Duodenal bile acids in infants with protracted diarrhoea

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SUMMARY Bile acids were estimated in the duodenum of infants with protracted diarrhoea and compared with those in a control group. Significantly lower levels of total bile acids were found in infants with protracted diarrhoea, a finding which may be due to ileal dysfunction. Low concentrations of total bile acids may contribute to the poor nutritional state of these patients by impairing the normal digestion and absorption of dietary fat and fat-soluble vitamins. The absence of deconjugated bile acids in the duodenal juice of most infants with protracted diarrhoea suggests that they do not contribute significantly to the pathophysiology of this disorder.

Protracted diarrhoea of infancy is a severe and debilitating disorder, characterised by the passage of at least 4 loose stools a day for more than 2 weeks. In some infants protracted diarrhoea is secondary to recognised gastrointestinal diseases (Larcher *et al.*, 1977), while in others the pathogenesis remains unknown. The patients become dehydrated and malnourished due to persistent fluid loss and to difficulty in maintaining an adequate oral intake of food. Impaired digestion and absorption, resulting from a damaged small intestinal mucosa, also contribute to the poor nutritional state. As recovery of small intestinal mucosal function may be delayed by malnutrition, a vicious cycle of events occurs which perpetuates the clinical problem, and intravenous nutrition may be needed to sustain life.

Bile acid studies on infants with protracted diarrhoea have shown two principal abnormalities, either of which could contribute to the pathophysiology of this disorder. Deconjugated bile acids were detected in the duodenal juice of some infants (Gracey *et al.*, 1969), while in experimental animals the same bile acids had cytopathic effects on the small intestinal mucosa (Dawson and Isselbacher, 1960), and inhibited the absorption of water, electrolytes, and monosaccharides from the lumen of the small intestine (Gracey *et al.*, 1971; Harries and Sladen, 1972). Increased faecal excretion of bile acids has also been shown in infants with protracted

diarrhoea (Balistreri *et al.*, 1977). If the loss is prolonged, it may reduce the size of the bile acid pool and lower the concentration of bile acids in the small intestine. As bile acids are essential for the optimal absorption of dietary fat and fat-soluble vitamins, a fall in concentrations below the level at which micelle formation occurs in the small intestine might further compromise the poor nutritional state of the patient. Bile acids in the duodenum of a control group of infants (group A) were therefore compared with those in a group of infants with protracted diarrhoea (group B).

Materials and methods

Patients. The infants were divided into two groups (Table 1). Infants with protracted diarrhoea (group

Table 1 *Clinical details of infants studied*

Cases	Age (weeks)	Sex	Diagnosis
<i>Group A (controls)</i>			
1	2	M	Inguinal hernia
2	5	F	Feeding problem
3	8	M	Congenital heart disease
4	8	M	Congenital heart disease
5	12	F	Feeding problem
<i>Group B (patients)</i>			
6	6	M	Protracted diarrhoea
7	6	M	Protracted diarrhoea + secondary glucose intolerance
8	8	M	Protracted diarrhoea + secondary lactose intolerance
9	8	F	Protracted diarrhoea
10	12	F	Protracted diarrhoea + secondary glucose intolerance

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B) had been passing at least 4 loose stools daily for more than 2 weeks, without the isolation of known enteropathogenic bacteria. Stools were tested for the presence of reducing substances using Clinitest tablets (Kerry and Anderson, 1964), and significant amounts ($>0.5\%$) were detected in 3 of them. The presence of glucose was confirmed by paper chromatography in 2 patients and lactose in one. All the infants in the control group (group A) needed tube feeding as a normal part of their medical care, and none had diarrhoea. As concentrations of duodenal bile acids have been shown to increase with age in early infancy (Challacombe *et al.*, 1975), these infants were of approximately similar ages as those in group B.

Sampling regimen. Duodenal juice was obtained by the use of a long polyethylene feeding catheter (Argyl, 5 FG, 91 cm), weighted with a gold bead (Rhea and Kilby, 1970). The distal end of the tube was allowed to pass into the duodenum by normal peristalsis and its position in the 2nd to 4th part of the duodenum was confirmed by screening. The first sample of duodenal juice aspirated was discarded to exclude contamination by gastric juice and the 2nd sample was placed in a sterile container and stored at -20°C until analysed. Samples were taken 2 hours after a full cream cows' milk formula feed in group A and 2 hours after a 5% glucose feed in group B. The

absence of significant differences in duodenal bile acid concentrations in infants 2 hours after either type of feed has been previously demonstrated (Challacombe *et al.*, 1975).

