Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 102 NOVEMBER 1959 No. 2

SECTION MEETINGS

DISTRICT OF COLUMBIA
George Washington University October 1, 1959
SOUTHERN
Tulane University September 24, 1959

Influence of Nicotinic Acid on Hepatic Cholesterol Synthesis in Rabbits.* (25214)

HUGH SCHADE AND PAUL SALTMAN (Introduced by Roslyn B. Alfin-Slater)
Dept. of Biochemistry and Nutrition, School of Medicine, University of S. California, Los Angeles

The clinical use of nicotinic acid to reduce serum cholesterol has been widely accepted (1,2). Although there seems reasonable agreement concerning the efficacy of this drug in lowering cholesterol concentration, there is no agreement as to the biochemical mechanisms operative in the process. Altschul(3) implies that nicotinic acid is converted to either diphospho- or triphosphopyridine nucleotide, which in turn, enhances the oxidative metabolism of cholesterol. It is interesting, however, that nicotinamide, the active moiety of these coenzymes, is ineffective in decreasing cholesterol levels(4). Kraupp *et al.*(5,6) propose that nicotinic acid activates the reticuloendothelial system which in turn alters chemical distribution of the cholesterol in the blood. Another hypothesis has been advanced (7) in which the release of heparin from depot stores by nicotinic acid leads to a depression of serum cholesterol. Merrill(8) has presented experiments which indicate that nicotinic acid enhances the rate of acetate-1-C^{14} incorporation into cholesterol. His finding of a 70-90% increase in rate of sterol synthesis by rat liver, *in vivo,* would demand even greater catabolism of cholesterol in order to produce the lowered blood levels. Recently Schön(9) demonstrated that *in vivo* formation of total hepatic cholesterol is depressed proportionally to the amount of nicotinic acid fed to rats. This inhibition of synthesis was attributed to a lack of acetyl-Coenzyme A needed for cholesterol as well as fatty acid synthesis. Since the effect of nicotinic acid in reducing serum cholesterol in rabbits closely parallels the human response, we have investigated the rate of its synthesis in rabbit liver slices from animals under different dietary regimens, by measurement of both total liver cholesterol as well as rate of incorporation of acetate-1-C^{14} into this compound. It will be shown in this report that nicotinic acid significantly reduces the rate of biosynthesis of cholesterol and a tentative mechanism is proposed.

Methods. Eight male rabbits were divided into 4 groups and fed the diets indicated below for a period of 6 months. Group I—Al-

* This research supported in part by grant from U.S.P.H.S. Facilities of the Allan Hancock Fn. were generously provided.

TABLE I. Effect of Dietary Nicotinic Acid and Cholesterol on Biogenesis of Cholesterol by Liver Slices.

Group	Diet	Total liver cholesterol (mg/g, wet wt)	Specific activity of digitonide (counts/min./mg)	Relative rate of cholesterol synthesis (counts/min./g liver)
I	Ration	11.8	440	5200
II	" + nicotinic acid	8.5	319	2710
III	" + cholesterol	34.6	93	3220
IV	" + cholesterol + nicotinic acid	31.2	66	2060

bers Rabbit Family Ration, Group II—idem. + .5% nicotinic acid, Group III—idem. + 2% cholesterol, Group IV—idem. + .5% nicotinic acid + 2% cholesterol. At the conclusion of the feeding period, the rabbits were sacrificed by decapitation, their liver extirpated, and immediately chilled in phosphate buffer, pH 7.4. A 20 g sample, wet weight, of each liver was homogenized in a Waring Blendor with 80 ml of a 30% alcoholic KOH solution (w/v), filtered, and brought to a final volume of 100 ml. Total liver cholesterol was determined on an 0.1 ml aliquot of each homogenate by the Henly modification of the Zlatkis procedure (10). The remaining portions of the livers were then sliced with a Stadie-Riggs hand microtome. Approximately 1 g of weighed slices was incubated at 37°C, with shaking in a phosphate buffer, pH 7.4, containing K_2HPO_4 0.067 *M*; KH_2PO_4 0.042 *M*; $MgCl_2$, 0.006 *M;* and acetate-1-C^{14} (specific activity approximately 1 mc/mM, 4 x 10^5 counts/min/ml) in a final volume of 5.0 ml. After 3 hours the slices and the medium were treated with 10 ml of sodium ethylate and brought to 85°C for 1 hour to saponify the lipid material. The non-saponifiable lipids were extracted 4 times with chloroform and the combined extracts taken to dryness with a stream of air. The residues were dissolved in 5 ml of a warm ethanol-acetone (1:1) solution and precipitated with a 1% digitonin in 80% ethanol solution. The digitonin precipitable fraction was removed by centrifugation, and resuspended in fresh ethanol-acetone solution. Aliquots were pipetted directly onto tared aluminum planchettes. The planchettes were dried in a stream of warm air, reweighed, and weight of the digitonide precipitate calculated. These samples were counted at infinite thinness in a gas flow counter.

