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The Composition of Bladder bile of Chicks Reared on Six Different Diets*)

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With 7 tables

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Young hamsters reared on a fat-free diet having an easily absorbable sugar as carbohydrate component develop cholesterol gallstones (1, 2).

The composition of the bladder bile of hamsters reared on such a diet (with glucose) has been examined with respect to cholesterol, lipid phosphorus, bile acids and pH and compared with the composition of the bladder bile of hamsters reared on diets affording complete or partial protection against gallstones in young individuals of this species (2).

Chicks do not develop gallstones when fed the "fat-free glucose diet" which causes formation of cholesterol gallstones in young hamsters.

The purpose of the present study was to examine the bladder bile of chicks reared on various diets, viz. 1. the "fat-free glucose diet", 2. a similar diet with rice starch instead of glucose, 3. a diet containing 5% cod liver oil, 4. a diet containing 5% hydrogenated palm kernel oil instead of the corresponding amount of glucose, 5. a diet in which the protein was represented by 15% casein + 8% gelatin instead of the usual 20% casein, and 6. a normal commercial ration for growing chicks.

Experimental

Six groups, each of 8 day-old male chicks (New Hampshire × White Leghorn) were used. They received for 3 days the stock diet (3) used in our hamster colony. Groups 1–5 were then given the experimental diets listed in table 1, whereas group 6 was given a normal commercial ration for growing chicks. The diets and tap water were available *ad libitum*.

After 42 days the chicks were sacrificed by decapitation at weekly intervals, one chick from each group on the same day, except when chicks died accidentially during the feeding period (chicks that died were autopsied, but their bile was not analyzed). Food but not water was removed about 16h before the chicks were sacrificed. Immediately after sacrifice autopsy was performed, the content of the gallbladder inspected and samples of bile taken out for analysis as in the experiments with hamsters (2). It was not necessary to pool bile from several chicks. The bile acid analyses were limited to taurine conjugates except in a few cases, including samples of bile from group 5, in which chromatograms for glycine conjugates were also carried out; no glycine conjugates were found.

The bile of all the chicks had a normal green color. In the chromatogram for taurine conjugated bile acids, biliverdin had the same R_f value as the dihydroxycholanic acids. By variation of the proportion between the components of the mobile phase the green biliverdin spot could be shifted to cover the taurocholic acid spot instead of the spot for taurodihydroxycholanic acids. It was thereby found that the overlapping with biliverdin had no influence on the results.

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Table 1. Experimental diets (g)

	Group 1	Group 2	Group 3	Group 4	Group 5
Casein, crude[1])	200	200	200	200	150
Gelatin					80
Glucose	743		693	693	713
Rice starch		743			
Salt mixture (U.S.P. XIII no. 2)	50	50	50	50	50
Vitamin mixture[2])	5	5	5	5	5
Choline chloride	2	2	2	2	2
Cod liver oil			50		
Hydrogenated palm kernel oil (mp 40–42 °C)				50	

[1]) From Dansk Mejeri Industri & Export Kompagni, Stege, Denmark.
[2]) See reference 1.
The diets were supplemented with 50 mg dl-α-tocopherol acetate (Ephynal, Roche) per kg. Vitamins A and D were given as an aqueous solution, cf. reference 1.

Results and Discussion

Tables 2–7 show the results of the bladder bile analyses and the weights of the individual chicks at the beginning and end of the experimental feeding[1]).

None of the chicks had gallstones.

As shown by the figures for initial and final weights of chicks, the diets used for groups 1–5 are not well suited for growth of chicks. The chicks receiving the commercial chicken mash grew at a normal rate.

The bile acids of chicks are present as taurine conjugates only (4, 5). The major component is chenodeoxycholic acid (6, 7). Cholic acid also occurs (8), but in much smaller amount. The system for chromatography of taurine conjugated bile acids used in our study separates cholic acid from dihydroxycholanic acid but does not separate individual dihydroxycholanic acids from each other. The latter are therefore recorded merely as "dihydroxycholanic acids", even though this fraction mostly consists of chenodeoxycholic acid.

