13. K. A. Narayan, Atherosclerosis, 13, 205 (1971).
14. W. Romen and W. D. Heine, Arch. Pathol. Anat. Abt. A, 356, 42 (1972).
15. T. Shimamura, Exp. Mol. Pathol., 13, 79 (1970).

TAUROCHOLIC ACID SYNTHESIS IN DOGS ON A HIGH CHOLESTEROL DIET

L. V. Kryukova

UDC 616.36-092.9-07: 616.36-008.839.31-073.9.16

Synthesis of ^{35}S-labeled taurocholic acid was studied in dogs with a gall bladder fistula kept on a high-cholesterol diet (2 g/kg). In the first period (1-2 months) of feeding on a high-cholesterol diet a marked increase in the synthesis of [^{35}S]taurocholic acid was observed. After 5-6 months, in the period of development of the pathological process in the liver, the synthesis and secretion of bile acids and the formation of a cholesterol residue in the bile were reduced. The serum cholesterol level in these dogs was increased but not significantly.

KEY WORDS: taurocholic acid; high-cholesterol diet.

The digestive tract plays an essential role in the synthesis, absorption, and subsequent conversion of cholesterol [9, 15]. The liver and intestine are evidently the main sources of endogenous cholesterol [5, 6, 11, 14]. If cholesterol synthesis in the liver is controlled through a system of negative feedback by the quantity of cholesterol taken in with the diet, cholesterol synthesis in the intestine, as has been shown for rats, must be independent of the amount of exogenous cholesterol ingested [10]. Exogenous cholesterol taken in with the diet has been shown to be converted in the liver into bile acids. An excessive intake of cholesterol is regulated by an increase in its conversion into bile acids and its excretion with the bile, by inhibition of cholesterol synthesis in the liver, by an increase in the excretion of cholesterol and its derivatives with the feces, and by restriction of its absorption from the digestive tract [7, 12, 15-19]. These changes are differently expressed in animals of different species, and this accounts for the differences in the degree of development of hypercholesteremia and of accumulation of cholesterol by the liver and other organs in experiments on animals receiving high-cholesterol diets. Administration of large doses of cholesterol to dogs led to a small increase in the serum cholesterol concentration, to marked accumulation of cholesterol in the liver tissue, and the increased formation of bile acids [13]. It was concluded that in animals of this species the main regulatory mechanism is the conversion of cholesterol in the liver into bile acids. However, it is not clear to what extent this mechanism operates in the presence of diseases of the liver.

The object of this investigation was to study synthesis of labeled taurocholic acid in dogs receiving a high-cholesterol diet at intervals during the development of hepatic pathology.

EXPERIMENTAL METHOD

Experiments were carried out on seven mongrel dogs with a gall gladder fistula and with the common bile duct ligated, five of which received a diet containing cholesterol (with the addition of bile of the same dog) in a dose of 2g/kg body weight daily for 6 months. On the day of the experiment the dog received 20 μCi [^{35}S]-methionine per os. The rate of excretion of radioactive label in the bile and of its incorporation into taurocholic acid was determined, and in some experiments parallel determinations were made of the cholic acid content in the bile. In a special series of experiments to determine the degree of conversion of cholesterol, entering from the intestine, into bile acids the method of isotope dilution was used. For this purpose, on the days after administration of [^{35}S]methionine bile was collected for 2-3 h, on the assumption that the quantity of radioactivity excreted in 1 ml does not change to any considerable degree, after which, when the activity of the bile was stable, the dog was given a cholesterol load and bile continued to be collected for 5 h and a

Laboratory of Pathophysiology, Central Research Institute of Gastroenterology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 6, pp. 679-682, June, 1978. Original article submitted July 12, 1977.

