Original Communications

Bile Acid Excretion in Man Following Administration of L3:5:3′ Triiodothyronine

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THE tendency toward an inverse relationship between the degree of thyroid activity and the serum cholesterol level was first reported by Epstein and Lande.[1] The extremely high cholesterol levels in myxedema have been of particular interest, and numerous studies have been concerned with the means whereby cholesterol metabolism is altered in response to thyroid activity. The conversion of cholesterol to bile acid in the dog was first shown by Bloch, Berg and Rittenberg,[2] while Siperstein and Murray[3] subsequently showed that in man a considerable portion of labeled cholesterol was recovered as bile acid in the bile.

METHODS

The present study was planned to investigate the effect of a thyroid hormone upon the excretion pattern of bile acid in man. L3:5:3′-tri-iodothyronine (Cytomel®) was employed because earlier studies had shown that its metabolic effects became evident a few hours after administration. Dosage varied from 200 to 500 μg; and the drug was given both orally and intravenously. Bile was collected either from postoperative T tube drainage following cholecystectomy or, in intact patients, by duodenal intubation. (In the latter instance, the gallbladder was stimulated to contract by instillation of a saturated solution of magnesium sulfate.) Intubations were made with the patients in the fasting state, and the first specimens obtained in the morning usually showed bile with the physical characteristics of a concentrated overnight accumulation. The patients were permitted clear liquids throughout the day and a little food at noon. The specimens taken in the late afternoon were obtained prior to supper.

Bile was stored until use at −20°c. It was subjected to alkaline hydrolysis at 121°c. (17 pounds per square inch) for a period of fifteen hours. Chromatography was then performed on Celite columns by the method of Matschiner *et al.*[4] The individual bile acids were determined spectrophotometrically and colorimetrically by modification of the methods of Mosbach et al.,[5] Irvin et al.[6] and Isaksson.[7] The method of Mosbach et al. permits quantitative determination of the total dihydroxycholanic acids and trihydroxycholanic acids. The method of Irvin et al. permits the specific determination of cholic acid. Chenodeoxycholic acid was determined specifically by the Isaksson method. Since the dihydroxycholanic acids of human bile consist of two main fractions, values for deoxycholic acid were obtained by subtracting chenodeoxycholic acid from dihydroxycholanic acids.§ Bile from two subjects (C. L. and V. C.) was subjected to paper chromatography using methods described by Sjövall.[9] By this method the trihydroxycholanic

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§ The colorimetric method described by Szalkowski and Mader[8] which is reported to be highly specific for deoxycholic acid was also employed. Analysis of bile by this method consistently gave values in excess of those obtained spectrophotometrically. This was particularly noticeable with stored specimens, and it is possible that absolutely fresh or lyopholized bile would not show these discrepancies.

acids and dihydroxycholanic acids conjugated with taurine and with glycine were identified. Since both of these subjects had been on T tube drainage for several days at the time bile was taken for analysis, their bile was considered to contain negligible amounts of deoxycholic acid, and dihydroxycholanic acid was considered equivalent to chenodeoxycholic acid.

RESULTS

Figures for total bile acid and the distribution of its three major constituents are given in Table I. Since bile obtained by intubation was diluted by duodenal contents and by the magnesium sulfate solution, quantitative data regarding bile acid excretion could not be obtained, but ratios among the different bile acids presumably were unaltered by dilution. Similarly, the patients who had choledochostomy were convalescing from recent operations and did not have total obstruction of the common bile duct at all periods of observation so that data regarding total bile acid excretion

TABLE I
Bile Acid Constituents of Bile Following Administration of L3:5:3′ Triiodothyronine