The analytical data for bladder bile of chicks on the "fat-free glucose diet" (table 2) are to be compared with the corresponding data for young hamsters on the same diet shown in tables 4 and 5 in our previous communication (2). The figures for cholesterol are of the same order of magnitude in the two species but seem to be more scattered in the chicks than in the hamsters. However, this may in some measure be due to the fact that the data for the chicks refer to individual animals, whereas most of the data for hamsters are obtained with pooled samples of bile from two animals.

Lipid phosphorus and the ratio lipid phosphorus:cholesterol are higher in the chicks than in the hamsters.

Total bile acids and the ratio total bile acids:cholesterol are also higher (about 7 times) in chicks than in hamsters.

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[1]) The average figures for the components of the bile are to be considered with the following reservation: In group 4 there is a continuous rise in cholesterol during the experimental period, and in some of the groups, e. g., nos. 1 and 5, a (less pronounced) tendency for lipid phosphorus to be higher at the beginning of the experiment than at the end.

Table 2.

Analytical data of bladder bile (millimolarities), initial and final body weights (g) of chicks in group 1 ("fat-free glucose diet")

Chick No.	Days on diet	Cholesterol	Lipid phosphorus	Total bile acids	Taurocholic acid	Taurodi-hydroxy-cholanic acids	Ratio dihydroxy-: trihydroxy-cholanic acids	Ratio total bile acids: cholesterol	Ratio lipid phosphorus: cholesterol	pH	Body weight initial	Body weight final
4014	42	4.99	66.8	437.6	11.62	426.0	36.7	87.6	13.4		50	241
4012	49	5.57	75.2	448.3	16.28	432.0	26.5	80.5	13.5		52	182
4011	56	8.16	76.5	334.3	24.65	309.6	12.6	40.8	9.4		45	161
4008	63	1.57	13.9	447.3	29.28	418.0	14.3	248.0	8.9		54	95
4009	70	2.18	27.4	434.2	12.22	422.0	34.5	199.0	12.6		44	234
4013	77	0.75	32.3	354.8	6.98	347.8	49.8	472.0	43.0	6.3	49	232
4010	84	4.42	30.3	310.5	15.12	295.4	19.5	70.2	6.9	6.3	55	141
		3.95^{7}	40.1^{7}	395.3^{7}	16.59^{7}	378.6^{7}	31.5^{7}	176.3^{7}	15.4^{7}			

Table 3.

Analytical data of bladder bile (millimolarities), initial and final body weights (g) of chicks in group 2 ("fat-free rice starch diet")

Chick No.	Days on diet	Cholesterol	Lipid phosphorus	Total bile acids	Taurocholic acid	Taurodi-hydroxy-cholanic acids	Ratio dihydroxy-: trihydroxy-cholanic acids	Ratio total bile acids: cholesterol	Ratio lipid phosphorus: cholesterol	pH	Body weight initial	Body weight final
4015	42	1.28	48.7	315.5	39.1	276.4	7.1	246.0	38.1		50	220
4016	49	2.18	32.4	192.8	58.2	134.6	2.3	88.5	14.9		44	172
4019	56	4.00	49.4	420.5	50.7	369.8	7.3	104.5	12.3		51	140
4021	63	6.53	88.4	357.0	43.8	313.2	7.2	54.5	13.6		51	149
4020	70	2.75	40.7	408.2	19.8	388.4	19.6	148.2	14.8		47	144
4018	77	5.21	55.8	410.0	12.0	398.0	33.2	78.8	10.7	5.8	55	132
4022	84	1.35	47.2	420.3	40.3	380.0	9.4	311.0	34.9	5.7	51	121
4017	91	4.66	24.0	328.7	17.1	311.6	18.2	70.6	5.2	6.1	47	126
		3.49^{8}	48.3^{8}	356.6^{8}	35.1^{8}	321.5^{8}	13.0^{8}	137.8^{8}	18.1^{8}			

Table 4.

