AHRENS, Jr., Anal. Chem. **31**, 307 (1959). — 4. BRO-RASMUSSEN, F. and W. HJARDE, Acta Chem. Scand. **11**, 34 (1957). — 5. BRO-RASMUSSEN, F. and W. HJARDE, Acta Chem. Scand. **11**, 44 (1957). — 6. STOWE, H. D., Arch. Biochem. Biophys. **103**, 42 (1963). — 7. BOLLIGER, H. R., in: E. STAHL, Dünnschicht-Chromatographie, p. 217–240 (Berlin 1962). — 8. DAM, H. and E. SØNDERGAARD, Acta Pharmacol. Toxicol. **9**, 131 (1953). — 9. DAM, H., S. HARTMANN, J. E. JACOBSEN and E. SØNDERGAARD, Acta Physiol. Scand. **41**, 149 (1957). — 10. RAHM, J. J., Z. SELINGER and R. T. HOLMAN, 6th Internat. Congr. Biochemistry, New York. Abstracts VII, 591 (1964). — 11. Moss, A. R. and J. C. DRUMMOND, Biochem. J. **32**, 1953 (1938). — 12. HARRIS, P. L. and M. I. LUDWIG, J. Biol. Chem. **180**, 611 (1949).

Authors' addresses:

Prof. Dr. HENRIK DAM, Ing. E. SØNDERGAARD and Ing. G. HØLMER, Department of Biochemistry and Nutrition, Polytechnic Institute, Østervoldgade 10, Copenhagen and cand. pharm. E. LEERBECK, The State Vitamin Laboratory, Blegdamsvej 21, Copenhagen (Denmark)

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen (Denmark)

Alimentary production of gallstones in hamsters

19. Composition of fistula bile from hamsters on rice starch diet

By INGE PRANGE, FREDE CHRISTENSEN and HENRIK DAM

With 2 tables

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In previous experiments (1, 2) we have examined the composition of bladder bile from hamsters reared on diets with different influence on gallstone formation in this species. The components analyzed were cholesterol, lipid-soluble phosphorus and bile acids.

In the present study we have examined the same components in fistula bile from hamsters shortly after the fistula was established and 17 to 21 hours later. The purpose was to examine the changes in composition occurring during this time interval.

In order to minimize possible influences originating from differences in absorption of fat from the intestine, a diet without added fat was used. Rice starch was chosen as carbohydrate component because previous experiments have shown that with a diet of this type the incidence of gallstones in young hamsters is low.

Experimental

The hamsters were young from our stock colony. They were placed in individual cages and reared on the experimental diet from the age of 36–41 days. The experimental diet was the previously described "rice starch diet" (1) with the modification that the salt mixture and vitamin mixture were those indicated in reference (3). (The salt mixture contains added trace elements. The vitamin mixture contains vitamins A and D in stabilized form, whereby the supply of these vitamins is simpler than in the previous study (1), where they were given separately in aqueous colloidal solution.)

Bile fistula was performed when the animals had been on the rice starch diet for a period of 40–49 days.

After the animal had fasted from the foregoing evening, laparotomy was performed under ether anesthesia. An opening was cut in the fundus of the gallbladder, and the content of the gallbladder was removed. A soft polyethylene catheter (about 0.7 mm outer diameter) was inserted into the gallbladder through the fundus. The one end of the catheter had been moulded into a flattened rim enabling the catheter to remain in position after the gallbladder had been tied around it. The ductus choledocus was ligated. The catheter was passed under the skin to the back and out through a small hole in the skin on the neck.

The first 20–25 mg of bile flowing out of the catheter were discarded. Thereafter the catheter was connected with a small glass ampule. The neck of the ampule was loosely fixed to the skin with catgut, and the animal was allowed to move around in the cage. Food and water were available all the time.

When in the course of 65–350 minutes an amount of about 400–800 mg of bile, "sample no. 1", had been collected, the ampule was shifted and the animal left in the cage until the following morning when the ampule was shifted again. The amount of bile collected over night, "the intermediate sample", was discarded after being weighed. During the following 170–325 minutes an amount of about 400–700 mg bile, "sample no. 2", was collected in a third ampule. Only samples no. 1 and 2 were analyzed.

Table 1 shows schematically the plan of collection of the bile samples.

