The Anomalous Solubility of Cholesterol in Oils.* (30753)

LEMUEL D. WRIGHT
Graduate School of Nutrition and Section of Biochemistry and Molecular Biology, Cornell University, Ithaca, N. Y.

Wright and Presberg recently showed(1,2) that the solubility of cholesterol in a typical hypercholesterolemic oil (coconut oil) may be decreased by the presence of certain dicarboxylic acids or by imidazole. Decreases in the solubility of cholesterol are accomplished by the formation of insoluble crystalline clathrates involving cholesterol and dicarboxylic acid or imidazole in a 1:1 molar ratio.

Solubility studies have now been extended to include similar studies with a variety of natural and synthetic oils. These studies indicate that, irrespective of the type of oil or the clathrate-forming agent employed, insoluble clathrates are formed only when the initial concentration of cholesterol is greater than one-half of saturation. A tentative explanation for the phenomenon observed, together with some implications with respect to cholesterol metabolism, especially with respect to possible mechanisms whereby cholesterol becomes deposited within the cardio-vascular system, such as occurs in atherosclerosis and related conditions, are presented here.

Materials and methods. Solubility studies were carried out as previously described(2). With each oil studied 2 levels of cholesterol were used, amounts just sufficient to saturate the oil with cholesterol, previously determined in a preliminary experiment, and amounts sufficient to one-half saturate the oil with cholesterol. The cholesterol-C^{14} used had approximately 290 dpm/mg. It was demonstrated by the method of solubility that the cholesterol contained approximately 5% of a radioactive impurity. Separate studies where the cholesterol was determined by the Liebermann-Burchard reaction demonstrated that the radioactive method of determination generally employed is a valid measure of cholesterol concentration. The sample of MCT (medium chain triglycerides) was obtained from the Drew Chemical Corp., New York City. The other oils were readily available commercial products.

Results and discussion. As indicated by the data of Fig. 1, the solubility of cholesterol in a variety of natural and synthetic oils is decreased by the presence of pimelic acid, methyl malonic acid, or imidazole as examples. Solubility of cholesterol in the oils studied varied over a 100% range from about 2.5% in tributyrin to about 5.0% in MCT. In general, at cholesterol levels between one-half and full saturation, for every increment in compound tested a corresponding amount of cholesterol was precipitated. Deviations from a theoretical line sometimes occurred such as with pimelic acid and tributyrin. Such deviations probably indicate that the cholesterol clathrate is not completely insoluble in the particular oil. With both dicarboxylic acids and imidazole, precipitation of cholesterol occurred until the cholesterol level in solution became one-half of saturation. No further precipitation occurred in any case with large increases in the level of added clathrate-forming agent.

As pointed out previously, the dicarboxylic acids and imidazole that are active in forming insoluble clathrates with cholesterol are relatively insoluble in oils. For example, the solubilities of pimelic acid, methyl malonic acid, and imidazole in coconut oil at 37°C are approximately: 0.0094, 0.046, and 0.075 mole per liter, respectively, where the solubility of cholesterol is approximately 0.118 mole per liter. These data permit the calculation of "solubility products" for the clathrates involving the products of the concentrations of cholesterol and clathrate-forming agents in saturated solutions of the clathrates. The "solubility products" for the clathrates of cholesterol and pimelic acid, methyl malonic acid, and imidazole are, respectively:

---

* Supported by research grant G-3327 from Nat. Science Foundation, by research grants AI-01891 and HE-04138 and career award K6-AM-16,612 from Nat. Inst. Health, and by funds made available through the State Univ. of New York.

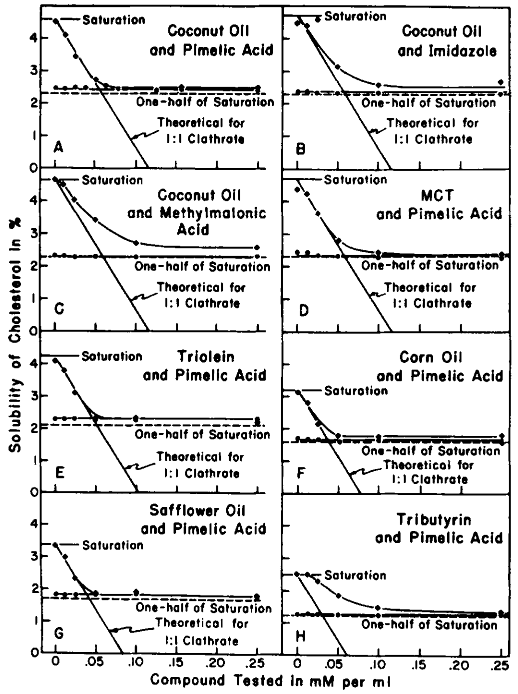


FIG. 1. Effect of dicarboxylic acids or imidazole on solubility of cholesterol in various oils. ♦—♦—♦ indicates that cholesterol was originally present in these tubes at a level of saturation in the oil. -●-●-●- indicates that cholesterol was originally present in these tubes at a level of one-half saturation in the oil.

