globin, averaged only 26%. However, the efficiency of conversion of from 8.3 to 11.75 g of hemoglobin subsequently given by slow intravenous infusion averaged 96%. It is suggested that some physiological, biochemical or artifactual process normally diverts a more or less constant amount of bilirubin from conversion to fecal urobilinogen measurable by Ehrlich's aldehyde reagent in collected stools even when the bilirubin to be converted is significantly augmented.

1. Watson, C. J., *Minn. Med.*, 1956, v39, 467 *et ff.*
2. Shen, S. C., *Arch. Int. Med.*, 1958, v101, 315.
3. Ham, T. H., *ibid.*, 1939, v64, 1271.
4. Ducci, H. J., Watson, C. J., *J. Lab. and Clin. Med.*, 1945, v30, 293.
5. Schwartz, S., Sborov, V., Watson, C. J., *Am. J. Clin. Path.*, 1944, v14, 598.
6. London, I. M., West, R., Shemin, D., Rittenberg, D., *J. Biol. Chem.*, 1950, v184, 351.
7. Gilbertsen, A. S., Lowry, P. T., Hawkinson, V., Watson, C. J., *J. Clin. Invest.*, 1959, v38, 1166.
8. Siedel, W., Möller, H., *Z. Physiol. Chem.*, 1939, v259, 113.
9. With, T. K., Biology of Bile Pigments, Arne Frost-Hansen, Copenhagen, 1954, p. 83.

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Effects of an Unsaturated and Saturated Lipid on Experimental Cholesterol Atherosis in Rabbits. (26078)

JOSEPH M. MERRILL (Introduced by Virgil Lequire)
Research Laboratory, Veterans Administration Hospital and Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tenn.

Much interest has been shown in the relationship between the serum cholesterol and the iodine value of the dietary fat. It has been reported that feeding rabbits a highly unsaturated lipid in a diet that contains cholesterol increases serum cholesterol and amount of cholesterol atherosis(1,2). The purposes of this paper are: (1) to report some effects of adding an unsaturated and a saturated lipid with 2% cholesterol to the diet of rabbits; (2) to advance an explanation for the results.

When either a saturated or unsaturated lipid was fed to rabbits eating a diet containing cholesterol, higher values of serum cholesterol were produced. Increased amounts of cholesterol were also found in the aortas. Highest cholesterol values were observed in rabbits consuming the most unsaturated lipid. With addition of either lipid to the diet, fecal excretion of Lieberman Burchard chromogens and 3-β hydroxy sterols fell.

Method. Adult male rabbits were divided into 4 groups. Group I, the control group, was fed stock rabbit chow (Purina Rabbit Chow Checkers), Group II, the same stock diet with 2% cholesterol; Group III, stock diet with 2% cholesterol and 10% coconut oil, and Group IV, stock diet with 2% cholesterol and 10% linoleic acid (Fisher). The iodine number of the coconut oil fed was 9, and of the linoleic acid 130. These diets were continued for 8 weeks. Body weight and total serum cholesterol were determined at weekly intervals(3). During a 24 hour period of each week of experimental diets, fecal collections were made from the rabbits in Groups II, III, IV and Lieberman-Burchard chromogens, 3-β hydroxy sterols, and bile acids were measured(4). After 8 weeks the animals were killed and liver and aorta removed. The 3 cm of the aorta just distal to the aortic valve and a sample of liver were taken for cholesterol analysis. These tissues were homogenized, extracted with chloroform and total cholesterol estimated by the Kingsley and Shaffert Technic(5).