Table 1. Plan of collection of bile samples, indicating number and weight of samples and length of time during which the samples were collected

Hamster no.	Sex *)	Days on diet	Duration of surgery **)	Time from completion of surgery to collection of sample no. 1	Sample no. 0 (discarded)	Sample no. 1		Intermediate sample (discarded)		Sample no. 2	
						weight	time	weigth	time	weight	time
			min	min	mg	mg	min	mg	min	mg	min
1250/55	m	40	20	5	c. 20	402	65	2595	1195	470	270
1250/20	m	41	20	10	c. 20	571	180	1877	1150	468	325
1250/110	f	42	15	10	c. 20	435	350	2746	1035	595	235
1251/42	m	43	15	10	c. 20	836	155	3898	1215	683	200
1251/35	m	43	20	10	c. 25	826	180	3931	1220	483	200
1251/111	m	47	20	20	c. 20	488	130	3806	1145	503	230
1251/46	m	47	20	10	c. 20	498	215	3313	1140	466	170
1251/144	m	49	25	5	c. 20	573	130	3778	1280	538	235
1251/25	f	49	20	10	c. 20	500	95	3561	1280	696	120

*) m = male; f = female.
**) Counted from beginning of the anaesthesia.

The analytical methods were the same as those used in our previous studies (1, 2) with the modification that the bile acids were determined on measured portions of bile weighed after freezedrying, whereafter the bile salts were extracted from the dry residue with alcohol and subjected to paper chromatography as previously.

Results

None of the animals had gallstones.

The results of the analyses are presented in table 2.

The time interval between the collection of bile samples no. 1 and no. 2 varied from 1035 to 1280 minutes, about 17 to 21 hours. The amount of bile secreted during the interval varied from 1877 to 3931 mg.

The changes of the data from sample no. 1 to sample no. 2 for one and the same animal were as follows:

The concentration of dry matter decreased somewhat except in two cases (nos. 1250/110 and 1251/46) where it remained constant[1]).

The concentration of total bile acids decreased except in one case (no. 1251/46) where it increased and was low already in sample no. 1.

The concentration of cholesterol decreased in 6 cases. In the other 3 cases (nos. 1250/110, 1251/42 and 1251/46) it increased and was low already in sample no. 1.

The concentration of lipid-soluble phosphorus increased in 5 cases, and decreased in the other 4 cases (nos. 1250/55, 1250/20, 1251/111 and 1251/25).

The ratio between total bile acids and cholesterol decreased, but in one case (no. 1251/46) the decrease was only slight.

The ratio between lipid-soluble phosphorus and cholesterol increased except in one case (no. 1251/42) where it remained unchanged, and in another case (no. 1251/46) where it showed a small decrease.

The ratio between total bile acids and lipid-soluble phosphorus decreased except in one case (no. 1251/46) where it increased slightly.

The ratio between glycine conjugation and taurine conjugation (G/T) decreased except in one case (no. 1250/20) where it remained unchanged and was low already in sample no. 1.

The ratio between dihydroxycholanic acids and trihydroxycholanic acids (Di/Tri) decreased except in one case (no. 1250/55) where it increased.

Discussion

Comparison of the present data with those found for bladder bile in our previous study (1) shows the following:

For samples no. 1, the individual values for total bile acids and the ratio G/T are lower than any of the values for these data previously found for bladder bile of hamsters on rice starch diet. With one exception, this also holds for the values of the ratio between total bile acids and lipid-soluble phosphorus, and with a few exceptions for the ratio between total bile acids and cholesterol.

For samples no. 2, the values for the above mentioned data, without exception, are lower than the corresponding data found for bladder bile of hamsters on rice starch diet, and generally the differences are more pronounced.

Comparison with the data for bladder bile of hamsters fed the "fat-free glucose diet", which produces a high incidence of cholesterol gallstones, shows that for samples no. 2, the values for the ratio between total bile acids and cholesterol occur in the range within which the corresponding data for bladder bile of hamsters on "fat-free glucose diet" were found, but the ratios between total bile acids and lipid-soluble phosphorus are much lower for samples no. 2 than for bladder bile of hamsters fed the "fat-free glucose diet".

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[1]) For three animals (nos. 1250/110, 1251/46 and 1251/25), the flow of bile (mg bile per minute) was slower during collection of sample no. 1 than during collection of sample no. 2. But only for the two first mentioned animals the secretion of dry matter (micrograms of dry matter per minute) was slower for sample no. 1 than for sample no. 2.

