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Duodenal bile acids in infancy

D. N. CHALLACOMBE, SUSAN EDKINS, and G. A. BROWN
From the Institute of Child Health, University of Birmingham

Challacombe, D. N., Edkins, S., and Brown, G. A. (1975). *Archives of Disease in Childhood*, **50**, 837. **Duodenal bile acids in infancy.** Duodenal bile acids have been estimated in three age groups in infancy from birth to 7 months, and age-related changes have been shown. The lowest concentrations of duodenal bile acids occurred in the youngest infants, and increasing concentrations were found with increasing age. Taurine conjugated bile acids predominated in early infancy, while in older infants bile acids were mainly conjugated with glycine. The probable presence of taurolithocholic acid in the duodenal bile of 2 newborns before the establishment of a gastrointestinal microflora remains to be confirmed, but could result either from transplacental passage of secondary bile acids or from endogenous synthesis by the fetal liver.

Bile acids are essential for the optimal absorption of dietary fat from the small intestine. They activate pancreatic lipase, solubilize the products of lipolysis, and promote the absorption of fatty acids and monoglycerides by the formation of mixed micelles.

Previous investigations on bile acids in the small intestine of normal infants have been performed on single fasting samples of duodenal juice (Encrantz, and Sjövall, 1959; Poley *et al.*, 1964) or on samples taken after a test meal (Norman, Strandvik, and Ojamäe, 1972; Signer *et al.*, 1974). The bile acid pool size has also been measured using a stable isotope technique (Watkins *et al.*, 1973).

In the present study bile acids were estimated in samples of duodenal juice taken at three fixed intervals in relation to time of feeding over a 24-hour period. The concentration of total bile acids and five individual bile acids has been measured in three groups of infants from birth to 7 months of age, with a view to determining age-related changes.

Materials and methods

Patients. Duodenal bile acids were measured in 34 infants who were patients in the Special Care Baby Unit of the Birmingham Maternity Hospital or the Birmingham Children's Hospital. All were being tube fed as a normal part of their medical care. None of the infants had disorders of the gastrointestinal tract, nor did they have clinical evidence of liver disease. They were arbitrarily divided into three age groups for the purpose of this study. Groups 1 and 2 were each subdivided into normal birthweight infants (>2·5 kg), and low birthweight (<2·5 kg).

Group 1. 12 infants from birth to 2 days of age (7 males and 5 females). 4 were of normal birthweight and 8 of low birthweight.

Group 2. 8 infants aged from 2 to 7 days (4 males and 4 females). 4 were of normal birthweight and 5 of low birthweight.

Group 3. 14 infants aged from 10 days to 7 months (7 males and 7 females). Infants in groups 1 and 2 were fed with expressed breast milk or full cream cow's milk formula while infants in group 3 were either on full cream cow's milk formula or had been partially weaned on to solids.

Sampling regimen. Duodenal juice was sampled using a sterile syringe and a sterile nasoduodenal tube (Argyle 5 FG, 91 cm in length), weighted with a gold bead (Rhea and Kilby, 1970). Details of the intubation technique have been previously described (Challacombe, Richardson, and Anderson, 1974).

Previous studies on the duodenal bile acids in premature infants (Signer *et al.*, 1974) have shown that mean bile acid concentrations fall immediately after a meal and return to approximately preprandial levels after 2–3 hours. In the present study infants in all groups were fed at 8.00 a.m. and the first sample of duodenal juice was aspirated 2 hours later at 10.00 a.m. They were next fed at noon, and at 2.00 p.m. a feed of 5% dextrose was given to all three groups. 2 hours after the dextrose, at 4.00 p.m., the second sample of

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duodenal juice was aspirated. The infants were then fed normally until midnight. On the following morning groups 1 and 2 had their first feed at 6.00 a.m., and the third sample of juice was aspirated at 9.00 a.m. A third sample was also collected from group 3 at 9.00 a.m., but these infants had fasted for the whole of the intervening period, i.e. from midnight. The three groups of infants were receiving different feeds appropriate to their age. The intention with the dextrose feed was to administer a standard feed, acceptable to infants in all three groups, for better comparison of the ensuing duodenal bile acid concentrations. The nasoduodenal feeding tube was left *in situ* for the whole 24 hours. The duodenal juice samples were transferred to sterile containers and stored at −20°C until analysed.