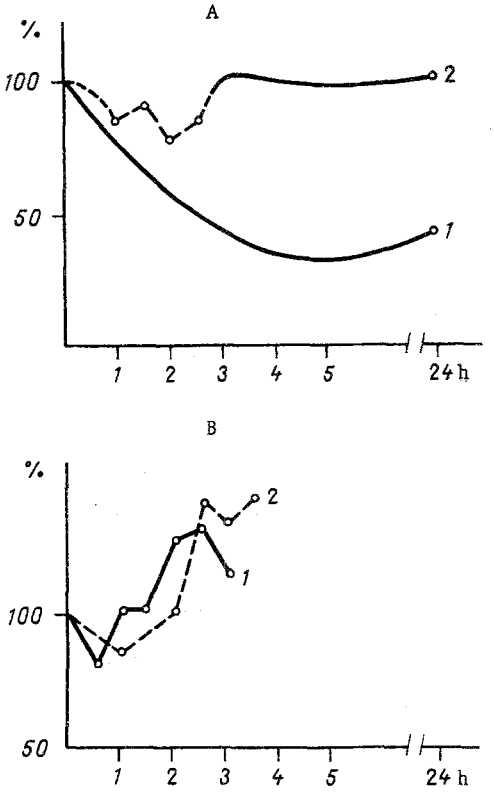

Fig. 1. Administration of cholesterol after stabilization of radioactivity of bile in dogs receiving high-cholesterol diet. A: 1) Control experiments; 2) after receiving high-cholesterol diet for 5 months (each curve plotted from mean data from three or four experiments). B: 1) Percentage of radioactivity excreted by dog Lad after 6.5 months; 2) percentage of cholic acid excreted during same period. Abscissa, time (in h); ordinate, cholic acid secreted (in percent of background values).

single portion was obtained after 24 h. The excretion of radioactivity in 1 ml bile was calculated as a percentage of the background level. The cholesterol concentrations in the blood serum and bile were determined periodically by means of the Liebermann–Burchard color reaction.

EXPERIMENTAL RESULTS

Dogs on which an operation had been performed to form a gall bladder fistula were used as the experimental model of the development of liver pathology. As a result of loss of some of the bile (despite regular feeding with their own bile) and the possible development of ascending infection through the biliary tract, such dogs are known [1] to develop functional and morphological disturbances gradually in the liver. In the later stages after the operation (10-12 months) the absorption and utilization of [^{35}S]methionine was observed to be disturbed in these animals and the ability of their liver to synthesize taurocholic acid, the principal bile acid in dogs, was reduced [2, 3].

In the present investigation it was shown in the group of control dogs that after administration of [^{35}S]-methionine to the dog per os radioactivity quickly entered the bile to reach a maximum 3-4 h after injection of the labeled amino acid.

Radiochromatographic analysis of the bile showed that during the first few hours of the experiment the principal compound of the bile containing radioactive sulfur was free methionine; a little later radioactive ^{35}S

was found in the protein of the bile and taurocholic acid. All the radioactivity was found in the taurocholic acid 24 h after injection of [^{35}S]methionine into the dog [4].

In dogs receiving a high-cholesterol diet for 1-2 months, a sharp increase in the secretion of radioactivity with the bile compared with the control dogs was observed in the experiments in which [^{35}S]methionine was introduced into the digestive tract. The relative percentage of radioactivity excreted in 1 ml bile exceeded 1000 units in some experiments, i.e., it was 3 to 4 times greater than normal. Fractionation of the components of an alcoholic extract of bile by paper chromatography (a system of butanol–glacial acetic acid–water 4:4:1) showed that 3-4 h after administration of [^{35}S]methionine 60% of the radioactive sulfur was incorporated into taurocholic acid. The results of a parallel biochemical determination of cholic acids in the bile of these dogs showed that their concentration was 2 or 3 times higher than the initial level. This fact is in agreement with data in the liberature, in which a fourfold increase in the secretion of bile acids was found in dogs receiving large doses of cholesterol with the diet [13]. The present results confirm the view that in animals of this species the mechanism of conversion of cholesterol into bile acids evidently plays the most important role in the general regulation of cholesterol metabolism.