Table 2. Analytical data of fistula bile from hamsters

Hamster no.	Sex	Sample no.	Dry matter %	Cholesterol mM	Lipid P mM	GC mM	GCD mM	GD mM	TC mM	TCD + TD mM	Total bile acids mM
1250/55	m	1	3.1	0.67	2.1	5.8	0.8	1.6	4.2	1.9	14.3
		2	2.0	0.19	1.3	0.2	0.2	0.04	0.8	0.5	1.7
1250/20	m	1	2.9	0.96	2.7	0.6	0.01	0.3	3.5	1.9	6.3
		2	2.3	0.49	1.8	0.2	0.07	0.02	1.2	0.4	1.9
1250/110	f	1	2.1	0.49	1.9	1.5	0.3	0.7	1.7	1.0	5.2
		2	2.0	0.51	2.9	0.9	0.2	0.2	2.9	0.4	4.6
1251/42	m	1	2.7	0.28	1.4	1.5	0.9	0.6	0.9	0.6	4.5
		2	1.8	0.38	1.9	0.2	0	0	2.2	0.3	2.7
1251/35	m	1	2.8	0.77	2.1	3.4	1.9	0.9	2.1	1.6	9.9
		2	2.2	0.56	2.6	0.3	0.2	0.4	4.1	0.7	5.7
1251/111	m	1	2.9	0.81	2.5	5.8	0.6	1.4	2.1	2.4	12.3
		2	1.9	0.40	2.2	0.4	0.8	0.1	2.2	0.5	4.0
1251/46	m	1	1.9	0.22	1.2	0.8	1.1	0.1	1.4	0.3	3.7
		2	1.94	0,33	1.7	0.3	0.5	0.2	3.9	0.3	5.2
1251/144	m	1	3.1	0.44	1.9	5.4	0.5	0.9	2.9	1.2	10.9
		2	2.0	0.37	2.0	0.3	0.1	0.2	3.0	0.5	4.1
1251/25	f	1	3.9	0.60	2.6	4.1	0.5	1.6	2.3	7.1	15.6
		2	2.1	0.28	1.4	0.3	0.2	0.03	1.3	1.1	2.9

*) GC = Glycocholic acid
GCD = Glycochenodeoxycholic acid
GD = Glycodeoxycholic acid
TC = Taurocholic acid
TCD + TD = Taurochenodeoxycholic acid + taurodeoxycholic acid

It is likely that the values for the ratio between total bile acids and cholesterol prevailing in samples no. 2 will favor precipitation of cholesterol when the bile is concentrated, but it is not known how the lower values for the ratio between total bile acids and lipid-soluble phosphorus and between glycine and taurine conjugation will influence the solubility of cholesterol.

The interruption of the supply of reabsorbed bile acids from the intestine to the liver seems to afford a simple explanation of the tendency of the ratios between total bile acids and cholesterol and between total bile acids and lipid-soluble phosphorus to decrease from samples no. 1 to samples no. 2.

The decrease of the ratio Di/Tri might be thought to be due to the interrupted supply of deoxycholic acid from intestine to liver. It is not possible, however, to find support for this assumption in the available data, since taurochenodeoxycholic acid could not be separated from taurodeoxycholic acid by the method used, and the data for glycochenodeoxycholic acid and glycodeoxycholic acid show a predominant decrease of the latter acid in only 5 of the 9 cases.

An explanation of the tendency of the ratio G/T to decline is not immediately on hand.

on rice starch diet without added fat*).

G mM	T mM	Di mM	Tri mM	G/T mM	Di/Tri	Total bile acids/cholesterol	Lipid P/cholesterol	Total bile acids/Lipid P
8.2	6.1	4.3	10.0	1.3	0.4	21.3	3.1	6.8
0.4	1.3	0.7	1.0	0.3	0.7	8.9	6.8	1.3
0.9	5.4	2.2	4.1	0.2	0.5	6.6	2.8	2.3
0.3	1.6	0.5	1.4	0.2	0.4	3.5	3.7	0.9
2.5	2.7	2.0	3.2	0.9	0.3	10.6	3.9	2.7
1.3	3.3	0.8	3.8	0.4	0.2	8.6	5.7	1.5
3.0	1.5	2.1	2.4	2.0	0.9	16.1	5.0	3.2
0.2	2.5	0.3	2.4	0.8	0.1	7.1	5.0	1.4
6.2	3.7	4.4	5.5	1.7	0.8	12.9	2.7	4.7
0.9	4.8	1.3	4.4	0.2	0.3	10.2	4.6	2.2
7.8	4.5	4.4	7.9	1.7	0.6	15.2	3.1	4.9
1.3	2.7	1.4	2.6	0.5	0.5	10.0	5.5	1.8
2.0	1.7	1.5	2.2	1.1	0.6	16.8	5.5	3.1
1.0	4.2	1.0	4.2	0.2	0.2	15.7	5.2	3.3
6.8	4.1	2.8	8.3	1.7	0.3	24.8	4.3	5.7
0.6	3.5	0.8	3.3	0.2	0.2	11.1	5.4	2.1
6.2	9.4	9.2	6.4	0.7	1.5	26.0	4.3	6.0
0.5	2.4	1.3	1.6	0.2	0.8	10.3	5.0	2.1

G = GC + GCD + GD
T = TC + TCD + TD
Total bile acids = G + T
Di = Dihydroxycholanic acids = GCD + GD + TCD + TD
Tri = Trihydroxycholanic acids = GC + TC

It is possible that the results of the present studies would have been more uniform, if interruption of the enterohepatic circulation could have been postponed until some days after the operation as in the studies of PERIC GOLIA and JONES (4, 5) on bile acids in guinea pigs, and in the studies of THUREBORN (6) on cholelithiasis in man. For practical reasons this could not be done in the present experiments.

