidence suggests that steatorrhoea in the stagnant loop syndrome is due to malabsorption of dietary fat, as opposed to production of endogenous or bacterial lipids (Donaldson, 1965). It does not appear to be due to bacterial inactivation of pancreatic lipase (Donaldson, 1965; Kim *et al.*, 1966). The small intestinal mucosa has been widely reported as normal by light microscopy (Paulk and Farrar, 1964; Donaldson, 1965; Rubin and Dobbins, 1965) and by electron microscopy (Kjaerheim and Nygaard, 1968; Tabaqchali *et al.*, 1968), though abnormalities have been reported by Ament *et al.* (1971).

Bacterial deconjugation of bile acids occurs in the small intestinal lumen of animals with surgically created blind loops (Donaldson, 1965; Kim *et al.*, 1966) and of patients

with the stagnant loop syndrome (Donaldson, 1965; Tabaqchali and Booth, 1966). Free (unconjugated) bile acids inhibit the uptake and esterification of fatty acids by small intestinal slices in vitro (Dawson and Isselbacher, 1960), but this does not appear to be the mechanism of fat malabsorption in vivo (Gallagher *et al.*, 1968; Cheney *et al.*, 1970; Clark *et al.*, 1970). Steatorrhoea in the stagnant loop syndrome is more likely to be due to a reduction in the concentration of bile acids in the aqueous phase of intestinal content to a level inadequate for micellar solubilization of dietary fat. A reduced incorporation of lipid into the micellar phase of intestinal content has been found in animals with surgically created blind loops (Kim *et al.*, 1966), and oral administration of conjugated bile acids has been shown to abolish the steatorrhoea in these animals, and in one patient with the stagnant loop syndrome (Tabaqchali *et al.*, 1968). Tabaqchali *et al.* (1968) reported a low concentration of conjugated bile acids in intestinal content from patients with the stagnant loop syndrome, but they studied fasting rather than postprandial samples, so that the significance of their results in relation to the solubilization of dietary fat is uncertain.

Two alternative mechanisms have been suggested to account for a reduced micellar concentration of bile acids. One is rapid absorption of free bile acids. Since these have a higher pK value (about 6) than bile acids conjugated with glycine (about 4) or with taurine (about 2), they are more rapidly absorbed than conjugated bile acids by passive nonionic diffusion at the pH of jejunal content (about 6), as has been shown in the rat by Dietschy *et al.* (1966) and in man by Hislop *et al.* (1967). The second suggested mechanism is that free bile acids may precipitate out of solution at the pH of intestinal content, since in-vitro experiments have shown that free bile acids precipitate at a pH below 6.5 to 7.0 (Small, 1971). Previous studies in patients with the stagnant loop syndrome have not paid attention to the physical state of bile acids in the intestinal aspirate.

In order to clarify further the role of altered bile acid metabolism in the pathogenesis of steatorrhoea in the stagnant loop syndrome, the postprandial concentration of free and conjugated bile acids has been measured in jejunal aspirate from nine patients with steatorrhoea due to the stagnant loop syndrome, and from 11 control subjects without gastrointestinal disease. Total bile acid concentration has also been measured in the micellar phase of intestinal aspirate obtained from both groups of subjects,

Material and Methods

Two groups of subjects were studied. Group 1 consisted of nine patients with steatorrhoea attributed to the stagnant loop syndrome. They all had a faecal fat excretion of more than 5 g fat daily in a three- to five-day collection on a diet of about 75 g fat daily, and a radiologically proved anatomical abnormality of the small intestine, of a sort that is known to be capable of giving rise to the stagnant loop syndrome. Four subjects had a small intestinal stricture, three had small intestinal diverticula, and two had a fistula between the small and large intestine. Postprandial jejunal aspirates from seven of the patients were cultured anaerobically, and an anaerobe count of 10^5 per ml or more was found in the upper or lower jejunal aspirate in six of these patients, and a count of 10^4 per ml in the seventh (Northfield *et al.*, 1973). Four of the nine patients had no other detectable cause for steatorrhoea apart from bacterial overgrowth. Three of these four patients were treated with ampicillin with reduction in faecal fat excretion. In the other five patients there was an alternative cause for steatorrhoea, but treatment resulted in a reduction in faecal fat excretion to normal (less than 5 g daily) in four patients, and from 27 to 8 g daily in the fifth. Treatment consisted of oral ampicillin (250 mg six-hourly for six days) in three patients, relief of subacute small intestinal obstruction by surgical resection in one patient, and rapid clinical and radiological relief of subacute small intestinal obstruction coincident with oral administration of azathioprine for Crohn's disease in the other. Group 2 formed the normal control group and consisted of 11 hospital inpatients, none of whom had known gastrointestinal disease or gastrointestinal symptoms. None of the subjects had been treated with antibiotics during the previous month, nor had they received any other drugs apart from night sedation during the previous week.