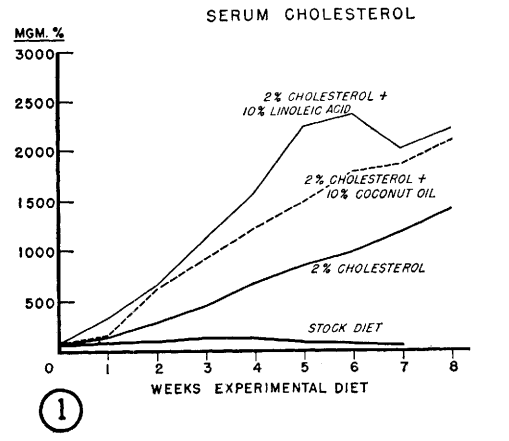
Results and discussion. The effect of the various diets on serum cholesterol is shown in Fig. 1. With addition of 2% cholesterol to stock diet the usual progressive rise in serum cholesterol was observed. With addition of either a saturated (coconut oil) or an unsaturated lipid (linoleic acid) to the diet con-

TABLE I. Effects of Unsaturated and Saturated Fats on Serum and Tissue Cholesterol.

Group	No. of animals	Final serum cholesterol, mg/100 ml	Aorta cholesterol	Liver cholesterol	% change TBW during exp. period, %
			mg/100 g fresh tissue		
I Controls	6	52 ± 6.2*	134 ± 16.7*	286 ± 27.9*	−11.0
II 2% cholesterol	12	1408 ± 138	211 ± 28.7	2280 ± 375	− 8.5
III *Idem* + 10% coconut oil	12	2005 ± 226	253 ± 30.7	2190 ± 445	− 1.3
IV " + linoleic acid	12	2390 ± 325	346 ± 64.2	6240 ± 875	−13.0

* ± S.E.

taining 2% cholesterol a further increase in average serum cholesterol was observed. Final average serum cholesterol for Group II was 1400 mg/100 ml; Group III, 2075 mg/100 ml; and in Group IV, 2200 mg/100 ml. It was concluded from these data that addition of a lipid to the diet containing cholesterol increased serum cholesterol whether the lipid was saturated or unsaturated.



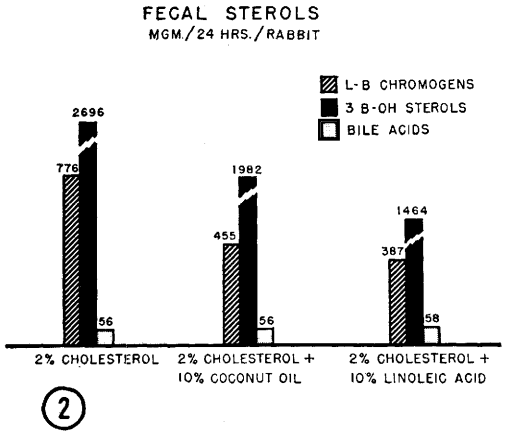


FIG. 1. Effect of 2% cholesterol with added lipids on serum cholesterol of rabbits.

FIG. 2. Effect of various diets on excretion of certain fecal sterols.

Table I summarizes results of the tissue analyses for cholesterol. The highest aortic tissue cholesterol was observed in those animals that consumed 2% cholesterol with the most unsaturated lipid. The rabbits that ate the diet containing cholesterol and coconut oil had the next highest average aortic tissue cholesterol. It was therefore apparent that addition of a lipid to the diet of rabbits increased aortic tissue cholesterol above that observed when an equivalent amount of cholesterol was eaten without added lipid.

Liver tissue studies (Table I) revealed the liver of the rabbits on the stock diet to contain 286 mg cholesterol/100 g of fresh tissue. Addition of 2% cholesterol to the diet caused an 8 fold increase in liver cholesterol. When coconut oil was added to the diet no further increase in liver cholesterol was found but addition of linoleic acid further increased liver cholesterol so that 6% of total weight of the liver was cholesterol.