Results. The data for total liver cholesterol as well as rate of incorporation of acetate-1-C^{14} into the digitonide precipitate are presented in Table I. Values for the relative rates of acetate incorporation into cholesterol per gram of liver are calculated from the product of the total cholesterol and specific activity of the digitonide in each case. It is evident, as often reported(11), that there is marked inhibition of cholesterol synthesis by exogenous dietary cholesterol. Further, there is a marked depression of cholesterol synthesis by dietary nicotinic acid both in the control and cholesterol fed animals. This latter finding is contrary to that of Merrill(8) who reported enhanced cholesterol synthesis by rat liver slices from animals fed nicotinic acid. Our data are, however, in agreement with those of Schön and permit a tentative explanation for the observed effect of nicotinic acid in lowering of serum cholesterol levels.

There is now considerable evidence to support a biochemical mechanism to account for the effect of nicotinic acid in lowering serum cholesterol. Reddi and Kodicek(12) using humans have demonstrated that the principal excretion product of intravenously injected nicotinic acid is nicotinuric acid. Miller(13) using hypercholesteremic patients and administering nicotinic acid orally found 20-25% of a 2 g/100 lb body weight dose was excreted as nicotinuric acid. The synthesis of nicotinuric acid has been described(14,15) as follows: nicotinic acid + glycine $\xrightarrow[\text{ATP}]{\text{Co A}}$ nicotinuric acid. Synthesis of cholesterol from acetate *via* mevalonic acid and the other at-

tendant reactions(16) is intimately linked to participation of Coenzyme A (Co A). Thus nicotinic acid could act by effectively competing with the cholesterol synthesizing system for a small pool of free Co A. Nicotinamide is excreted as N^1-methyl nicotinamide, which is formed by reactions not requiring Co A. This may explain the inactivity of orally administered nicotinamide in reducing serum cholesterol. Confirmation for a hypothesis involving competition for Co A is presented by Wagner-Jauregg(17) who demonstrated that acetylation of sulfanilamide by a pigeon liver extract is inhibited by nicotinic acid and other compounds requiring Co A for detoxication. Schön's(9) demonstration of graded inhibition of cholesterol and total lipid synthesis in rats by increasing doses of nicotinic acid indicates diminished lipogenesis from acetyl-Co A. α-Phenylbutyric as well as diphenylbutyric acid, compounds requiring Co A in detoxication reactions, also effectively blocked lipid synthesis. Steinberg and Fredrickson (18) have also demonstrated inhibition of cholesterol synthesis from acetate-1-C^{14} *in vitro,* but failed to correlate their results with a competition for Co A.

Summary. 1) The mechanism by which ingestion of large amounts of nicotinic acid lowers serum cholesterol in rabbits has been investigated. The rate at which cholesterol is synthesized from acetate-1-C^{14} by liver slices from animals on control or cholesterol supplemented diets, with or without nicotinic acid, has been measured. 2) There is marked inhibition of the rate of cholesterol synthesis by liver slices from animals fed nicotinic acid on both control and supplemented diets. Since the principal detoxication product of large doses of nicotinic acid is nicotinuric acid, it is possible that this inhibition of cholesterol synthesis occurs as a direct result of competition of lipid synthesizing and detoxication systems for a limiting amount of Co A in the liver cell.

1. New and Non Official Drugs, *J. Am. Med. Assn.,* 1958, v168, 1773.

2. Parsons, W. B., Jr., Flinn, J. H., *Circulation,* 1958, v18, 489.

3. Altschul, R., *J. Am. Med. Assn.,* 1958, v166, 822.

4. Parsons, W. B., Jr., Flinn, J. H., *ibid.,* 1957, v165, 234.

5. Kraupp, O., Schnetz, E., Stormann, H., *Z. ges. exp. Med.,* 1958, v129, 601.

6. Foreign Letters, *J. Am. Med. Assn.,* 1958, v167, 19.

7. O'Reilly, P. O., *Can. Med. Assn. J.,* 1958, v78, 402.

8. Merrill, J. M., *Circulation Research,* 1958, v6, 482.

9. Schön, H., *Nature,* 1958, v82, 534.

10. Henly, A. A., *The Analyst,* 1958, v82, 286.

11. Mukherjee, S., Alfin-Slater, R. B., *Arch. Biochem. and Biophys.,* 1958, v73, 350.

12. Reddi, K. K., Kodicek, E., *Biochem. J.,* 1953, v53, 286.

13. Miller, O. N., Hamilton, J. G., Goldsmith, G. A., *Circulation,* 1958, v18, 489.

14. Jones, K. M., Elliot, W. H., *Biochem. et Biophys. Acta,* 1954, v14, 586.

15. Sundaram, T. K., Sarma, P. S., *ibid.,* 1956, v22, 547.

16. Dituri, F., Rabinowitz, J. L., Hullin, R. P., Gurin, S., *J. Biol. Chem.,* 1957, v229, 825.

17. Wagner-Jauregg, T., *Experientia,* 1957, v13, 277.

18. Steinberg, D., Fredrickson, D. S., *Ann. N. Y. Acad. Sci.,* 1956, v64, 579.

Received July 31, 1959. P.S.E.B.M., 1959, v102.