Patient, Sex, Age (yr.) and Dose	Time Specimen Obtained	Bile Acids				
		Total (mg./ml.)	Cholic (%)	Chenodeoxycholic (%)	Deoxycholic (%)	CD:C
Intubation Cases						
C. B., M, 72, 500 μg. given orally at 9 A.M.	9:00 A.M.	0.43	69.1	17.3	13.6	0.25
	11:30 A.M.	0.50	79.5	6.6	13.9	0.08
	5:30 P.M.	0.22	56.8	22.3	20.9	0.39
	9:30 A.M.	1.13	57.7	26.5	15.8	0.46
B. B., F, 24, 300 μg. given orally at 9 A.M.	9:00 A.M.	5.20	24.2	46.2	29.6	1.91
	11:00 A.M.	0.84	53.9	18.0	28.1	0.33
C. C., M, 44, 200 μg. given orally at 9 A.M.	9:00 A.M.	1.63	21.4	55.5	23.1	2.59
	11:00 A.M.	1.46	23.9	58.1	18.0	2.43
	5:00 P.M.	2.64	21.3	71.2	7.5	3.34
L. B., M, 51, 300 μg. given orally at 9 A.M.	9:00 A.M.	2.32	33.6	65.1	1.3	1.94
	11:00 A.M.	3.38	44.1	47.0	8.9	1.07
	5:00 P.M.	1.61	40.7	51.4	7.9	1.26
F. G., M, 64, 500 μg. given orally at 9:30 A.M.	9:30 A.M.	0.42	59.8	27.2	13.0	0.46
	5:00 P.M.	0.06	74.2	19.4	6.4	0.26
	10:00 A.M.	0.35	66.2	21.3	12.5	0.32
L. S., M, 53, 500 μg. given orally at 9:30 A.M.	9:30 A.M.	4.17	39.1	40.0	20.9	1.02
	6:00 P.M.	0.05	42.5	36.2	21.3	0.85
	8:30 A.M.	6.75	34.1	43.3	22.6	1.27
R. F., M, 51, 500 μg. given orally at 11 A.M.	11:00 A.M.	0.71	39.8	60.2	0	1.51
	3:00 P.M.	0.02	57.7	42.3	0	0.73
B. A., M, 26, 500 μg. given orally at 3 P.M.	10:15 A.M.	6.45	61.7	31.8	6.5	0.52
	1:00 P.M.	0.56	61.5	26.9	11.6	0.44
	5:00 P.M.	0.02	84.2	10.5	5.3	0.13
W. H., M, 48, no medication given	9:00 A.M.	4.31	55.3	35.3	9.4	0.64
	12:00	2.48	54.8	45.2	0	0.83
	4:15 P.M.	1.43	55.2	26.1	18.7	0.47
L. B., M, 51, no medication given	9:00 A.M.	4.21	21.1	51.3	27.6	2.43
	1:30 P.M.	4.30	28.8	64.3	6.9	2.23
	5:00 P.M.	3.19	25.0	60.1	14.9	2.40
W. I., M, 36, no medication given	9:00 A.M.	1.04	28.7	38.5	32.8	1.34
	11:00 A.M.	1.57	28.9	42.8	28.3	1.48
	1:15 P.M.	0.20	22.3	39.6	38.1	1.78
	3:00 P.M.	0.03	36.7	60.0	3.3	1.64

(*Continued next page*)

TABLE I (*Continued*)

