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Studies on human Bile

IV. Influence of ingestion of cholesterol in the form of eggs on the composition of bile in healthy subjects

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With 12 tables

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The fact that ingestion of cholesterol in an easily absorbable form such as egg yolk tends to increase serum cholesterol in man is well established (1), but the possible influence of dietary cholesterol on the composition of human bile does not seem to have been examined.

The experiments described in the present paper were carried out in order to ascertain whether daily intake of a substantial amount of cholesterol in the form of whole eggs markedly influences the composition of bile in healthy subjects especially with respect to the molar percentages of each of the main bile acids in the total bile acids and the molar ratios between total bile acids and cholesterol, between lipid-soluble phosphorus and cholesterol, and between total bile acids and lipid-soluble phosphorus.

Experimental

Nine healthy volunteers, 19 to 22 years of age, received diets furnishing from 5 to 10 eggs per day through two consecutive periods of 3 weeks. The constituents of the daily rations were adjusted so as to keep the total amounts of protein and fat constant at 20 and 42 calorie per cent respectively. Further, the daily intake of calories was adjusted with a view to enable each volunteer to maintain constant body weight during the experiment.

Preparation and administration of the diets were undertaken by the Diet Kitchen of the Medical Department A of the University Hospital.

Examples of the diets used are shown in table 1 A. The volunteers were allowed to drink water, tea and coffee without sugar and cream. Other food items than those shown in the table were not introduced in any of the diets.

It was not considered necessary to begin the experiments with a period in which the subjects received a fixed diet without eggs, because continuous intake of 5 to 10 eggs per day is quite unusual and furnishes a much larger amount of cholesterol than does any commonly used diet. Further, previous experiments (2) had failed to show any significant change of the data to be determined in the present study as a consequence of replacing butter with a high-linoleic acid margarine.

Immediately before, and 3 and 6 weeks after beginning of the egg regimen, samples of duodenal bile were collected fasting after intravenous injection of cholecystokinin. Those
fractions of the bile samples having a pH higher than 6.9 were analyzed for bile acids, cholesterol and lipid-soluble phosphorus.

The methods for collection and analysis of the bile samples were as described in our previous studies (2, 3).

Simultaneously with the collection of bile, blood samples were taken for determination of serum total cholesterol.

Collection of bile was performed in the Gastro-enterological Laboratory, determination of serum cholesterol in the Central Laboratory of the University Hospital. Analysis of bile was undertaken in the Department of Biochemistry and Nutrition of the Polytechnic Institute to which the bile samples were speedily transferred.

The eggs used were chicken eggs of the Danish type „C“, weighing from 55 to 60 g with shell. Their content of cholesterol was found to be 206 mg per egg. Estimates of the volunteers' daily intake of egg-cholesterol were based on this figure.

The cholesterol content was determined by hard-boiling of 6 eggs, cooling and division of each yolk in quarters by weight. One quarter from each yolk were combined and treated with 100 ml 60% KOH (w/v) on boiling water bath for 3 hours. The non-saponifiable fraction was shaken out quantitatively with ether. After washing with alkaline water and distilled water to neutrality, the ethereal solution was dried with anhydrous sodium sulfate, evaporated to dryness and dissolved in chloroform. The LIEBEMANN-BURCHARD reaction was performed on an aliquot of the solution as described previously (4) whereafter the absorbancy at 625 nm was measured and compared with the absorbancy obtained with a standard of pure cholesterol. Earlier one of us (5) had found a content of 239.5 mg total cholesterol per egg for chicken eggs of the size used in the present experiment. The commonly used food tables by SOUCI, FACHMANN & KRAUT (6) indicate 238 mg cholesterol for eggs of the size in question.

Results and Discussion

The results are shown in tables 1–12.¹)

Table 1 shows the amounts of egg cholesterol consumed and the variation of serum cholesterol during the egg regimen.

Table 2 shows pH, per cent Dry Matter, and the millimolarities of Cholesterol (C), Lipid-soluble Phosphorus (P), and Total Bile Acids (TBA) in the bile samples before and after 3 and 6 weeks of egg diet. TBA is taken as the sum of Glycocholic Acid (GC), Glycochenodeoxycholic Acid (GCD), Glycodeoxycholic Acid (GD), Taurocholic Acid (TC), and Taurochenodeoxycholic plus Taurodeoxycholic Acids (TCD + TD). (If it had been possible to determine other bile acids that are present in small amounts, the value for TBA would have been a little higher).

Tables 3–7 show the molar percentages of GC, GCD, GD, TC and TCD + TD in TBA.

Tables 8 and 9 show the molar ratios between Glycine-conjugation and Taurine-conjugation (G/T), and between Dihydroxycholanoic Acids and Trihydroxycholanoic Acids (Di/Tri), respectively.

Tables 10–12 show the other ratios to be considered.

From table 1 it is obvious that the egg diet produced a more or less pronounced increase of serum total cholesterol, apparently without marked relation to the quantity of cholesterol consumed per kg body weight. The mean value of serum cholesterol was higher after 6 weeks than after 3 weeks. For one of the volunteers (LVM), whose

¹) In tables 1 and 3–12, figures in parenthesis indicate per cent of the values before the egg diet.