Analytical data of bladder bile (millimolarities), initial and final body weigths (g) of chicks in group 3 (the "glucose diet" with 5% cod liver oil)

Chick No.	Days on diet	Cholesterol	Lipid phosphorus	Total bile acids	Taurocholic acid	Taurodi-hydroxy-cholanic acids	Ratio dihydroxy-: trihydroxy-cholanic acids	Ratio total bile acids: cholesterol	Ratio lipid phosphorus: cholesterol	pH	Body weight initial	Body weight final
4029	42	2.92	88.1	344.9	20.70	324.2	15.7	118.0	30.2		46	190
4030	49	2.68	63.8	357.3	9.32	348.0	37.3	133.3	23.8		47	195
4028	56	2.88	43.8	415.0	41.40	373.6	9.0	144.0	15.2		49	122
4026	63	7.58	104.8	386.3	34.90	351.4	10.1	51.0	13.8		49	183
4024	70	5.59	98.0	321.2	12.22	309.0	25.3	57.4	17.5		53	205
4027	77	2.99	58.5	283.6	50.00	233.6	4.7	94.5	19.5	5.8	50	141
		4.11[6]	76.2[6]	351.4[6]	28.09[6]	323.3[6]	17.0[6]	99.7[6]	20.0[6]			

Table 5.

Analytical data of bladder bile (millimolarities), initial and final body weights (g) of chicks in group 4 (the "glucose diet" with 5% hydrogenated palm kernel oil)

Chick No.	Days on diet	Cholesterol	Lipid phosphorus	Total bile acids	Taurocholic acid	Taurodi-hydroxy-cholanic acids	Ratio dihydroxy-: trihydroxy-cholanic acids	Ratio total bile acids: cholesterol	Ratio lipid phosphorus: cholesterol	pH	Body weight initial	Body weight final
4035	42	1.71	96.5	376.5	16.48	360.0	21.8	220.0	56.4		53	245
4033	49	2.03	55.7	387.5	29.10	358.4	12.3	190.5	27.4		47	192
4032	56	2.95	94.5	361.8	26.80	335.0	12.5	122.5	32.0		52	187
4037	63	3.15	111.0	397.6	14.19	383.4	27.0	126.0	35.2		51	152
4038	70	4.14	107.2	358.2	24.60	333.6	13.6	86.5	25.9		50	184
4034	77	6.21	70.5	363.5	9.50	354.0	37.3	58.5	11.4	5.7	53	167
		3.37[6]	89.2[6]	374.2[6]	20.11[6]	354.0[6]	20.8[6]	134.0[6]	31.4[6]			

Table 6.

Analytical data of bladder bile (millimolarities), initial and final body weights (g) of chicks in group 5 (the diet with 15% casein and 8% gelatin)

Chick No.	Days on diet	Cholesterol	Lipid phosphorus	Total bile acids	Taurocholic acid	Taurodihydroxycholanic acids	Ratio dihydroxy-: trihydroxy-cholanic acids	Ratio total bile acids: cholesterol	Ratio lipid phosphorus: cholesterol	pH	Body weight	
											initial	final
4045	42	3.34	87.3	323.5	34.10	289.4	8.5	96.8	26.2		51	170
4042	49	3.32	78.6	279.5	68.90	210.6	3.1	84.3	23.7		54	217
4039	56	5.15	74.6	391.9	46.10	345.8	7.5	76.1	14.5		44	238
4046	63	3.04	86.7	226.3	26.50	199.8	7.5	74.4	28.5		57	277
4044	70	2.76	60.0	326.0	99.40	226.6	2.3	118.1	21.8		49	324
4040	77	3.04	41.6	364.6	72.40	292.3	4.0	120.0	13.7	6.0	43	223
4041	84	2.69	34.3	324.1	40.50	283.6	7.0	120.5	12.8	6.2	47	334
4043	91	3.26	23.0						7.1	6.1	50	245
		3.33^{8}	60.8^{8}	319.4^{7}	55.41^{7}	264.0^{7}	5.7^{7}	98.6^{7}	18.5^{8}			

Table 7.