Bile acid analysis. Qualitative assessment of the bile acid content of duodenal juice was made using the thin layer chromatographic method of Panveliwalla *et al.* (1970). Duodenal juice was loaded directly on the plates without pretreatment, and after development improved definition of the bile acid components was obtained by using a spray of 10 g/100 ml phosphomolybdic acid in ethanol, rather than iodine vapour as described in the published method.

Quantitation of individual bile acids was made using a duplicate thin layer plate on which specimen tracks were alternated with tracks loaded with a mixture of reference standards (Maybridge Research Chemicals, Launceston, Cornwall). After development of the plates the specimen tracks were masked and the reference standards visualized by spraying with phosphomolybdic acid. The specimen tracks were then segmented by reference to the visualized standard tracks and individual bile acids from the duodenal samples were recovered by scraping off and vacuuming the appropriate areas of silica gel into stoppered tubes. After heating with concentrated sulphuric acid, fluorescence was measured in a Farrand spectrophotofluorimeter, using activation and emission wavelengths of 470 nm and 500 nm, respectively. Quantitation was achieved by comparison with standard bile acids, separated on and scraped from the same plate. Results were expressed as mmol bile acid/l duodenal juice. Five bile acids were measured in this way, namely glycocholic acid (GC), glycine conjugated dihydroxy bile acids (glycochenodeoxycholic acid+glycodeoxycholic acid, GCDC), taurocholic acid (TC), taurine conjugated dihydroxy bile acids (taurochenodeoxycholic acid+taurodeoxycholic acid, TCDC), and taurolithocholic acid (TLC). Total bile acid concentrations were derived by summing the individual values.

In a series of 12 duodenal juice samples, total bile acid concentrations estimated by this summation technique were in good agreement with total concentrations determined by the 3-α-hydroxysteroid dehydrogenase enzyme assay (Iwata and Yamasaki, 1964). The mean levels estimated by each method were 6·36 mmol/l (SD 3·03 mmol) and 6·27 mmol/l (SD 3·07 mmol) for the summation and enzymatic methods, respectively. The difference between the means was not significant when assessed by Student's 't' test ($P>0·9$). Glycolithocholic acid was not estimated since none of the infants investigated produced a chormatographic spot with glycolithocholic mobility. Separate estimates of the taurine and glycine conjugated dihydroxy bile acids were not possible due to nonseparation by the method used.

Recoveries of bile acids by this method ranged from 90–98%, with a mean of 94% (Panveliwalla *et al.*, 1970), and precision determined in this laboratory by duplicate analysis of samples gave a coefficient of variation of 10%.

Results

Differences between arithmetic means were examined for significance in all comparisons using Student's 't' test. Where the differences are stated to be significant the P value was $<0·05$.

Diurnal variation.

Group 1. (Under 2 days, Fig. 1, 2, Table I.) The mean total bile acid concentrations for the three sampling times were all similar and the small differences between the means were not significant. All values were low when compared with concentrations reported in adults (Sjövall, 1960). Concentrations of individual bile acids were also relatively constant and so therefore were the ratios of glycine to taurine conjugated bile acids and the trihydroxy to dihydroxy bile acids. Mean total bile acid concentrations in the low birthweight subgroup of group 1 (Table IV), were not significantly different from the values found in the normal birthweight infants.

