notes on methodology

Enterohepatic circulation in man. A simple method for the determination of duodenal bile acids

Roel J. Vonk,[1] C. M. Frank Kneepkens,[2] Rick Havinga, Folkert Kuipers, and Charles M. A. Bijleveld

Department of Pediactrics, University Hospital, Groningen, The Netherlands

Summary A method has been developed for easy sampling of duodenal bile acids. For this purpose Entero-Test® was used, an encapsulated nylon thread originally used to estimate enteral parasites. This capsule is swallowed by a fasting subject and one end of the thread is taped at a corner of the mouth. Four hours after swallowing the thread, it is withdrawn and bile acids are eluted with buffer. The solution is applied to a Sep-Pak C18 cartridge to extract bile acids, which are subsequently analyzed by capillary gas-liquid chromatography and liquid chromatography. In vitro analyses showed that there was no preferential binding to the thread of any bile acid and that binding was pH-independent. A high correlation (r = 0.98) was found between direct analyses of bile and analyses by Entero-Test after in vitro incubation. The values obtained by the Entero-Test were similar to those of duodenal bile simultaneously collected with the normal intubation technique (r = 0.99). Duodenal bile acid composition showed a daily variation. In 11 healthy volunteers the following bile acid composition of unstimulated duodenal juice was found (mean ± SD; %): choleate 44 ± 12 (glycine/taurine ratio 1.8), chenodeoxycholate: 29 ± 6 (G/T ratio 2.3); deoxycholate: 25 ± 11 (G/T ratio 5.7), lithocholate: 1, ursodeoxycholate: < 1. The described technique turned out to be an easily applicable method for determination of duodenal bile acids in man. This enables longitudinal studies concerning the factors that determine the bile acid pool composition and its relevance to various diseases. — **Vonk, R. J., C. M. F. Kneepkens, R. Havinga, F. Kuipers, and C. M. A. Bijleveld.** Enterohepatic circulation in man. A simple method for the determination of duodenal bile acids. *J. Lipid Res.* 1986. **27:** 901-904.

Supplementary key words bile acids • duodenal intubation

Bile acids undergo enterohepatic circulation. The composition of the bile acid pool (primary and secondary bile acids, taurine and glycine conjugates) depends on hepatic metabolism, bacterial metabolism in the intestinal lumen, and the efficiency of intestinal absorption (1, 2). Factors affecting bile acid pool composition in man, e.g., diet and drugs, are difficult to investigate because of the relative inaccessibility of the gastrointestinal tract. Duodenal intubation to collect bile is a cumbersome technique especially for use in children; therefore we developed an easy system for the determination of duodenal bile acids using the commercially available Entero-Test® string to collect unstimulated duodenal juice. This method enables longitudinal investigations of bile acid pool composition because serial samples can readily be obtained with minimal discomfort.

METHODS

Entero-Test® was purchased from HDC Corp., Mountain View, CA. Two types of Entero-Test are available, one for use in children and one for adults; they differ in the length of the thread and the size of the capsule. Entero-Test consists of an encapsulated nylon thread, which is swallowed with water and one end is taped at a corner of the mouth. The capsule dissolves in the stomach and the thread, which is weighted at its distal end, passes into the duodenum. Four hours after being swallowed by a subject in a fasting state, the thread is withdrawn and stored at −20°C until analysis. No special instructions were given to the patients or healthy volunteers concerning their dietary intake and drug consumption preceding the test or their behavior during the test except to maintain their fasting state.

Positioning of the thread in the duodenum was checked by measuring the pH after withdrawal, using the pH stick which is supplied with the Entero-Test, but in most instances it was apparent from the bright yellow color.

Subjects

Eleven healthy volunteers (20-40 years, five females and six males) participated in this study, as well as two pediatric patients: two brothers with intermittent cholestasis (13 and 16 years). A number of the participants underwent the test more than once (indicated in the Results section).

The clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Analyses

Bile acids were eluted from the thread by four consecutive washings with 0.5 M phosphate buffer, pH 7.0, and extracted from the buffer using Sep-Pak C18 cartridges (Waters Inc., Milford, MO). Bile acids were analyzed by capillary gas-liquid chromatography and liquid chro-

[1] Address reprint requests to: Dr. R. J. Vonk, Dept. of Pediatrics, Bloemsingel 10, 9712 KZ Groningen, The Netherlands.
[2] Present address: Department of Pediatrics, Free University Hospital, Amsterdam, The Netherlands.

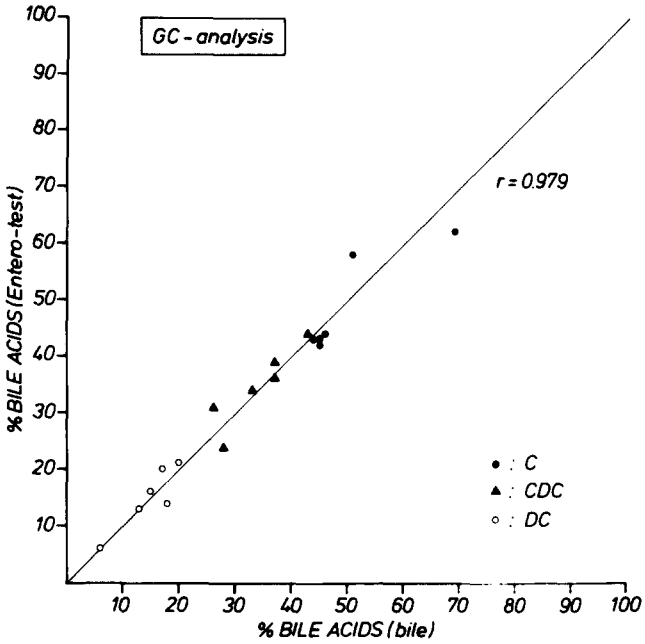

Fig. 1. Comparison between direct analyses and indirect analyses by means of the Entero-Test® of human duodenal juice. For quantitation, gas chromatography was used.

matography. Gas-liquid chromatography was performed according to Setchell and Matsui (3) using an HP 5880 gas chromatograph equipped with a CP Sil 5 and a CP Sil 19 column (Chrompak BV, Middelburg, The Netherlands). Liquid chromatography was performed according to Ruben and van Berge Henegouwen (4) with a Varian 5000 liquid chromatograph and a Waters C 18 μBondapack RCM column. Sep-Pak C18 cartridges were rinsed with a 10% acetone solution after activation prior to use.

RESULTS

In vitro binding tests were performed to analyze the binding capacity of the thread and to see whether preferential binding of any bile acid occurred. A solution of the following bile acids was prepared in 0.5 M phosphate buffer containing 4% albumin (pH 7.0): taurocholate (TC), glycocholate (GC), taurochenodeoxycholate (TCDC), glycochenodeoxycholate (GCDC), taurodeoxycholate (TDC), glycodeoxycholate (GDC), taurolithocholate (TLC), and glycolithocholate (GLC). Except for the lithocholate (LC) conjugates, all bile acids were used in equal amounts to a final concentration of 10, 20, 40, and 80 mM. LC conjugates were added to all solutions to a 3% concentration. The correlation was calculated between the results from the direct analysis of the solution and the results from the analysis by Entero-Test. For the 10, 20, 40, and 80 mM bile acid solutions, the correlation coefficients were 0.83, 0.93, 0.99, and 0.99, respectively. When a pH of 6.0 instead of pH 7.0 was used, the correlation coefficients were 0.92, 0.99, 0.85, and 0.82, respectively, and with pH 8.0 the coefficients were 0.95, 0.93, 0.87, and 0.91, respectively. When the thread was incubated in a bile acid solution of pH 7.0 and afterwards passed through absorbent cotton wetted with pH 2 buffer, no change in the relative composition of the bile acid solution could be detected. After withdrawing the thread from the intestine, the bile acids were eluted with 0.5 M phosphate buffer. The diluted suspension was applied directly to a Sep-Pak C18 cartridge to extract bile acids (5). This rapid procedure resulted in an almost complete removal of the bile acids from the thread; additional washings with methanol yielded only 1–2% more.