Our results agree largely with those of THUREBORN as far as the decrease of the ratio between total bile acids and cholesterol is concerned. But in our study, the ratio between lipidsoluble phosphorus and cholesterol tended to increase, whereas THUREBORN found decrease of this ratio. The ratios G/T and Di/Tri did not show a regular variation in THUREBORN's study, whereas in our studies these ratios tended to decrease. Whether these differences are due to species characteristics, to differences in diet or to other causes is not known.

Summary

Young hamsters were reared on an artificial diet with rice starch as carbohydrate and without added fat.

Bile fistula was performed under ether anesthesia when the animals had been on the diet for 40–49 days.

The content of the gallbladder and the first 20–25 mg of bile flowing out of the catheter were discarded.

The following 400–800 mg of bile, designated as sample no. 1, and a similar quantity of bile, designated as sample no. 2, collected after a time interval of 17–21 hours, were weighed and analyzed, whereas the bile collected during the time interval was discarded after being weighed.

Total solids, cholesterol, lipid-soluble phosphorus and bile acids were determined in the samples from 9 animals.

The prevailing changes from sample no. 1 to sample no. 2 were:

Decrease of the concentrations of total solids and of total bile acids, decrease of the ratios between total bile acids and cholesterol, between total bile acids and lipid-soluble phosphorus, between glycine conjugated bile acids and taurine conjugated bile acids, and between dihydroxycholanic acids and trihydroxycholanic acids; increase of the ratio between lipid-soluble phosphorus and cholesterol.

Zusammenfassung

Junge Hamster wurden mit einer künstlichen Nahrung ohne Fettzusatz und mit Reisstärke als Kohlenhydrat gefüttert.

Nach 40 bis 49 Tagen wurde, unter Äthernarkose, eine Gallenfistel angelegt.

Der Inhalt der Gallenblase und die ersten 20–25 mg der aus dem Katheter ausfließenden Galle wurden entfernt aber nicht analysiert. Die darauffolgenden 400–800 mg Galle wurden gesammelt und als Probe Nummer 1 bezeichnet. Nach einem Zeitintervall von 17–21 Stunden wurde eine ähnliche Menge von Galle gesammelt und als Probe Nummer 2 bezeichnet. Beide Gallenproben 1 und 2 wurden gewogen und analysiert, während die in dem Zeitintervall gesammelte Galle nur gewogen wurde.

Gesamttrockensubstanz, Cholesterin, lipid-löslicher Phosphor und Gallensäuren wurden in den Gallenproben von 9 Tieren bestimmt.

Die vorherrschenden Veränderungen von Probe Nummer 1 zu Probe Nummer 2 waren:

Abnahme der Konzentration von Trockensubstanz und von Gesamtgallensäuren, Abnahme der Verhältnisse zwischen Gesamtgallensäuren und Cholesterin, zwischen Gesamtgallensäuren und lipid-löslichem Phosphor, zwischen glycin-konjugierten Gallensäuren und taurin-konjugierten Gallensäuren und zwischen Dihydroxycholansäuren und Trihydroxycholansäuren; Zunahme des Verhältnisses zwischen lipid-löslichem Phosphor und Cholesterin.

References

1. Prange, I., F. Christensen and H. Dam, Z. Ernährungswiss. **3**, 59–78 (1962). — 2. Prange, I., F. Christensen and H. Dam, Z. Ernährungswiss. **4**, 193–207 (1964). — 3. Dam, H., I. Prange and F. Christensen, Z. Ernährungswiss. **6**, 97–106 (1965). — 4. Peric Golia, L. and R. S. Jones, Proc. Soc. Exp. Biol. Med. **105**, 337–339 (1960). — 5. Peric Golia, L. and R. S. Jones. Proc. Soc. Exp. Biol. Med. **106**, 177–180 (1961). — 6. Thureborn, E., Human Hepatic Bile, Composition Changes Due to Altered Hepatic Circulation. Monograph. Supplement 303 to Acta Chirurg. Scand. (Stockholm 1962).

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Author's address:

Prof. Dr. Henrik Dam, Department of Biochemistry and Nutrition, Polytechnic Institute, Østervoldgade 10 III, Copenhagen (Denmark)