Group 2. (2–7 days, Fig. 3, 4, Table II). Mean total bile acid concentrations for the three

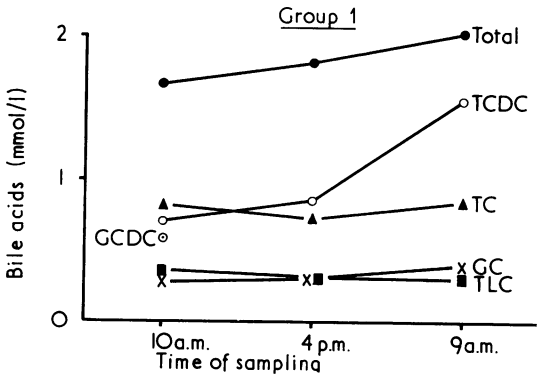

FIG. 1.—*Variation in mean bile acid concentrations in group 1 at the three sampling times. TCDC, taurine conjugated dihydroxy bile acids; TC, taurocholic acid; GC, glycocholic acid; TLC, taurolithocholic acid; GCDC, glycine conjugated dihydroxy bile acids.*

TABLE I

Duodenal bile acids (mmol/l) in group 1: 12 infants under 2 days of age

	10.00 a.m.	4.00 p.m.	9.00 a.m.
Total bile acids			
Mean	1·65	1·8	1·97
SD	1·1	1·7	1·85
Glycine/taurine conjugates			
Mean	0·09	0·5	0·17
SD	0·03	0·6	0·14
Trihydroxy/dihydroxy bile acids			
Mean	1·8	2·06	1·0
SD	1·3	2·3	0·43
Taurocholic			
Mean	0·78	0·69	0·78
SD	0·36	0·4	0·5
Taurochenodeoxycholic			
Mean	0·68	0·84	1·47
SD	0·4	1·03	1·16
Taurolithocholic			
Mean	0·32	0·29	0·28
SD	0·17	0·22	0·14
Glycocholic			
Mean	0·28	0·28	0·34
SD	0·15	0·12	0·2
Glycochenodeoxycholic	0·55 (1 sample)	—	—

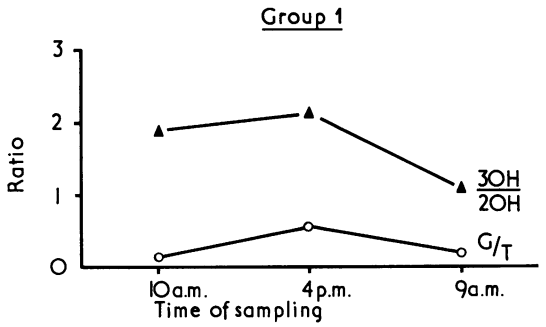

FIG. 2.—*Variation in mean bile acid ratios in group 1 at the three sampling times. 30H/20H, ratio of the sum of the trihydroxy-bile acid concentrations (taurocholic, glycocholic, and cholic acids) to dihydroxy bile acid concentrations (taurochenodeoxycholic, glycochenodeoxycholic, and deoxycholic acids). G/T, ratio of the total glycine conjugates to total taurine conjugates.*

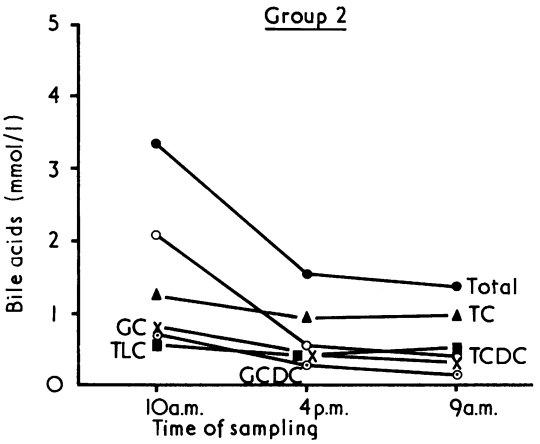

FIG. 3.—*Variation in mean bile acid concentrations in group 2 at the three sampling times.*

sampling times in this group were also not significantly different nor were they significantly different from the means found in group 1. Mean total bile acid concentrations in the low birthweight infants in this group were also similar to those found in the normal birthweight infants.

Group 3. (10 days to 7 months, Fig. 5, 6, Table III). Total bile acid concentrations in this group were much higher, with values comparable to those quoted for adults. Though the mean total bile acid concentration for the third samples were higher than that found at the other sampling times, the value was not significantly different. Individual bile acids were also high in the third sample but the glycine/taurine and trihydroxy/dihydroxy bile acid ratios at all sampling times remained relatively constant

The absence of significant differences in bile acid concentrations at the sampling times chosen suggest that the bile acid secretory response to ingested milk feeds does not change markedly during the day. A similar response to ingestion of a glucose feed has justified its use as a standard feed acceptable to infants of different ages who are normally on differing feed regimens.