Analytical data of bladder bile (millimolarities), initial and final body weights (g) of chicks in group 6 (reared on a normal commercial chicken ration)

Chick No.	Days on diet	Cholesterol	Lipid phosphorus	Total bile acids	Taurocholic acid	Taurodihydroxycholanic acids	Ratio dihydroxy-: trihydroxy-cholanic acids	Ratio total bile acids: cholesterol	Ratio lipid phosphorus: cholesterol	pH	Body weight	
											initial	final
4049	42	1.59	70.8	334.1	82.50	251.6	3.1	210.0	44.5		51	770
4053	49	7.83	74.0	267.7	64.70	203.0	3.1	34.4	9.5		64	880
4050	56	2.62	64.8	368.0	66.00	302.0	4.6	141.0	24.7		54	1020
4048	63	1.25	49.7	326.7	50.70	276.0	5.4	261.0	39.8		65	1220
4055	84	3.71	48.3	346.7	117.50	229.2	2.0	93.6	13.1	6.3	47	1610
4141	84	0.82	34.2	398.2	44.20	354.0	8.0	485.0	41.7	6.5	ca. 49	1530
4052	89	2.71	65.6	430.8	40.80	390.0	9.6	158.6	24.2	6.3	42	1320
		2.93^{7}	58.2^{7}	353.2^{7}	66.63^{7}	286.5^{7}	5.1^{7}	197.6^{7}	28.2^{7}			

The ratio dihydroxycholanic:trihydroxycholanic acids is about 30 times as high in the chicks as in the hamsters.

These differences parallel the differences between the two species with respect to gallstone formation, i. e., the marked tendency to formation of cholesterol gallstones in young hamsters on the "fat-free glucose diet" and the absence of gallstones in chicks.

In the cases where pH was determined, the values were lower than previously found for hamsters. This seems to hold for chicks on all the diets in the present experiment.

The chicks on the "fat-free rice starch diet" do not show a higher content of total bile acids or a higher ratio total bile acids:cholesterol than the chicks on the "fat-free glucose diet", such as it might have been expected from the corresponding data in the hamster experiment [tables 5 and 9 in our previous communication (2)]. The most marked difference between the chicks on these two diets is the lower ratio dihydroxy-:trihydroxycholanic acids in the "rice starch diet".

Compared with the "fat-free glucose diet", the two diets containing 5% of fat have caused an increase in the content of lipid phosphorus of the bladder bile, but they have not increased the total amount of bile acids. Cod liver oil has not lowered the cholesterol concentration. In the group fed hydrogenated palm kernel oil, the cholesterol concentrations begin (at 42 days) with low values and seem to increase steadily throughout the feeding period.

The diet in which the protein is represented by 15% casein and 8% gelatin instead of 20% casein seems to have caused less scattering of the cholesterol values. The ratio dihydroxy-:trihydroxycholanic acids is lower than in the group receiving the "fat-free glucose diet" with 20% casein. In two cases (chicks nos. 4042 and 4044) chromatography for glycine conjugated bile acids was carried out, but no such conjugates were found. Thus, the supply of glycine through the gelatin does not seem to have altered the conjugation.

When, finally, the normal commercial chicken diet is compared with the "fat-free glucose diet", it is apparent that the cholesterol values of chicks on the normal diet are lower except for one animal (no. 4053), which behaved differently from the others by having a much higher value for cholesterol and a somewhat lower value for total bile acids than the other chicks in the group.

The ratio lipid phosphorus:cholesterol is higher and the ratio dihydroxy-:trihydroxycholanic acids lower on the normal chicken diet than on the "fat-free glucose diet".