Patient, Sex, Age (yr.) and Dose	Time Specimen Obtained	Bile Acids				
		Total (mg./ml.)	Cholic (%)	Chenodeoxycholic (%)	Deoxycholic (%)	CD:C
Choledochostomy Cases						
Z. C., F, 52, 500 μg. given intravenously at 9 A.M.	5:00–7:00 A.M.	1.85	71.9	28.1	0	0.39
	7:00–9:00 A.M.	2.44	70.6	29.4	0	0.42
	9:00–11:00 A.M.	2.25	74.8	23.8	1.4	0.32
	11:00–1:00 P.M.	2.06	71.7	27.1	1.2	0.38
	1:00–3:00 P.M.	1.73	66.8	33.2	0	0.50
	3:00–5:00 P.M.	1.90	66.9	33.1	0	0.50
	5:00–7:00 P.M.	1.77	65.9	34.1	0	0.52
	7:00–9:00 P.M.	1.87	56.8	43.2	0	0.76
	9:00–11:00 P.M.	2.84	59.9	40.1	0	0.67
	11:00–1:00 A.M.	2.74	63.5	36.5	0	0.58
	1:00–3:00 A.M.	2.14	60.0	40.0	0	0.67
	3:00–5:00 A.M.	2.15	61.5	38.5	0	0.63
R. P., F, 68, 500 μg. given intravenously at 11 A.M.	8:00–9:00 A.M.	9.70	79.6	20.4	0	0.26
	10:00–11:00 A.M.	3.81	69.5	30.5	0	0.44
	11:00–12:00	3.68	69.2	30.8	0	0.45
	12:00–1:00 P.M.	3.10	59.8	40.2	0	0.67
	1:00–2:00 P.M.	3.25	60.0	38.2	1.8	0.64
	2:00–3:00 P.M.	3.46	71.8	28.2	0	0.39
	3:00–4:00 P.M.	3.79	60.3	39.7	0	0.66
W. F., M, 81 400 μg. given intravenously at 9:15 A.M.	5:30–8:10 A.M.	1.92	60	40	0	0.67
	8:10–9:15 A.M.	1.39	58	42	0	0.72
	9:15–11:00 A.M.	1.32	60	40	0	0.67
	11:00–1:00 P.M.	1.19	60	39	1	0.65
	1:00–3:00 P.M.	1.18	61	37	2	0.61
	3:00–5:00 P.M.	1.38	60	40	0	0.67
E. T., F, 66 500 μg. given orally at 10 A.M.	9:00–10:00 A.M.	0.83	46	41	13	0.89
	10:00–11:00 A.M.	0.80	49	38	13	0.78
	11:00–12:00	0.74	44	49	7	1.11
	12:00–1:00 P.M.	0.64	40	50	10	1.25
	1:00–2:00 P.M.	0.68	41	41	18	1.00
	3800–4:00 P.M.	0.81	42	36	22	0.86
E. T., F, 66, 500 μg. given intravenously at 9 A.M.	7:00–8:00 A.M.	9.0	69	31	0	0.45
	8:00–9:00 A.M.	6.37	71	29	0	0.41
	9:00–10:00 A.M.	6.48	70	30	0	0.43
	10:00–11:00 A.M.	8.64	70	30	0	0.43
	11:00–1:00 P.M.	6.81	72	28	0	0.39
	1:00–2:00 P.M.	5.64	73	26	1	0.36
	2:00–4:00 P.M.	6.02	71	29	0	0.41

TABLE I (*Continued*)

Patient, Sex, Age (yr.) and Dose	Time of Specimen	Total Bile Acids (mg./ml.)	Glyco-cholic (%)	Glyco-chenodeoxy-cholic (%)	Tauro-cholic (%)	Tauro-chenodeoxy-cholic (%)	Cheno: Cholic	Glycine: Taurine
Choledochostomy Cases (Continued)								
V. C., F, 58, 500 μg. given orally at 10 A.M.	9:00–10:00 A.M.	0.48	17	9	44	30	0.64	0.35
	10:00–11:00 A.M.	0.43	14	10	44	32	0.72	0.32
	11:00–12:00	0.48	3	8	55	34	0.72	0.12
	12:00–1:00 P.M.	0.53	20	14	36	30	0.79	0.52
	1:00–2:00 P.M.	0.24	22	14	24	40	1.17	0.56
	2:00–3:00 P.M.	0.91	17	7	54	22	0.41	0.32
	3:00–4:00 P.M.	0.78	18	8	50	24	0.47	0.35
C. L., F, 40, 500 μg. given orally at 10 A.M.	7:00–8:00 A.M.	1.93	43	20	26	11	0.45	1.70
	8:00–9:00 A.M.	2.59	46	20	22	12	0.47	1.94
	9:00–10:00 A.M.	1.86	45	22	22	11	0.49	2.03
	10:00–11:00 A.M.	4.32	54	28	13	5	0.49	4.56
	11:00–12:00	7.68	25	16	48	11	0.37	0.70
	12:00–1:00 P.M.	5.99	26	22	40	12	0.61	0.92
	1:00–2:00 P.M.	2.50	30	14	34	22	0.61	0.79
	2:00–3:00 P.M.	—	—	—	—	—	—	—
	3:00–4:00 P.M.	1.80	41	27	19	13	0.67	2.10