To continue the analysis of this observed increase in the secretion of bile acids under the influence of a high-cholesterol diet experiments were carried out by the method of dilution of the radioactive label. The results of counting the radioactivity of the bile secreted in a fasting state by the dogs (24 h after injection of [^{35}S]methionine showed that the level of radioactivity fluctuated within narrow limits for 2-3 h. Administration of a single cholesterol load (2 g/kg) against the background of stable radioactivity of the bile during the first period when the dogs were kept on an experimental diet led always to a regular decrease in the radioactivity of the bile by 50% and more (Fig. 1A). The excretion of radioactivity with the bile still remained depressed 24 h after cholesterol loading. This decrease in the radioactivity of the bile may be due to two causes: dilution of the [^{35}S]taurocholate label by unlabeled taurocholic acid, formed de novo in large quantities from the excess of cholesterol entering the body or as a result of a decrease in the secretion of the principal components of the bile, including bile acids. The first hypothesis is evidently confirmed by the increase in the cholic acid concentration in the secreted bile, determined in these experiments by a biochemical method, and also the increased synthesis of [^{35}S]taurocholic acid, mentioned above, in dogs during the first months of administration of a high-cholesterol diet. In dogs kept for a long time (4-5 months) on a high-cholesterol diet, the dilution of the ^{35}S label mentioned above was not observed (Fig. 1A). In some cases cholesterol loading led after 6 months in the experimental animals to a small increase both in the cholic acid concentration in the bile and in the content of radioactivity in 1 ml of secreted bile (Fig. 1B). In this case additional ^{35}S may probably have entered the liver and some increase in the incorporation of label into the bile acids may have occurred. However, the overall ability of the liver to synthesize and secrete bile acids was sharply reduced after 6 months. The decrease in the utilization of exogenous cholesterol for bile acid synthesis may be connected with a decrease in its absorption from the intestine, and also with an increase in the excretion of cholesterol by the intestine [7]. Incidentally, an excessive intake of cholesterol caused little change in its blood concentration for a long time, and only after 5-6 months was it increased slightly (from 100 ± 7 to 140 ± 2 mg%; $P < 0.01$). The healthy liver can evidently cope for quite a long time with a high intake of exogenous cholesterol and it convert it into bile acids. The development of liver pathology in the dogs gradually disturbed this ability of the liver to synthesize bile acids. Meanwhile the moderate character of the hypercholesteremia can possibly be explained on the grounds that, with a decrease in the concentration of bile acids in the bile secreted by these dogs the absorption of cholesterol from the intestine may also have been reduced. The disturbance of the secretory function of the liver was manifested also as a change in the chemical composition of the bile. In this period an abundant gray deposit appeared in the bile of the experimental dogs at a time of a reduction in the secretion of the principal components of the bile. Chemical tests showed that cholesterol accounted for 90% of the composition of this deposit.

Administration of a high-cholesterol diet to dogs during the development of liver pathology thus led to a gradual decrease in the conversion of cholesterol into bile acids, to a disturbance of the secretion of the principal components of the bile, and to the precipitation of cholesterol deposits, together with a moderate increase in the serum cholesterol level.

LITERATURE CITED

1. K. S. Zamychkina, E. A. Rudik-Gnutova, D. E. Grodzenskii, et al., Med. Radiol., No. 3, 63 (1956).
2. K. S. Zamychkina, L. V. Kryukova, and R. S. Rubinov, in: Proceedings of the 16th Scientific Conference of Physiologists of the South of the RSFSR with the Participation of Biochemists and Pharmacologists [in Russian], Ordzhonikidze (1967), p. 145.