Generally, the chicks on all the diets examined show higher values for the ratios lipid phosphorus:cholesterol, total bile acids:cholesterol and dihydroxy-:trihydroxycholanic acids than the hamsters on the "fat-free glucose diet". These ratios are, generally, even higher than those for the hamsters on the "curative diet".

This circumstance tends to stabilize the solution of cholesterol in the bile of the chicks. The lower pH may to some extent act in the opposite direction. However, the final result is a much better stabilization of the chicken bile, in which formation of gallstones has never been observed.

The results strengthen the assumption that the occurrence of cholesterol gallstones in young hamsters reared on the "fat-free glucose diet" is due largely to the low ratios lipid phosphorus:cholesterol and total bile acids:cholesterol, although the influence of additional factors cannot be excluded.

Summary

Cholesterol, lipid phosphorus, bile acids and, in some cases, p_H were determined in the bladder bile of young chicks reared for maximally 91 days on the following diets:

1. "A fat-free" diet in which the protein was furnished as casein (20%) and the carbohydrate as glucose (74.3%). (This diet causes formation of cholesterol gallstones when fed to young hamsters.)

2. A diet similar to 1., but with rice starch instead of glucose. (This diet largely protects against gallstone formation in young hamsters.)

3. A diet similar to 1. in which 5% cod liver oil replaced 5% glucose.

4. A diet similar to 1. ind which 5% hydrogenated palm kernel oil replaced 5% glucose.

5. A diet similar to 1. but containing 15% casein, 8% gelatin and 71,3% glucose.

6. A normal commercial ration for growing chicks.

Compared with the composition of bladder bile of young hamsters reared on the cholesterol gallstone-producing diet, the chicks in all groups had much higher values for the ratios total bile acids:cholesterol and dihydroxy-:trihydroxycholanic acids and also a higher value for the ratio lipid phosphorus:cholesterol, whereby the absence of gallstone formation in the chicks apparently is explained. Replacement of glucose by rice starch did not increase the ratio total bile acids:cholesterol, such as it was found in hamsters. Introduction of 5% cod liver oil or hydrogenated palm kernel oil caused an increase of lipid phosphorus. On the diet with hydrogenated palm kernel oil, the cholesterol values seemed to increase and the ratio total bile acids: cholesterol to decrease during the feeding period. The diet with 15% casein and 8% gelatin did not cause formation of glycine conjugated bile acids.

In spite of the fact that the chicks on the artificial diets 1–5 did not grow at a normal rate the composition of their bladder bile was not extremely different from that of the chicks on the commercial chicken ration. The order of magnitude of the cholesterol content was about the same as in hamsters.

Bladder bile p_H was somewhat lower in the chicks than in the hamsters.

Zusammenfassung

Gruppen von Küken wurden von einem Alter von 3 Tagen an während maximal 91 Tagen mit 6 verschiedenen Diäten gefüttert, und ihre Blasengalle auf Cholesterin, Lipid-Phosphor, Gallensäuren und in einigen Fällen p_H analysiert.

Die Diäten waren:

Für Gruppe 1: eine künstliche fettfreie Nahrung mit Kasein (20%) als Protein-Komponente und Glukose (74.3%) als Kohlenhydrat-Komponente. Diese Nahrung ruft bei jungen Hamstern Cholesterin-Gallensteine hervor.

Für Gruppe 2: Eine Nahrung von derselben Zusammensetzung aber mit Reisstärke anstatt Glukose als Kohlenhydrat-Komponente. Diese Nahrung schützt junge Hamster weitgehend gegen Cholesterin-Gallenstein-Bildung.

Für Gruppe 3: Eine Nahrung wie für Gruppe 1, in welcher 5% Dorschlebertran anstatt der gleichen Menge Glukose eingemischt war.

Für Gruppe 4: Eine Nahrung wie für Gruppe 1, in welcher 5% gehärtetes Palmenkernöl anstatt der gleichen Menge Glukose eingemischt war.