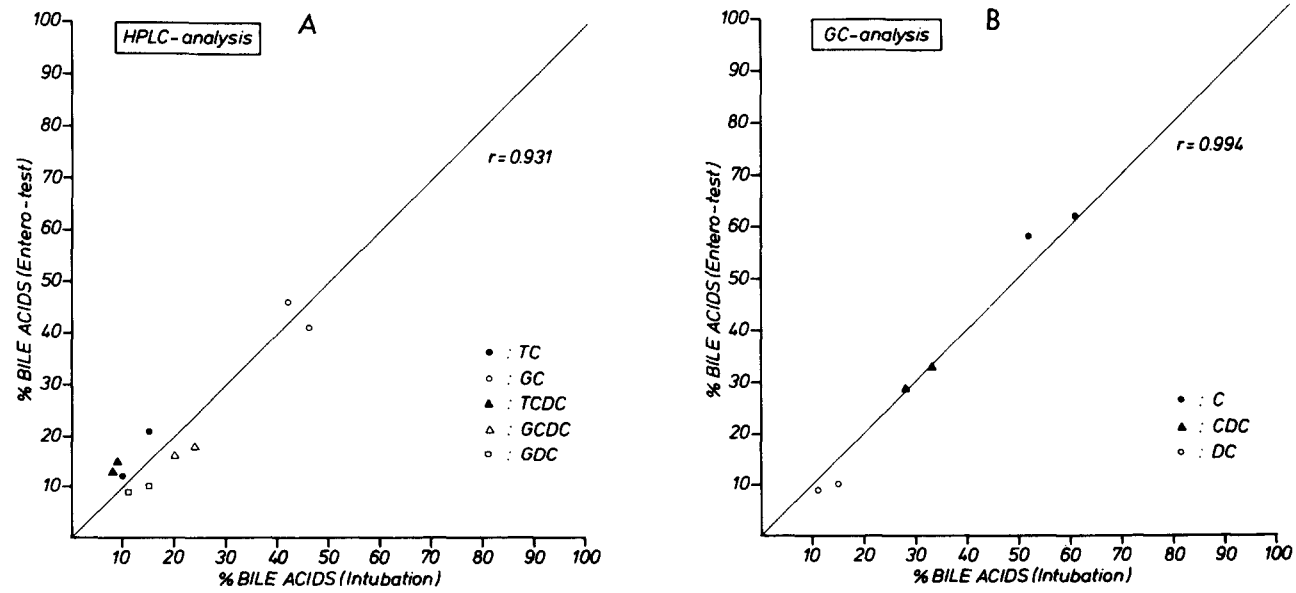

Fig. 2. Comparison of the bile acid pool composition in two healthy volunteers by means of the standard intubation technique and the Entero-Test®. Bile acids were analyzed by liquid chromatography (A) and capillary gas chromatography (B).

TABLE 1. Day to day variation in duodenal bile acid composition

Subject	Day	C	CDC	DC	LC	UDC
				%		
A	1	44	28	26	1	1
	2	38	30	29	2	1
	3	52	22	24	1	2
B	1	64	31	6		
	2	63	32	6		
	3	60	31	9		
C	1	25	46	29		
	3	27	51	22		
D	1	49	23	26	2	
	3	30	27	43		
E	1	19	53	22	7	
	3	40	38	20	2	

Day to day variation in the duodenal bile acid composition (%) in three healthy volunteers and two patients (D and E) with intermittent cholestasis (measured during anicteric periods). Entero-Test® was applied at three consecutive days (A, B) or on the first and third day (C, D, and E).

To investigate whether preferential binding of any bile acid from bile occurred, the threads were incubated with bile obtained from patients after intubation. Bile acid composition was analyzed directly as well as indirectly in five cases. The correlation coefficient between direct and indirect analyses was 0.98 for the gas–liquid chromatographic analysis (**Fig. 1**) as well as for the liquid chromatographic analysis. When the Entero-Test was used in five healthy volunteers and the bile was analyzed by liquid chromatography and gas–liquid chromatography, a correlation coefficient of 0.99 was found between the results obtained by both analytical techniques. This means that both techniques are superimposable.