Bile acid analysis. Bile acids were estimated using sulphuric acid fluorescence after separation by thin layer chromatography (Panveliwalla *et al.*, 1970). Total and individual bile acids were measured in samples of duodenal bile from both groups of infants. Separate estimates of the individual taurine and glycine conjugated dihydroxy bile acids were not made because it was not possible to separate them by the chromatographic method.

Results

The results of bile acid analyses are shown in Table 2. The difference between mean values of total bile acids, taurine, and glycine conjugates, and individual bile acids in group A and group B were examined for statistical significance using Student's *t* test. Differences were regarded as significant when *P* values were <0.05 .

Concentrations of total bile acids were significantly lower in infants in group B ($P<0.01$). This finding was mainly due to lower mean concentrations of taurocholic ($P<0.01$) and glycocholic acids ($P<0.05$). Ratios of total glycine conjugates to total

Table 2 Duodenal bile acids (mmol/l)

Cases	Total bile acids	Conjugates			Deconju- gated bile acids	Tauro- cholic acid	Taurine conjugated dihydroxy bile acids	Tauro- lithocholic acid	Glycocholic acid	Glycine conjugated dihydroxy bile acids
		Glycine	Taurine	Glycine						
				Taurine						
Group A (controls)										
1	5.4	1.4	3.9	0.3	—	1.6	2.3	ND	1.0	0.5
2	7.1	4.0	3.0	1.3	—	1.4	1.1	0.5	3.1	1.0
3	9.1	6.3	2.8	2.2	—	1.4	1.1	0.3	3.1	3.2
4	6.1	5.0	1.0	5.0	—	0.4	0.3	0.4	3.6	1.4
5	9.8	6.4	3.3	1.9	—	1.3	1.3	0.7	3.9	2.5
Mean	7.5	4.6	2.8	2.2	—	1.2	1.2	0.4	2.9	1.7
SD	1.9	2.0	1.1	1.7	—	0.5	0.7	0.2	1.1	1.1
Coefficient of variation (%)	25	43	38	80	—	38	57	66	39	64
Group B patients										
6	2.1	1.6	0.6	2.6	—	0.5	0.1	ND	1.6	ND
7	7.1	4.9	2.2	2.2	—	0.5	1.3	0.4	2.4	2.5
8	2.8	1.6	0.2	8.0	0.9	ND	0.2	ND	1.3	0.4
9	1.4	0.6	0.8	0.7	—	0.2	0.3	0.2	0.7	ND
10	2.1	1.0	1.1	0.9	—	0.4	0.5	0.2	0.6	0.4
Mean	3.1	1.9	1.0	2.9	—	0.3	0.5	0.2	1.3	0.6
SD	2.3	1.7	0.7	2.9	—	0.2	0.5	0.2	0.7	1.0
Coefficient of variation (%)	74	89	75	103	—	62	100	100	53	173
t	3.3	2.3	3.2	0.46	—	3.8	1.9	1.6	2.7	1.6
P	<0.01	<0.05	<0.01	>0.3	—	<0.01	>0.05	>0.1	<0.05	>0.1

ND = not detected.

Conversion: SI to traditional units—Taurocholic acid: 1 mmol/l $\approx$ 51.5 mg/100 ml. Taurine conjugated dihydroxy bile acids: 1 mmol/l $\approx$ 49.9 mg/100 ml. Tauro-lithocholic acid: 1 mmol/l $\approx$ 43.8 mg/100 ml. Glycocholic acid: 1 mmol/l $\approx$ 46.5 mg/100 ml. Glycine conjugated dihydroxy bile acids: 1 mmol/l $\approx$ 44.9 mg/100 ml.

taurine conjugates (G/T) were not significantly different in the two groups. Mean concentrations of taurine conjugated dihydroxy bile acids, glycine conjugated dihydroxy bile acids, and tauroolithocholic acid were also not significantly different. In group B taurocholic acid was present in 4 out of 5, tauroolithocholic acid in 3 out of 5, and glycine conjugated dihydroxy bile acids in 3 out of 5 infants. In group A all bile acids were present with the exception of tauroolithocholic acid which was not detected in one patient. Deconjugated bile acids were not detected in infants in group A, but cholic acid (0.13 mmol/l; 5.3 mg/100 ml), chenodeoxycholic acid (0.57 mmol/l; 22.3 mg/100 ml), and lithocholic acid (0.25 mmol/l; 9.4 mg/100 ml) were found in the duodenal juice of one infant in group B who had secondary lactose intolerance. In this infant, who has been previously reported (Challacombe *et al.*, 1974a), *Bacteroides* sp. (obligate anaerobes with known bile acid deconjugating properties *in vitro*) were isolated from the duodenal juice.