Postprandial jejunal samples were obtained by a modified rapid transit tube (Wiggins *et al.*, 1967). The position of the aspiration site was checked radiologically at the duodenojejunal flexure in all subjects. Upper and lower jejunal samples were obtained 30-90 minutes postprandially from sites 0-45 cm and 45-90 cm beyond this site.

Intestinal aspirate was separated into oil phase, micellar phase, and sediment by ultracentrifugation (Hofmann and Borgstrom, 1964) at 100,000 g for three hours at 37°C. Bile acids were extracted into methanol, and qualitative analysis of whole content and of separate phases after ultracentrifugation was carried out by thin layer chromatography (Eneroth, 1963; Anderson and Haslewood, 1970). Quantitative measurements of total bile acid concentration were carried out on whole content and on micellar phase of intestinal aspirate, using the 3 α -hydroxysteroid dehydrogenase enzyme assay (Iwata and Yamasaki, 1964). In small intestinal samples containing free bile acids, the concentrations of free and conjugated bile acids were measured individually by this method in the whole aspirate after elution of the appropriate streaks from a thin layer chromatogram (Northfield *et al.*, 1973). The pH of intestinal aspirate was measured by a radiometer electrode system.

Results

Eight of the nine patients with the stagnant loop syndrome (group 1) had detectable free bile acids in the upper jejunal aspirate, and the ninth had free bile acids in the lower jejunal aspirate. None of the 11 control subjects (group 2) had free bile acids in the upper jejunal aspirate, but two of them had a low concentration of free bile acids in the lower jejunal aspirate. In both groups of subjects, there was no significant difference in the concentration of conjugated and total bile acids between the upper and lower jejunal aspirates, and the mean jejunal concentration has therefore been used for comparing the concentra-

tions in the two groups of subjects (see table). There was no significant difference in total acid concentration in the whole aspirate, but group 1 patients had a reduced concentration of conjugated bile acids in the whole aspirate, and of total bile acids in the micellar phase of intestinal aspirate.

Mean ($\pm$ S.D.) jejunal Bile Acid Concentrations (μ mol/ml) in the Two Groups

Group (n)	Free	Conjugated	Total	Micellar* (% of Total)
1. Stagnant loop syndrome (9)	3.4 $\pm$ 5.8	3.4 $\pm$ 3.6	6.9 $\pm$ 4.9	3.4 $\pm$ 1.5 (60 $\pm$ 27)
2. Normal Controls (11)	0.02 $\pm$ 0.06	7.4 $\pm$ 2.4	7.4 $\pm$ 2.3	5.4 $\pm$ 2.3 (78 $\pm$ 24)
Significance	P < 0.0005	P < 0.005	N.S.	P < 0.05

*Values for micellar bile acid concentration are based on six subjects only from each group.
N.S. = Not significant.

After ultracentrifugation of samples from two patients the micellar phase was decanted, and the sediment at the bottom of the tube was eluted with methanol. Thin layer chromatography of the methanolic eluate showed the presence of free cholic, chenodeoxycholic, and deoxycholic acid in both samples. Glycine dihydroxy bile acids were also detected in the sediment from one of the samples, but virtually no taurine conjugates or glycocholic acid were detected, despite the fact that they were present in the micellar phase from both samples.

The mean pH ($\pm$ S.D.) of jejunal aspirate was 6.0 $\pm$ 0.4 in group 1 and 5.7 $\pm$ 0.6 in group 2.

Discussion

Though bile acid deconjugation occurs in the normal human ileum (Northfield *et al.*, 1970), free (unconjugated) bile acids were absent in upper jejunal aspirate from all control subjects in this study, and were present in trace amounts only in two lower jejunal aspirates. A much higher concentration of free bile acids was found in jejunal aspirates from patients with the stagnant loop syndrome.