In two healthy volunteers (subjects A and K), a comparison was made between the results obtained by the standard intubation technique of sampling bile and the Entero-Test (**Fig. 2**). For this purpose an Entero-Test capsule was introduced in the duodenum together with a standard duodenal tube. The correlation coefficient between the bile acid analyses of both tests was 0.99 which indicates that the same results are obtained by Entero-Test and the classical intubation technique.

The day-to-day variation in the bile acid pool composition is shown in **Table 1.** In three healthy volunteers and in two patients with intermittent cholestasis in an anicteric period (D and E), the Entero-Test was applied two or three times within a period of 3 days. Distinct variations were observed in both patients and in one of the volunteers (A), but in the other two volunteers relatively little variation occurred. The results for 11 healthy volunteers are shown in **Table 2.** The mean percentage of the various bile acids was not significantly different for males and females.

Futhermore, it was found that the ratio of glycine-conjugated bile acids to taurine-conjugated bile acids (G/T ratio) differed between the bile acid species. The G/T ratio for cholate was 1.8 ± 1.1; for chenodeoxycholate, 2.3 ± 1.4; and for deoxycholate, 5.7 ± 3.3.

DISCUSSION

The study of the bile acid pool composition in man is rather complicated. On one hand, when serum bile acid profiles are measured, only a minor part of the pool is analyzed and it is known that the various bile acids behave differently with respect to the hepatic clearance (6,

TABLE 2. Bile acid composition in duodenal juice

Subject	Sex	C	CDC	DC	LC	UDC	μmol of Bile Acid per Thread
				%			
F	F	59	26	15			3.10
G	F	45	24	31			0.52
H	F	54	28	16			3.41
I	F	22	21	53	2	1	18.90
Mean ± SD	F	47 ± 15	24 ± 3	27 ± 16	1 ± 1	1 ± 1	7.2 ± 7.4
B	M	42	30	22	6		0.34
C	M	47	37	15			0.36
J	M	28	33	39			0.73
K	M	47	31	21	1		0.63
A	M	59	24	16	1		2.54
L	M	28	42	31			1.61
M	M	41	34	25			1.27
Mean ± SD	M	42 ± 11	33 ± 6	24 ± 8	1 ± 2		1.1 ± 0.8
Mean ± SD	F + M	44 ± 12	29 ± 6	25 ± 11	1 ± 2		3.6 ± 5.5
Carey (16)		35	35	24	6		

Bile acid composition (%) in duodenal juice obtained with Entero-Test® in 11 healthy volunteers and the mean values ± SD for females, males, and the total group of participants. Values from Carey (16) are given for comparison. The total amount of bile acids attached to the thread is also indicated. C, cholic acid; CDC, chenodeoxycholic acid; DC, deoxycholic acid; LC, lithocholic acid; UDC, ursodeoxycholic acid.

7). On the other hand, sampling bile by duodenal intubation is a burdensome technique, especially for children.

We studied the possibility of using Entero-Test as a suitable alternative, a device originally developed for the diagnosis of duodenal parasites (8–10). Entero-Test has also been used for measuring intestinal microflora (11) and hemorrhage (12). While our study was in progress, the usefulness of the Entero-Test in the evaluation of cholestatic jaundice in infancy was described (13, 14). Furthermore, Whitney and Burlingame (15) used Entero-Test for determination of duodenal bile acid composition with specialized mass spectrometric techniques.

In our studies, in vitro tests revealed that no preferential binding of conjugated primary and the major conjugated secondary bile acids to the thread occurred. The pH did not influence the binding pattern, not even when a thread saturated with buffer pH 7.0 was passed through a piece of absorbent cotton with pH 2, which mimics the gastric passage after withdrawal. The mechanism by which binding occurs is probably an adsorption of fluid by the thread. Approximately 14 μl of fluid was bound to 1 cm of the thread. Only minor differences were observed when the Entero-Test was compared with the duodenal intubation technique. This means that for sampling duodenal bile acids the intubation technique can be replaced by the Entero-Test.