TABLE II

Duodenal bile acids (mmol/l) in group 2: 8 infants aged 2–7 days

	10.00 a.m.	4.00 p.m.	9.00 a.m.
Total bile acids			
Mean	3·33	1·49	1·36
SD	3·0	1·12	1·15
Glycine/taurine conjugates			
Mean	0·37	0·25	0·29
SD	0·17	0·09	0·18
Trihydroxy/dihydroxy bile acids			
Mean	1·06	1·45	2·04
SD	0·38	0·32	1·0
Taurocholic			
Mean	1·17	0·89	0·88
SD	0·7	0·62	0·88
Taurochenodeoxycholic			
Mean	2·08	0·47	0·35
SD	1·45	0·29	0·23
Taurolithocholic			
Mean	0·6	0·38	0·37
SD	0·06	0·24	0·39
Glycocholic			
Mean	0·72	0·34	0·26
SD	0·48	0·12	0·06
Glycochenodeoxycholic			
Mean	0·64	0·3 (1 sample)	0·1 (1 sample)
SD	0·22		

TABLE III

Duodenal bile acids (mmol/l) in group 3: 14 infants aged 10 days to 7 months

	10.00 a.m.	4.00 p.m.	9.00 a.m.
Total bile acids			
Mean	8·47	7·33	13·55
SD	5·87	7·94	8·99
Glycine/taurine conjugates			
Mean	1·88	2·92	2·12
SD	1·32	3·05	1·01
Trihydroxy/dihydroxy bile acids			
Mean	2·09	2·18	2·08
SD	1·16	1·86	1·7
Taurocholic			
Mean	2·09	1·17	2·15
SD	1·6	1·05	1·5
Taurochenodeoxycholic			
Mean	1·66	1·44	1·77
SD	1·87	2·76	1·5
Taurolithocholic			
Mean	0·7	0·75	0·94
SD	0·3	1·12	0·56
Glycocholic			
Mean	3·00	2·52	3·9
SD	1·52	1·92	2·9
Glycochenodeoxycholic			
Mean	1·49	2·52	3·9
SD	1·49	2·3	3·3

Variation with age. Since there was no significant difference found in any of the 3 groups, in total bile acid concentrations obtained at the three sampling times, overall mean total bile acid concentrations were calculated from all samples collected from each group. Comparison of this overall mean for groups 1 and 2 showed no significant difference, but the overall mean for group 3 was significantly greater ($P < 0·001$), than the overall means for both groups 1 and 2.

The mean concentrations of both taurine and glycine conjugated bile acids both increased with age, but the taurine conjugates increased more slowly. Taurine conjugates were predominant in groups 1 and 2, while glycine conjugates predominated in group 3. Taurine conjugated bile acids

TABLE IV

Total duodenal bile acids (mmol/l) in infants of low birthweight and normal birthweight in groups 1 and 2

	Group 1	Group 2
Normal birthweight		
No. of infants	4	3
Mean	1·2	2·66
SD	1·07	2·67
Low birthweight		
No. of infants	8	5
Mean	2·06	2·22
SD	1·6	2·27

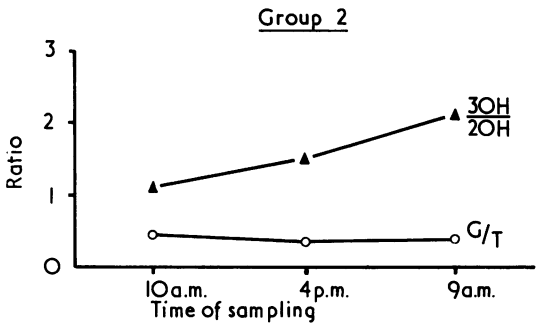

FIG. 4.—*Variation in mean bile acid ratios in group 2 at the three sampling times. 30H/20H and G/T, see legend to Fig. 2.*