Steatorrhoea in these patients could not be accounted for by rapid absorption of these free bile acids leading to a reduced total bile acid concentration. It could, however, be accounted for on the basis of intraluminal precipitation of free bile acids, since the bile acid concentration was reduced in the micellar phase of jejunal content. Free bile acids precipitate out of solution in vitro at a pH below 6.5-7.0 (Small, 1971) and the pH range for jejunal content from both groups of subjects was 4.5 to 7.0. This explanation is supported by the finding of free bile acids in the sediment after ultracentrifugation of samples from two patients. The sediment contained a negligible quantity of taurine conjugates, which have a very low precipitation pH, and of glycocholic acid (precipitation pH 4.3), but one sample contained glycine conjugates of the dihydroxy bile acids (precipitation pH 4.8-5.0). Taurine conjugates solubilize free bile acids below the precipitation pH if the molar ratio is more than 4:8:1 (Small, 1971), and a similar effect is found with glycine conjugates; but the molar ratio was considerably less than this in most of the samples from patients with the stagnant loop syndrome averaging 1:1.

The presence of free bile acids was associated with a reduced jejunal concentration of conjugated bile acids in the stagnant loop syndrome. In-vitro studies suggest that the concentration of conjugated bile acids must exceed 4 μ mol/ml in order to provide the number of micelles necessary to dissolve the hydrolytic products of a normal dietary load (Badley *et al.*, 1969). The mean concentrations of both conjugated and micellar bile acids were below this level in patients with the stagnant loop syndrome but were considerably higher than this level in controls.

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Quantitative Skeletal Histology in Untreated End-stage Renal Failure

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Summary

Forty-six patients with end-stage renal failure were subjected to iliac crest biopsy before the initiation of a dialysis programme and regardless of the presence of skeletal symptoms. Quantitative studies of undecalcified sections showed osteoporosis in 11 patients, osteosclerosis in 10, and osteomalacia (alone or in combination with other lesions) in 14. Semiquantitative studies showed osteitis fibrosa (alone or in combination with other lesions) in 29. The various abnormalities occurred alone or in combination with one another and, to a large extent, independently of serum biochemistry.

Radiological examination failed to diagnose the histological abnormality in 12 of 13 patients with osteomalacia and in 10 of 25 patients with osteitis fibrosa. These abnormalities were commoner in women, in patients with pyelonephritis, and in patients with documented renal failure of long standing. Bone volume changes could not be correlated with any clinical parameters.

Skeletal findings in untreated patients should be taken into account when the effects of chronic dialysis or renal transplantation or both are being considered.

Introduction

The association between renal failure and skeletal disease has been recognized for almost a century (Lucas, 1883) and the literature contains many reviews of the subject (Claireaux, 1953; Stanbury, 1957, 1968; Kleeman *et al.*, 1969; Katz *et al.*, 1969). In recent years the emphasis has shifted to the study of

skeletal lesions among patients receiving chronic haemodialysis (O'Riordan *et al.*, 1970; Jowsey *et al.*, 1972) and among recipients of renal homografts (Hall *et al.*, 1969; Johnson *et al.*, 1971; Harrington *et al.*, 1971). As such patients are likely to have composite lesions (those due to their renal failure and those due to treatment) we have attempted to identify, by quantitative methods, what abnormalities were present before the commencement of a dialysis/transplantation programme.

Patients and Methods

Forty-six patients were subjected to iliac crest biopsy at the time of the creation of an arteriovenous fistula and before the initiation of maintenance haemodialysis for end-stage renal failure. All had serum creatinine levels in excess of 12 mg/100 ml and none had undergone more than two peritoneal dialyses before the performance of bone biopsy. There was no selection of patients according to skeletal symptoms (which were present in two patients) or according to serum biochemistry (other than nitrogenous retention).

The various underlying renal disorders are shown in table I. There were 18 patients (15 male, 3 female) with chronic glomerulonephritis. This diagnosis was made on the basis of histological findings (Heptinstall, 1968) in nine cases and on the basis of a characteristic history of acute glomerulonephritis and/or the nephrotic syndrome before the onset of renal failure in three cases. In six patients the diagnosis of chronic glomerulonephritis was based on normal pyelographic appearances, the

TABLE I—Underlying Renal Disorders in 46 Patients Subjected to Bone Biopsy

	M.	F.	Mean Age (years)	Mean Duration of Renal Failure* (months)
Glomerulonephritis ..	15	3	36	38
Pyelonephritis ..	2	16	39	33
Malignant hypertension ..	4	2	31	5
Polycystic ..	0	2	53	19
Hereditary ..	0	1	46	48
Undiagnosed ..	0	1	42	25
Total	21	25	38	31

*Renal failure was dated from the time of the first documented blood urea nitrogen of 31 mg/100 ml or more.

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