Lipid Components of Bile from Choline Deficient Rats.* (30147)

IBERT C. WELLS AND JEROME M. BUCKLEY
Department of Biochemistry, Creighton University School of Medicine, Omaha, Nebr.

Choline deficiency produces a prompt and marked decrease in blood lipids of the rat (1,2) and fatty degeneration of the liver in this and in most of the other species examined. These observations support the conclusion that the mobilization of liver lipid is impaired in choline deficiency.

Bile contains lipids derived from the liver. It was the purpose of this work to ascertain whether the lipids in bile decreased during choline deficiency as the blood lipids do or whether the bile might serve, in this deficiency state, as an excretory route for the lipids accumulating in the liver.

A partial examination of bile from choline deficient rats has been reported by Colwell (3). His analyses of 6 days' collections of bile revealed a mild decrease in concentrations of total lipid and cholesterol. During the collection period, food intake decreased and infection may have occurred. In the present study, the time of bile collection has been restricted to the 24 hours immediately after bile duct cannulation to minimize the effects of the variable factors of food consumption and infection.

Materials and methods. Male, Holtzman† rats (95-110 g) were divided into 2 groups. The control group was fed *ad lib* pulverized Purina laboratory chow‡ for 3-10 days and the experimental group was fed *ad lib* for 5-7 days the choline-free, 18% casein, 21% fat diet previously described(4). The control rats gained weight steadily. The experimental animals gained weight initially, then their body weights either became constant or decreased.

The rats were anesthetized with Pentobarbital U.S.P. and their bile ducts cannulated with beveled polyethylene tubing under aseptic conditions as described by Bocklage *et al* (5). The operated animals were placed in restraining cages and provided with diet, water and 5% sodium bicarbonate solution (5). Bile was collected for 18-24 hours immediately after the operation and the bile samples were frozen and stored. The animals survived the operation from 2-7 days and had variable losses of body weight.

Portions of the bile samples were extracted with propanol(6) and total esters were determined in these extracts by the method of Rapport and Alonzo(7). Total cholesterol and phospholipids were extracted by treatment of the remainder of the bile samples with chloroform and methanol(8); bile acid conjugates were left in the aqueous methanol fractions. Cholesterol and its esters were determined by the procedure of Rosenthal and Jud(9); lipid phosphorus in the chloroform extracts was determined by the method of Bartlett(10); bile acid conjugates were determined as cholic acid(11).

Results and discussion. The control and choline deficient rats produced the same volume of bile (Table I). However, the concentrations of total esters, bile acid conjugates, total cholesterol and lipid phosphorus were significantly less in the bile from choline deficient rats. The decrease in concentration of total cholesterol was entirely accounted for by the absence of cholesterol esters; the concentrations of free cholesterol were identical in the bile from normal and choline deficient rats.

Our results indicate that free and esterified cholesterol occur in normal bile in the same ratio as reported for serum(2) and that essentially only free cholesterol occurs in bile from choline deficient rats. The report by Colwell (3) indicated that about 10% of the cholesterol in normal bile is esterified and that esterification of cholesterol in bile was not affected by choline deficiency. This differ-

* This investigation was supported in part by USPHS Research Grant AM-05235 from Inst. of Arthritis and Metab. Dis.

† Holtzman Co., Madison, Wis.

‡ Ralston Purina Co., St. Louis, Mo. Purina laboratory chow contains not less than 23.0% protein, not less than 4.5% fat, not more than 6.0% fiber and not more than 9.0% ash. It is supplemented with choline chloride, vit. A, D and E, vit. B and trace minerals.

TABLE I. Analyses of Bile from Normal and Choline Deficient Rats.*

Rats	Volume produced (ml)	Total esters (mg olive oil)	Conjugated bile acids (mg cholic acid)	Total cholesterol (mg)	Free cholesterol (mg)	Lipid phosphorus (mg)
Control	7.3 ± 1.0† (12)‡	22.3 ± 1.8 (11)	14.2 ± 1.3 (10)	1.89 ± .06 (9)	.49 ± .04 (9)	.752 ± .079 (10)
Choline deficient	7.2 ± .8 (12)	11.6 ± 1.1§ (12)	6.8 ± 1.2§ (12)	.59 ± .07§ (10)	.55 ± .14 (11)	.222 ± .020§ (12)

* All quantities are expressed in the units indicated per 100 g body wt per 24 hr.
† Average ± standard error of mean.
‡ Number of samples analyzed is given in parentheses.
§ Student's "t" test; $P < .01$.

ence may be explained by the prolonged period of bile collection used in his study, since the liver lipids of the normal rats increased during this period to a level resembling that in choline deficient rats. Also, some of the cholesterol esters may have been hydrolyzed by the hydrochloric acid used during the isolation of the bile lipids(3).

Since the normal ratio of free to esterified cholesterol occurs in the serum of choline deficient rats(2), the present findings that choline deficient rats excrete no esterified cholesterol in their bile but excrete the same amount of free cholesterol as normal rats provide an explanation for the observation by Best *et al*(12) that the amounts of glycerides and cholesterol esters increased in parallel in the livers of choline deficient rats.

Summary. The results obtained by the analyses of bile, collected from normal and choline deficient male rats during the first 24 hours after cannulation of the bile duct, indicate that the volume of bile produced is not changed but the concentrations of lipid components are generally decreased. Esterified cholesterol was absent and free cholesterol was in the same concentration as normal in the deficient bile. These findings may explain the previously observed accumulation of cholesterol esters in the livers of choline deficient rats.

1. Lombardi, B., Ugazio, G., Raick, A., Fed. Proc., 1964, v23, 126.
2. Tinoco, J., Shannon, A., Lyman, R. L., J. Lipid Research, 1964, v5, 57.
3. Colwell, A. R., Jr., Am. J. Physiol., 1951, v164, 274.
4. Wells, I. C., J. Biol. Chem., 1956, v222, 931.
5. Bocklage, B. C., Nicholas, H. J., Doisy, E. A., Jr., Elliott, W. H., Thayer, S. A., Doisy, E. A., ibid., 1953, v202, 27.
6. Connerty, H. V., Briggs, A. R., Eaton, E. H., Jr., Clin. Chem., 1961, v7, 37.
7. Rapport, M. M., Alonzo, N., J. Biol. Chem., 1955, v217, 193.
8. Bligh, E. G., Dyer, W. J., Canad. J. Biochem. Physiol., 1959, v37, 911.
9. Rosenthal, H. L., Jud, L., J. Lab. and Clin. Med., 1958, v51, 143.
10. Bartlett, G. R., J. Biol. Chem., 1959, v234, 466.
11. Reinhold, J. G., Wilson, D. W., ibid., 1932, v96, 637.
12. Best, C. H., Lucas, C. C., Patterson, J. M., Ridout, J. H., Biochem. J., 1946, v40, 368.

Received February 5, 1965. P.S.E.B.M., 1965, v119.