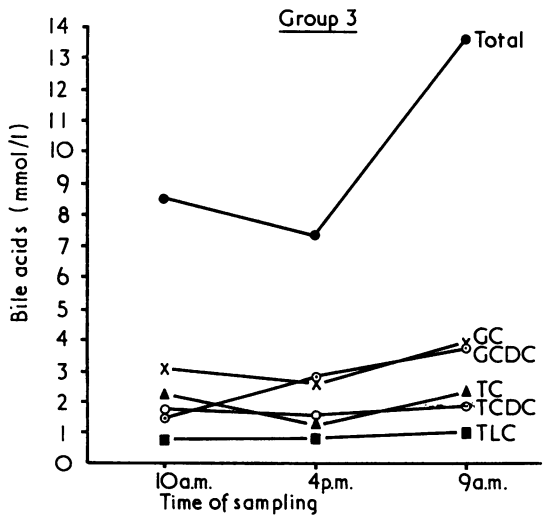

FIG. 5.—*Variation in mean bile acid concentrations in group 3 at the three sampling times.*

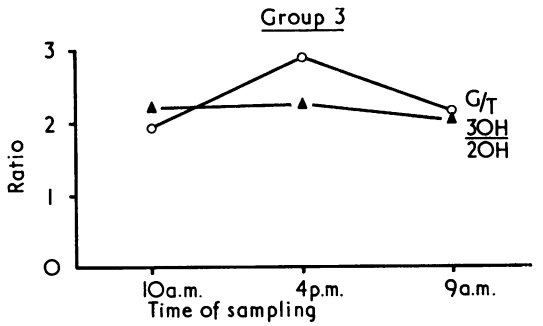

FIG. 6.—*Variation in mean bile acid ratios in group 3 at the three sampling times. 30H/20H and G/T, see legend to Fig. 2.*

were the only bile acids present in 16 out of 33 samples in group 1, and 8 out of 23 samples in group 2, while all samples in group 3 contained taurine conjugates. Glycine conjugated bile acids were the only bile acids present in 1 out of 33 samples in group 1, in 4 out of 23 samples in group 2, while all samples in group 3 contained glycine conjugates. This change in bile acid conjugation was reflected in the ratio of glycine to taurine conjugates which was low in groups 1 and 2 and higher in group 3. The trihydroxy : dihydroxy bile acid ratio was similar in all three groups.

The deconjugated bile acids, cholic, deoxycholic, chenodeoxycholic, and lithocholic acids, were not detected in any samples of duodenal juice from any infant in the three groups.

A bile acid spot with a chromatographic mobility identical to the taurolithocholic acid standard was shown in 18 out of 33 samples in group 1, in 10 out of 23 samples in group 2, and in all 42 samples from group 3. The concentration of this bile acid increased with age in the infants studied (Challacombe and Edkins, 1972).

Discussion

In this study the concentrations of total bile acids and of five individual bile acids were measured in the duodenal juice in infancy. The results show qualitative and quantitative changes which appear to be age related.

Bile acids are necessary for the optimal absorption of dietary fat and the coefficient of absorption of fat in premature and term infants fed on cow's milk is low during the neonatal period (Fomon, 1967). Malabsorption of fat in infancy may therefore be

the result of both low concentrations of bile acids in the duodenum (Norman *et al.*, 1972; Signer *et al.*, 1974), and of a reduced bile acid pool (Watkins *et al.*, 1973). Impaired solubilization of the products of lipolysis by bile acids leading to diminished absorption of fat may also explain the finding of monoglycerides in the faeces of young infants (Watkins, *et al.*, 1974). Though fat absorption improves in older infants, appreciable loss of fat in the faeces may still occur (Fomon, 1967). Increased absorption of dietary fat in older infants may therefore be related to rising concentrations of duodenal bile acids as shown in this study. Further studies relating fat absorption to duodenal bile acid concentrations in older infants will be necessary to confirm this suggestion.