A possible explanation for the effect of the added lipid on serum and tissue cholesterol was found in studies on excretion of fecal sterols (Fig. 2). Animals eating the diet of 2% cholesterol excreted approximately 85% more Lieberman-Burchard chromogens than did animals eating the diets with added fat. Animals eating the diet containing 2% cholesterol excreted 56% more 3-β hydroxy sterols than did animals whose diet contained added fat. Despite these differences in excretion of cholesterol and 3-β OH sterols, bile acid excretion remained essentially the same in the 3 groups of animals studied. These results suggest that addition of either a saturated or an unsaturated lipid to the diet of the rabbit containing cholesterol decreases excretion of cholesterol. This probably means that addition of lipid to the diet in-

creased absorption of the cholesterol and that a considerable portion of this cholesterol was retained. The fact that the animals eating the linoleic acid excreted the least amount of 3-β hydroxy sterols and Lieberman-Burchard chromogens and had the highest concentration of cholesterol in the liver suggests that linoleic acid was the more effective of the two lipids in promoting cholesterol absorption.

Summary. Adding fat to the diet of the rabbit which contains cholesterol increased serum cholesterol. The increased serum cholesterol was associated with increased aortic tissue cholesterol. It is proposed that addition of the lipid to the diet increased absorption and retention of cholesterol and this intensified the cholesterol atherosis.

1. Rona, G., Chappel, C. I., Gaudry, R., *Can. J. Biochem. Physiol.*, 1959, 37.
2. Merrill, J. M., *Fed. Proc.*, 1959, v18, 1948.
3. Pearson, S., Stern, S., McGavack, T. H., *J. Clin. Endocrinol.*, 1952, v12, 1245.
4. Merrill, J. M., *Cir. Res.*, 1959, v7, 709.
5. Kingsley, G. R., Schaffert, R. R., *J. Biol. Chem.*, 1949, v180, 315.

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In vivo Tumor Inhibitory Activity of Normal Human Serum.* (26079)

ERNEST P. NOBLE AND AUSTIN S. WEISBERGER
Department of Medicine and Biochemistry, Western Reserve University, Cleveland, Ohio

During the past few years increasing interest has been focused on the effect of serum and its components on normal and malignant animal cells. Willheim *et al.*(1,2) have shown that normal human serum is able to digest Ehrlich ascites and other tumor cells. Serum obtained from schizophrenic patients was shown by Federoff(3) to be toxic to strain L cells. Bolande and co-workers(4,5), have demonstrated cytotoxic action of serum for HeLa cells, U-12 cells of human origin, Ehrlich ascites and Sarcoma 180 cells. Björklund(6) has presented evidence for presence of a cytotoxic factor in normal serum coupled to an inhibitor capable of rapid and complete destruction of human tumors *in vitro*. The studies presented here, initiated 3 years ago, deal with comparisons of *in vivo* tumor inhibitory activity of sera from normal individuals and sera from patients with diseased states on Sarcoma 180 cells. It was found that normal human serum exerted a greater inhibitory effect on Sarcoma 180 ascites tumor growth than sera from patients with leukemia and related diseases. Attempts were also made at partial isolation and characterization of the normal human serum components toxic to the tumor cells.

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Materials and methods. Donor CFW mice with Sarcoma 180 tumor in the ascitic form were sacrificed under ether anaesthesia, 6-8 ml of non-hemorrhagic peritoneal fluid was withdrawn and immediately placed in 1.0 ml saline and 0.2 ml heparin (1000 USP units/ml). A cell count was made on an aliquot of the tumor suspension, and the remainder diluted with cold pH 7.4 isotonic saline so that each inoculum of 1 ml contained 2 x 10^6 cells.

Blood was withdrawn from healthy subjects and from patients prior to initiation of therapy. Rabbit, guinea pig, mouse and rat blood were obtained by cardiac puncture; and bovine, porcine, calf and lamb blood collected from the jugular vein. Defibrination was immediately carried out by agitating blood with glass beads and the cellular elements centrifuged at 3000 g in the cold. The supernatant was aspirated and frozen at −70°C. All subsequent handling of the sera and their fractions was carried out rapidly between 0 and 3°C.

A 90% saturated ammonium sulfate solution of normal human serum (NHS) was prepared by addition of solid ammonium sulfate,