Discussion

Concentrations of total bile acids were significantly lower in the duodenum of infants in group B compared with group A. Morphological and functional changes in the bowel may interrupt the enterohepatic circulation of bile acids leading to increased bile acid excretion in the faeces. The liver initially compensates for the increased faecal loss by increased synthesis, but eventually synthesis is inadequate, leading to a depleted bile acid pool and to low levels of bile acids in the duodenum. There is some experimental evidence in favour of this proposed mechanism. The oral or intravenous administration of radioactively-labelled bile acids to adults with diffuse ileal disease leads to rapid excretion of the radioactive label in the faeces (Meihoff and Kern, 1968). Oral administration of C¹⁴-cholic acid to infants with ileal resections and to infants with protracted diarrhoea also results in increased faecal excretion of this label (Balistreri *et al.*, 1977). The presence of ileal dysfunction in protracted diarrhoea is further suggested by abnormal Schilling tests for vitamin B12 absorption (Balistreri *et al.*, 1977). Impaired ileal function may be the result of associated mucosal damage. Severe mucosal changes have been reported in the upper small intestine (Greene *et al.*, 1975) and these may be sufficiently extensive to involve the terminal ileum where most bile acid reabsorption should occur. Ileal dysfunction may also contribute to the protracted diarrhoea in these patients, as excessive amounts of dihydroxy bile acids reaching the colon have been shown to provoke excessive

water secretion by the colonic mucosa (Mekhjian *et al.*, 1971).

A higher ratio of glycine to taurine conjugates in duodenal bile is usually present in adults with terminal ileal disorders (Heaton *et al.*, 1968), a finding which may be due to the relative scarcity of taurine or of its precursor cysteine, in the face of an increased demand for bile acid conjugation. Mean values of the glycine/taurine ratio were not significantly raised in patients with protracted diarrhoea compared with the controls. As bile acids are mainly conjugated with taurine in early infancy, failure to demonstrate consistently raised glycine/taurine ratios in patients with protracted diarrhoea may be due to an increased availability of taurine for conjugation.

Trihydroxy bile acid concentrations were significantly lower in infants in group B compared with group A, and mean concentrations of dihydroxy bile acids were also low, but the values did not reach statistical significance. In adults with ileal resections the absorption of both dihydroxy and trihydroxy bile acids has been shown to be equally impaired (Low-Beer *et al.*, 1974). Although ileal dysfunction may be one explanation for the bile acid findings reported in protracted diarrhoea, further study of the enterohepatic circulation and faecal excretion of bile acids in this disorder will be necessary. Bacterial overgrowth in the upper small intestine (Gracey and Stone, 1972; Challacombe *et al.*, 1974b), a rapid small intestinal transit time, or impaired synthesis of bile acids by the liver due to malnutrition, could each influence bile acid metabolism in infants with protracted diarrhoea.

The deconjugated bile acids, deoxycholic and chenodeoxycholic acids, have been shown to have cytopathic and metabolic-inhibitory effects on the small intestine of experimental animals (Dawson and Isselbacher, 1960; Low-Beer *et al.*, 1970; Gracey *et al.*, 1971; Harries and Sladen, 1972), and these bile acids could have similar effects in patients with protracted diarrhoea and monosaccharide malabsorption (Gracey *et al.*, 1969). Cholic, chenodeoxycholic, and lithocholic acids were detected in small quantities in only one infant with protracted diarrhoea, an infant who was also intolerant of oral lactose. These findings suggest that deconjugated bile acids were not important in the pathophysiology of protracted diarrhoea in our patients, but samples were taken from a single site only in the duodenum, and sampling from other sites will be necessary before the contribution of deconjugated bile acids can be fully evaluated. Concentrations of total bile acids in the duodenum of 4 of the 5 infants in group B were below a level of 4 mmol/l. Badley *et al.* (1969) have shown that total bile acid concentrations in the upper small intestine should exceed this value

for optimal fat absorption to occur. The digestion and absorption of dietary fat and fat-soluble vitamins may therefore be impaired in patients with protracted diarrhoea, and absorption and nutrition may be improved by the use of medium chain triglycerides, which are well absorbed in the absence of bile acids.

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