0.0094 × 0.059 = 0.00055; 0.046 × 0.059 = 0.0027; and 0.075 × 0.059 = 0.0044. Thus in the case of coconut oil alone these "solubility products" vary over an 8-fold range (0.0044/0.00055 = 8) and are exactly proportional to the solubility of the clathrate-forming agent. It seems a statistical improbability that with a number of clathrate-forming agents and with a number of natural and synthetic oils that the "solubility products" would be such that in every case cholesterol is precipitated only at a concentration greater than exactly one-half of saturation. Furthermore, at nearly saturated solutions of cholesterol and low levels of clathrate-forming agents precipitation of clathrates occurs where the product of cholesterol concentration and clathrate-forming agent is less than the "solubility product." Clearly a simple "solubility product" relationship is not the explanation for the phenomenon observed.

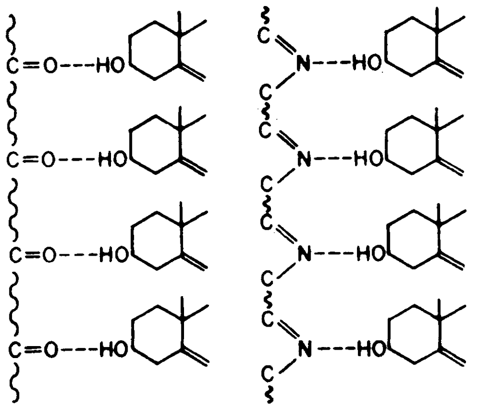
A second possibility that might explain the present results is that clathrate formation occurs only between dissolved cholesterol and undissolved clathrate-forming agent. In such a case the formation of clathrates would be a function of the cholesterol concentration alone. It is conceivable that this critical concentration could vary directly with the solubility of cholesterol in an oil and actually be one-half of the solubility of cholesterol in the particular oil. An objection to this explanation is that with several clathrate-forming agents, for example imidazole (Fig. 1, B), clathrate formation occurs at concentrations of clathrate-forming agent below the level of saturation of the clathrate-forming agent in the oil.

A third possible explanation for the present results is that the clathrates form at all levels of cholesterol concentration, are insoluble at cholesterol concentrations greater than one-half of saturation, and are soluble at cholesterol concentrations that are one-half of saturation or less. This possibility was explored by carrying out the following sequence of experiments.

The clathrate of cholesterol and pimelic acid was first prepared. The clathrate, together with the excess pimelic acid was then centrifuged out. Sufficient additional cholesterol was then added to the supernatant solution to bring the "total" cholesterol concentration to saturation. No further clathrate formation occurred indicating that the cholesterol remaining in solution after the precipitation of clathrate is not present as "soluble clathrate." The actual counts were as follows: supernatant solution following the equilibration of coconut oil with cholesterol-C^{14} just sufficient to saturate the oil, 6340 cpm/g; supernatant solution following the equilibration of coconut oil with cholesterol-C^{14} just sufficient to saturate the oil together with a large excess of pimelic acid, 3480 cpm/g; supernatant solution following the equilibration of the previous supernatant solution after addition of sufficient cholesterol-C^{14} to raise the "total" cholesterol to full saturation, 6890 cpm/g.

A fourth possible explanation for the present results is that cholesterol is present in oils at saturation in two states of dispersion, a less stable form that yields insoluble clathrates with appropriate dicarboxylic acid or imidazole and a more stable form that does not yield clathrates. The results would suggest that at saturation the two forms are present in equal amounts. Obviously some physico-chemical determination must be employed to substantiate or refute this explanation with certainty.

It would appear from the specificity studies that have been carried out(1,2) that the clathrate-forming agent must be of such a molecular size that the susceptible cholesterol molecules are held at a favorable spacing for crystallization, presumably involving hydrogen bonding, to occur. This concept is shown diagrammatically as follows:



Although it is unlikely *in vivo* that tissue concentrations of free dicarboxylic acids or imidazole would ever exist that would be involved in clathrate formation, it is conceivable that hydrogen bond formation between cholesterol and tissue components containing regularly spaced atoms capable of sharing the hydroxyl hydrogens of cholesterol could occur. If such a phenomenon is the explanation for the precipitation of cholesterol under certain pathological conditions, it would appear that insoluble clathrate formation would be restricted to free cholesterol occurring in tissue lipids over and above one-half of saturation. Practical methods for the prevention of undesirable cholesterol deposits might be directed to means of keeping the cholesterol concentration of body fats below the level of one-half of saturation.

Summary. Insoluble-clathrate formation occurs between cholesterol and certain dicarboxylic acids or imidazole in a number of natural or synthetic triglycerides only when the initial concentration of cholesterol is in excess of one-half of saturation. The possibility that cholesterol is present in oils in two separate states of dispersion is discussed.

1. Wright, L. D., Presberg, J. A., Fed. Proc., 1963, v22, 269.

2. ———, Proc. Soc. Exp. Biol. and Med., 1964, v115, 497.

Received September 9, 1965. P.S.E.B.M., 1966, v121.

A Deficient Binding Mechanism for Norepinephrine in Hearts of Scorbutic Guinea Pigs. (30754)

NGEM B. THOA,* RICHARD J. WURTMAN AND JULIUS AXELROD
(Introduced by Sidney S. Chernick)
Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, Md.

It has been demonstrated that the induction of scurvy in guinea pigs is associated with hyperresponsiveness to the pressor and cardiac inotropic effects of injected catecholamines(1).

Such hyperresponsiveness could be the consequence of at least 3 different mechanisms: *1*) A larger fraction of the circulating exogenous catecholamine could be delivered to the cardiovascular receptors(2), due to the vascular changes produced in scurvy. *2*) The

---

* Visiting Scientist. Present address: Dept. of Pharmacology, Howard Univ. Med. School, Washington, D.C.