3. K. S. Zamychkina and L. V. Kryukova, Byull. Éksp. Biol. Med., No. 3, 60 (1967).
4. K. S. Zamychkina, Vest. Akad. Med. Nauk SSSR, No. 12, 20 (1968).
5. S. D. Konyushko, "Pattern of cholesterol excretion in different parts of the intestine under normal and pathological conditions," Author's Abstract of Candidate's Dissertation, Moscow (1971).
6. M. S. Martsevich, S. I. Filipovich, L. A. Shekun, et al., Vest. Akad. Med. Nauk SSSR, No. 12, 11 (1968).
7. M. S. Martsevich, in: Activity of the Digestive System and its Regulation under Normal and Pathological Conditions [in Russian], Moscow (1969), p. 119.
8. M. S. Martsevich, Byull Éksp. Biol. Med., No. 10, 43 (1973).
9. K. A. Tret'yakova, Usp. Sovrem. Biol., 77, 35 (1974).
10. F. Chevallier, Bull. Soc. Chim. Biol. (Paris), 48, 715 (1977).
11. F. Chevallier and C. Lutton, Rev. Europ. Étud. Clin. Biol., 16, 16 (1971).
12. H. Danielsson, in: Advances in Lipid Research, Vol. 1, New York (1963), p. 335.
13. K. J. Ho and C. B. Taylor, Arch. Pathol., 86, 585 (1968).
14. S. Hotta and I. L. Chaikoff, Arch. Biochem., 56, 28 (1955).
15. C. Lutton, Digestion, 14, 342 (1976).

ROLE OF MEMBRANE-BOUND CALCIUM IN CHANGES IN ATPase ACTIVITY, PERMEABILITY, AND STRUCTURAL STATE OF THE HUMAN ERYTHROCYTE MEMBRANE

S. N. Orlov, A. S. Shevchenko, and Yu. V. Postnov

UDC 612.111.014.46:546.41

Experiments were carried out on reconstituted erythrocytes obtained by rapid reversible hemolysis. The free Ca^{2+} concentration in the reconstituted erythrocytes was maintained by means of Ca-EGTA and Ca-citrate buffers. The ouabain-inhibited component of ATPase activity with high affinity for Ca^{2+} ($K_{0.5}$ = 4 μM) and a change in the passive and active permeability for K^+ in the region of free Ca^{2+} concentrations up to 10 μM could be found only by modifying the content of membrane-bound Ca^{2+}. Reducing its content on the inner side of the membrane of the reconstituted erythrocytes was accompanied by a change in the hydrophobicity of the hydrocarbon regions of the membrane. It is suggested that Ca^{2+}-induced changes in the structural state of the erythrocyte membrane may be the direct cause of the change in ATPase activity with high affinity for Ca^{2+} and in permeability for monovalent cations.

KEY WORDS: membrane-bound calcium; erythrocyte membrane; ATPase activity; permeability.

The presence of a Ca^{2+} pump, responsible for the distribution of Ca^{2+} between the plasma and intracellular medium in the ratio of 1000:1, is now firmly established [13]. Nevertheless, the mechanism of the effect of Ca^{2+} on the structural and functional state of the erythrocyte membrane in vivo still remains unexplained. Inhibition of Na,K-ATPase activity by high Ca^{2+} concentrations (100 μM), found in erythrocyte ghosts [7], and the corresponding increase in passive permeability for K^+ [8] do not in fact take place in intact erythrocytes in which the free intracellular Ca^{2+} concentration is maintained below 1 μM [12].

On the other hand, the concentration of Ca^{2+} bound with the inner side of the erythrocyte membrane is higher than the free intracellular Ca^{2+} concentration (Ca_f^{2+}) by more than an order of magnitude, and amounts to 8 μmoles/liter of cells [11]. An investigation was accordingly undertaken to study the role of membrane-bound Ca^{2+} in changes in the structural and functional state of the erythrocyte membrane.

Central Research Laboratory, Fourth Main Board, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 6, pp. 682-685, June, 1978. Original article submitted July 19, 1977.

0007-4888/78/8506-0745$07.50