A remarkable daily variation in bile acid pool composition was found in two patients with intermittent cholestasis (measured during an anicteric period). The results of bile acid analysis in a group of 11 volunteers indicate that there is also a large interindividual variation in the bile acid pool composition in healthy persons. The mean values, however, confirm data from others (16). The differences in the ratio of glycine-conjugated bile acids to taurine-conjugated bile acids are remarkable. The increased ratio glycodeoxycholate/taurodeoxycholate can theoretically be explained by a more efficient conservation of glycodeoxycholate in the enterohepatic circulation compared to the tauro-conjugate. However, until now, there was a lack of experimental data on this subject.

From the presented data it is clear that the Entero-Test is a suitable device for sampling duodenal bile acids. It is an easy, non-burdensome method, also useful for children. With this device it is possible to study the influence of diet and drugs on the bile acid pool composition and, moreover, to perform longitudinal studies concerning the relation between bile acids and pathophysiology of the liver and intestine.

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REFERENCES

1. Hofmann, A. F. 1984. Chemistry and enterohepatic circulation of bile acids. *Hepatology.* **4:** 4S–14S.
2. Matern, S., and W. Gerok. 1979. Pathophysiology of the enterohepatic circulation of bile acids. *Rev. Physiol. Biochem. Pharmacol.* **85:** 125–203.
3. Setchell, K. D. R., and A. Matsui. 1983. Serum bile acid analysis. *Clin. Chim. Acta.* **128:** 1–17.
4. Ruben, A. T., and G. P. van Berge Henegouwen. 1982. A simple reverse phase high pressure liquid chromatographic determination of conjugated bile acids in serum and bile using a novel compression separation system. *Clin. Chim. Acta.* **119:** 41–50.
5. Setchell, K. D. R., and J. Worthington. 1982. A rapid method for the quantitative extraction of bile acids and their conjugates from serum using commercially available reverse-phase octadecylsilane bonded silica cartridges. *Clin. Chim. Acta.* **125:** 135–144.
6. Aldini, R., A. Roda, A. M. M. Labate, G. Cappelleri, E. Roda, and L. Barbara. 1982. Hepatic bile acid uptake: effect of conjugation, hydroxyl and keto groups, and albumin binding. *J. Lipid Res.* **23:** 1167–1173.
7. Iga, T., and C. D. Klaassen. 1982. Hepatic extraction of bile acids in rats. *Biochem. Pharmacol.* **31:** 205–209.
8. Rosenthal, P., and W. M. Liebman. 1980. Comparative study of stool examinations, duodenal aspiration, and pediatric Entero-Test for giardiasis in children. *J. Pediatr.* **96:** 278–279.
9. Bezjak, B. 1972. Evaluation of a new technique for sampling duodenal contents in parasitologic diagnosis. *Am. J. Dig. Dis.* **17:** 848–850.
10. Beal, C. B., P. Viens, R. G. L. Grant, and J. M. Hughes. 1970. A new technique for sampling duodenal contents. *Am. J. Trop. Med. Hyg.* **19:** 349–352.
11. Colon, A. R. 1976. Sampling of duodenal contents by nylon line. *J. Pediatr.* **89:** 513–514.
12. Babb, R. R., and C. B. Beal. 1974. Use of a duodenal capsule for localization of upper gastrointestinal hemorrhage. *Gut.* **15:** 492–493.
13. Rosenthal, P., W. M. Liebman, F. R. Sinatra, J. A. Perman, and M. T. Thaler. 1985. String test in evaluation of cholestatic jaundice in infancy. *J. Pediatr.* **107:** 253–255.
14. Rosenthal, P. 1985. Collection of duodenal bile in infants and children by the string test. *J. Pediatr. Gastroenterol. Nutr.* **4:** 284–285.
15. Whitney, J. O., and A. L. Burlingame. 1985. Mass spectrometric analysis of bile acids in neonatal liver disease. *In* Mass Spectrometry of Large Molecules. S. Facchetti, editor. Elsevier, Amsterdam. 185–207.
16. Carey, M. C. 1982. The enterohepatic circulation. *In* The Liver: Biology and Pathobiology. I. Arias, H. Popper, D. Schachter, and D. A. Shafritz, editors. Raven Press, New York. 429–465.