were not truly quantitative.

Table II shows the deviations from the initial control in the chenodeoxycholic acid to cholic acid (CD:C) ratio for the treated subjects and the maximum deviation from the mean in the untreated subjects. One subject (B. A.) is listed both as a control and as a treated subject, since two bile specimens were obtained from him prior to medication. Figure 1 shows the percentage change in the CD:C ratio plotted against time for each patient studied by intubation. In this figure the CD:C ratio of the initial specimen was arbitrarily taken as 100 per cent, and deviations were plotted from this point. The maximal effect in the treated subjects appears to occur approximately two hours following administration of the drug. No fall in the CD:C ratio is noted in the control subjects, and there is in fact a tendency for their CD:C ratio to rise during the period of observation.

COMMENTS

The significant and somewhat unexpected result of this study was the early fall in excretion of chenodeoxycholic acid relative to cholic acid, which was observed in the intubation cases. Eriksson[10] reported on the bile acid excretion of rate with bile fistulas made hyperthyroid by the feeding of desiccated thyroid.

TABLE II
Effect of L3:5:3′ Triiodothyronine upon Human Bile Acid Composition

Patient, Sex, Age (yr.) and Dose	Clinical Impression	Maximum Decreased CD:C Observed (%)	Time of Observation (hr.)	Maximum Increased CD:C Observed (%)	Time of Observation (hr.)	Total Study Time (hr.)
C. B., M, 72, 500 μg. given orally	Duodenal ulcer	66.9	2.5	83.2	24.5	24.5
B. B., F, 24, 300 μg. given orally	Functional bowel disease	82.6	2			2
C. C., M, 44, 200 μg. given orally	Chronic discoid lupus erythematosus	6.2	2	29.8	8	8
L. B., M, 51, 300 μg. given orally	Diabetes, parathyroid adenoma	55.1	2			8
F. G., M, 64, 500 μg. given orally	Arteriosclerotic heart disease	42.8	7.5			24.5
L. S., M, 53, 500 μg. given orally	Functional bowel disease	16.7	8.5	24.7	23	23
R. F., M, 51, 500 μg. given orally	Duodenal ulcer	51.6	4			4
B. A., M, 26, 500 μg. given orally	Duodenal ulcer	71.0	2			2
B. A., M, 26, no medication given	Duodenal ulcer	17.3	3			3
W. H., M, 48, no medication given	Duodenal ulcer	25.8	7	28.8	3	7
L. B., M, 51, no medication given	Diabetes, parathyroid adenoma	8.2	4.5			8
W. I., M, 36, no medication given	Coronary occlusion			31.1	4	6
Z. C., F, 52, 500 μg. given intravenously	Cholecystectomy, 17 days postoperatively	23.4	1	83.4	11	24
R. P., F, 68, 500 μg. given intravenously	Cholecystectomy, 4 days postoperatively			53.4	2	8
E. T., F, 66, 500 μg. given orally	Cholecystectomy, 2 days postoperatively	13.7	1	42.0	3	7
E. T., F, 66, 500 μg. given intravenously	Cholecystectomy, 7 days postoperatively	12.0	3	3.6	1	9
W. F., M, 81, 400 μg. given orally	Cholecystectomy, 5 days postoperatively	16.5	5			11.5
C. L., F, 40, 500 μg. given orally	Cholecystectomy, 5 days postoperatively; taurine 3.0 gm. four times a day for 3 days	21.3	2	42.6	6	7
V. C., F, 58, 500 μg. given orally	Cholecystectomy, 8 days postoperatively; taurine 3.0 gm. four times a day for 3 days	35.9	5	82.8	4	7