Für Gruppe 5: Eine Nahrung wie für Gruppe 1 mit der Veränderung, daß die Protein-Komponente aus Kasein (15%) und Gelatine (8%) bestand.

Für Gruppe 6: Ein normales, handelsübliches Futtergemisch für wachsende Küken. Gallensteine kamen in keiner der Gruppen vor.

Verglichen mit der (früher mitgeteilten) Zusammensetzung der Blasengalle von jungen Hamstern, welche mit der Cholesterin-Gallensteine hervorrufenden Nahrung gefüttert wurden, zeigten sämtliche Gruppen von Küken viel höhere Werte der Verhältnisse Gesamt-

Gallensäuren: Cholesterin und Dihydroxycholansäuren: Trihydroxycholansäuren und auch höhere Werte des Verhältnisses Lipid Phosphor: Cholesterin, wodurch das Ausbleiben der Cholesterin-Gallenstein-Bildung bei Küken anscheinend erklärt ist.

Der Austausch von Glukose mit Reisstärke (Gruppe 2) führte nicht zu Erhöhung des Verhältnisses Gesamt-Gallensäuren: Cholesterin wie in früheren Versuchen mit Hamstern.

Einführung von 5% Fett (Gruppe 3 und 4) erhöhte den Gehalt an Lipid-Phosphor.

In Gruppe 4 (gehärtetes Palmenkernöl) stieg der Cholesteringehalt anscheinend während der Fütterungsperiode an, während das Verhältnis Gesamt-Gallensäuren: Cholesterin abnahm.

Die gelatinehaltige Nahrung (Gruppe 5) führte nicht zur Bildung von Glyzin-konjugierten Gallensäuren.

Die Küken der Gruppen 1–5 wuchsen bedeutend schlechter als diejenigen der Gruppe 6, welche die normale Futtermischung erhielten. Nichts destoweniger waren die Abweichungen in der Zusammensetzung ihrer Blasengalle von der der normal-wachsenden Küken nicht extrem.

Das p_H der Blasengalle war etwas niedriger bei den Küken als bei den Hamstern.

References

1. Dam, H. and Christensen, F., Z. Ernährungswiss. 2, 154 (1962). — 2. Prange, I., Christensen, F. and Dam, H., Z. Ernährungswiss., 3, 59 (1962). — 3. Dam, H. and Christensen, F., Z. Ernährungswiss. 2, 36 (1961). — 4. Haslewood, G. A. D. and Sjövall, J., Biochem. J. 57, 126 (1954). — 5. Anderson, J. G., Haslewood, G. A. D. and Wotton, J. D. P., Biochem. J. 67, 323 (1957). — 6. Windaus, A., Bohne, A. and Schwarzkopf, E., Z. physiol. Chem. 140, 167 (1924). — 7. Windaus, A. and van Schoor, A., Z. physiol. Chem. 157, 177 (1926). — 8. Yamasaki, K., J. Biochem. (Tokyo) 18, 323 (1933).

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Zur Frage möglicher Beeinträchtigung der ernährungsphysiologischen Eigenschaften von Erdnußöl durch längeres Erhitzen

Von Karl Bernhard und Heribert Wagner

Mit 3 Tabellen

(Eingegangen am 23. September 1962)

Von verschiedener Seite wurden Beeinflussung von Wachstum und Gesundheit von Versuchstieren (zumeist Ratten) beobachtet, denen auf hohe Temperaturen erhitzte Fette oder Öle mit einer normalen Diät verfüttert wurden. Die Art des Erhitzens ist dabei nicht gleichgültig und kann zu nachweisbaren strukturellen Veränderungen führen, welche die ernährungsphysiologischen Eigenschaften eines so behandelten Produktes herabsetzen und als Ursache im Tierversuch beobachtbarer Schädigungen aufzufassen sind (1–3).

Das längere Erhitzen von Fetten in Gegenwart von Sauerstoff praktizieren zahlreiche Gaststätten. Es schien angezeigt, durch einige Versuche zu einer Ab-