For optimal fat absorption from the small intestine, Badley, Murphy, and Bouchier (1969) suggested that the concentration of bile acids in the upper small intestine should exceed 4 mmol/l, the lower limit of duodenal bile acids found in normal adults after a meal. Though samples in the present investigation were taken 2 hours after a meal during the fat absorption phase, the total bile acid concentrations in the low birthweight infants in groups 1 and 2 were below 4 mmol/l in 33 out of 36 samples (91%). The concentration of bile acids from the normal birthweight infants in groups 1 and 2 also failed to reach this value in 18 out of 20 samples (90%). However, in group 3 the concentration of total bile acids was only below this value in 18 out of 42 samples of duodenal juice (23%).

Signer *et al.* (1974) also found low levels of duodenal bile acids in premature infants after a test meal. The infants fed on human milk had lower levels of duodenal bile acids than infants fed on cow's milk, yet absorbed fat better. 23% of the infants in our group 3, who were fed on cow's milk, also had low duodenal bile acid concentrations (<4 mmol/l) and it is likely that fat absorption from breast milk will continue to be superior to fat absorption from cow's milk, even in older infants.

Bile acids have been shown to be principally conjugated with taurine in groups 1 and 2 and with glycine in group 3. The following explanations for early predominance of taurine conjugation have been suggested by Jacobsen and Smith (1968).

(1) Greater amounts of endogenous taurine may be available for conjugation with bile acids in the liver in early infancy. Plasma taurine levels in the newborn are 2–3 times higher than plasma levels in the mother (Ghadimi and Pecora, 1964), and the levels fall to those found in adults during the first 2 weeks of life (Dickinson, Rosenblum, and Hamilton, 1965). The taurine content of fetal liver is also greater than that of the adult liver (Ryan and Carver, 1966).

(2) The concentration of taurine in the blood of premature infants is higher than that found in term infants (Ghadimi and Pecora, 1964). The predominance of taurine conjugates in early infancy may therefore be due to immaturity of taurine degrading pathways of the liver (Schreier, 1962).

(3) Taurine degradation may be dependent on the availability of dietary taurine during the neonatal period. Oral administration of taurine to normal adults enhanced conjugation of bile acids with taurine (Sjövall, 1959), whereas glycine administration did not improve glycine conjugation. However, dietary taurine is unlikely to be an important factor, as human milk contains higher levels of taurine than cow's milk, yet no significant differences in conjugation were found with infants fed on different types of milk (Encrantz and Sjövall, 1959).

(4) Degradation of taurine to inorganic sulphate has been shown in the rat to be dependent on the presence of a suitable intestinal microflora (Boquet and Fromageot, 1965). This metabolic pathway may not be available in early infancy before the establishment of a normal intestinal microflora, if indeed it exists at all in humans.

(5) Conjugation with glycine is a relatively recent phylogenetic development. and is characteristic of herbivorous mammals. Taurine conjugation occurs very early in the evolutionary scale (Haslewood, 1962), and the predominance of taurine conjugates in early infancy may be an example of biochemical atavism.

The presence of a chromatographic spot with a similar mobility to the taurolithocholic acid standard, in the duodenal juice of many of the infants studied, has also been reported in two infants by Poley *et al.* (1964) and in 8 infants by Norman *et al.* (1972). In two of the newborns in the present study, this bile acid was detected in duodenal bile before an aerobic or anaerobic microflora had become established in the upper gastrointestinal tract or in the faeces.

Confirmation of the identity of this chromatographic spot as taurolithocholic acid by gas liquid chromatography and mass spectrometry will be necessary. In animal experiments lithocholic acid increased the mitotic activity of the bile duct epithelium, leading initially to bile duct hyperplasia, and eventually to cirrhosis (Palmer and Hruban, 1966). The accumulation of lithocholic acid in the fetal liver, either as a result of transplacental passage or endogenous synthesis, could cause abnormal development of bile ducts and contribute to the causes of liver disease in infancy.

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Correspondence to Dr. D. N. Challacombe, Children's Research Unit, Taunton and Somerset Hospital, Musgrove Park Branch, Taunton, Somerset TA1 5DA.