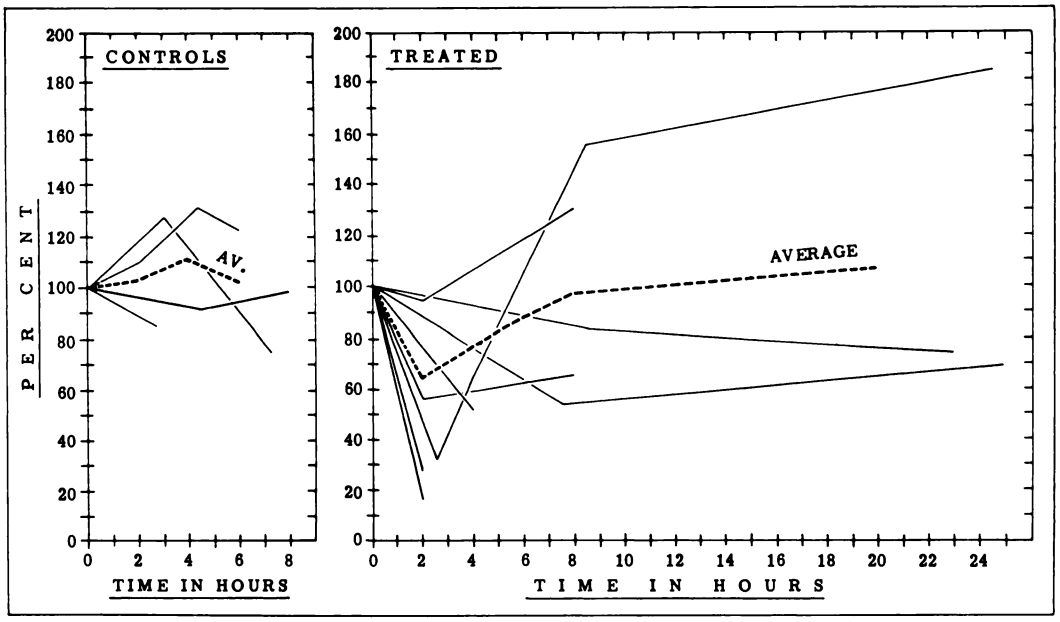


FIG. 1. Percentage of chenodeoxycholic to cholic acid in untreated subjects and in those given L3:5:3′ triiodothyronine. Bile was obtained by duodenal aspiration. Ratio of chenodeoxycholic to cholic acid in the initial bile specimens was taken as 100 per cent with subsequent deviations plotted from this point.

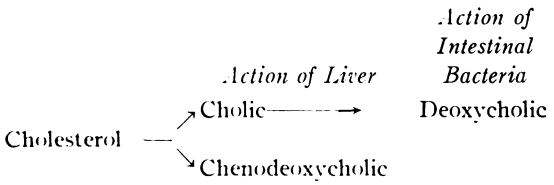
In his animals the proportion of chenodeoxycholic acid rose, while the total bile acid output was not significantly changed. Whether these diametrically opposed results represent species difference or a specific property of L3:5:3′ triiodothyronine in contradistinction to desiccated thyroid, or are a function of differences in rate of action is not presently known. The increase of the CD:C ratio to a value greater than the initial value of this ratio in bile specimens obtained twelve to twenty-four hours following administration of L3:5:3′ triiodothyronine suggests that Eriksson may have missed the earlier effect. More recently Hellström and Sjövall[11] described the effects of thyroid hormone on the bile acids in man. Their study emphasized primarily the increase in taurine conjugation of bile acids following the administration of thyroid to patients with myxedema who were followed over periods of weeks or months. Several different hormones were used, and no consistent effect on the CD:C ratio was observed under these conditions.

The relative lack of effect of L3:5:3′ triiodothyronine after either oral or intravenous administration to patients with choledochostomy drainage was unexpected, since it had been hoped to obtain some information from these patients concerning quantitative excretion of the different bile acids. Continuous external drainage of bile through a choledochostomy has been shown to increase the total bile acid output greatly.[12] A possible explanation for our inability to observe an unequivocal change in CD:C ratio following the administration of L3:5:3′ triiodothyronine to patients with choledochostomy may be that the specific hormonal effect is to cause a relative increase in excretion of cholic acid. If the liver is already excreting cholic acid at its maximal rate, administration of L3:5:3′ triiodothronine could not increase it.

A second possibility that might explain the insignificant effects upon the CD:C ratio in patients with choledochostomy is that these patients have suffered some manner of hepatic damage either from anesthesia, surgical trauma or from prolonged obstruction of the common duct. The transient diminution in bile acid excretion immediately following surgery has been documented previously, and this does not typically last more than two days.[12] Not all

of the patients with a choledochostomy studied by us were jaundiced at the time of operation, and results of liver function tests in others showed elevations only in serum bilirubin and alkaline phosphatase; therefore, parenchymal liver disease could not be considered a significant factor in altering bile acid metabolism. At the time (postoperatively) our studies were made, jaundice was minimal or absent.

Oxidation of cholesterol to bile acid in man is at present thought to occur in the following manner:



This schema accounts for those bile acids most frequently found in high concentration. Deoxycholic acid is of particular interest, since its presence in human bile is thought to result exclusively from the re-excretion of deoxycholic acid produced by intestinal bacteria which has been resorbed from the lower intestinal tract.[13] In consequence, it is not found in established bile fistulas. Conceivably deoxycholic or some other bile acid resulting from bacterial action could influence the CD:C ratio. The presence of considerable deoxycholic acid in bile from a patient with fistula (E. T.) at the time of her first test, without a notable CD:C ratio effect, and the variable amounts of deoxycholic acid in bile from our intubated subjects suggest that this is not likely.

Established bile fistulas have been shown to contain bile acid conjugated almost entirely with glycine, and in this respect they contrast with bile from intact adult subjects which typically contains about 20 to 30 per cent of taurine-conjugated bile acid.[14] With the thought that taurine conjugation of bile acids might in some manner mediate the change in CD:C ratio following administration of thyroid hormone, two subjects (C. L. and V. C.) were fed taurine, 3.0 gm. a day in divided dosage, during the period of bile collection and during the three previous days. In these subjects considerable taurine conjugation resulted, but the change in CD:C ratio noted in the intubation cases was not observed. The alteration in CD:C ratio in the intact subject seems clear-cut and runs counter to the trend seen in multiple bile acid determinations made during the day in control subjects not receiving medication. The other bile acid ratios show no consistent pattern of change.

CONCLUSIONS

Changes in the pattern of bile acid excretion in man following administration of L3:5:3′ triiodothronine are reported. In the intact person a significant diminution was observed in the ratio of chenodeoxycholic acid to cholic acid (CD:C) in bile obtained by duodenal intubation. Similar change was not noted in bile obtained by choledochostomy drainage. Maximal change in bile acid ratios was observed approximately two hours following administration of L3:5:3′ triiodothyronine.

SUMMARY

Bile obtained by duodenal intubation of human subjects was analyzed for its bile acid content. Specific bile acids studied were cholic, chenodeoxycholic and deoxycholic. Following administration of L3:5:3′ triiodothronine a decrease was noted in the ratio of chenodeoxycholic to cholic acid (CD:C). This change was maximal at two hours. It was not noted in bile obtained by choledochostomy drainage from patients with recent cholecystectomies.

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