Solubility of Cholesterol in Various Fats and Oils.* (29172)

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Wilkens *et al*(1) have suggested that the extent of solubility of cholesterol in various fats may be a factor in assessing the cholesteremic properties of each particular fat. Good correlations have been derived between the solubility of cholesterol in various fats, the iodine number of the fats in question and their effects on serum cholesterol levels. A facile correspondence between solubility of cholesterol in a given fat *in vitro* with its cholesteremic properties *in vivo* is questionable in the face of the complex scheme of cholesterol absorption(2). Recent reports on the hypocholesteremic properties of medium chain triglyceride (MCT)(3,4), a liquid fat composed almost exclusively of C_8 and C_{10} triglycerides (iodine number 0.2), also casts doubt on the validity of Wilkens' hypothesis. The possibility arises that the solubility of cholesterol in various fats is a function of the composition of the fat and that, of the fats investigated earlier in dietary experiments, coconut oil contained the fatty acids of shortest chain length. We have carried out a systematic investigation into the solubility of cholesterol in natural and synthetic triglycerides which is the basis of this report.

Our findings that addition of free fatty acids will increase the atherogenicity for rabbits of the usual cholesterol-corn oil regimen (5) also prompted an investigation into the effects of specific fatty acids.

Materials and methods. The technique used was similar to that used by Wright and Presberg(6,7) in somewhat analogous studies. A mixture of 100 mg of cholesterol-4-C^{14} of known specific activity and 2 ml of the oil was shaken for 18 hours in a 25 ml Erlenmeyer flask kept at 37°C in a Dubnoff shaker. The suspension was centrifuged in a 37°C room and an aliquot of the clear supernatant fat drawn off and transferred to a previously weighed vial used for assay in a liquid scintillation spectrometer (TriCarb, Packard Instrument Co., La Grange, Ill.). Radioactive assay was carried out using 0.6% 2.5-diphenyloxazole (PPO) and 0.02% dimethyl 1,4-bis-2-(5-phenyloxazolyl)benzene (dimethyl POPOP) in toluene. The solution of scintillators was added directly to the vial into which the solution of cholesterol in fat had been weighed. Since most of the oils used have some color, the quenching of each oil was determined by weighing a quantity equivalent to that taken for radioactive assay of dissolved cholesterol into a vial, adding 1 ml of a benzene solution of the cholesterol-4-C^{14} used and assaying the radioactivity of this solution.

The cholesterol-4-C^{14} used was prepared by adding radioactive cholesterol of high specific activity (New England Nuclear Corp., Boston, Mass.) to a solution of cholesterol in ethanol, and recrystallizing the resultant radioactive cholesterol to constant specific activity. The cholesterol-4-C^{14} used in this study had a specific activity of 650 cpm/mg.

When the effect of fatty acids was determined, a solution of the pure fatty acid in either corn oil or coconut oil was prepared. Stearic and arachidic acid were not completely soluble in these oils at the 2% level at room temperature and these suspensions were warmed immediately before use. All the fatty acids used were purchased from Calbiochem, Inc., Los Angeles, Calif.

For calculation of the solubility of cholesterol in each oil, the counts recovered, corrected for quenching, were divided by the specific activity of the cholesterol to give mg recovered and this figure divided by the weight of assayed sample. The results were duplicable in all instances.

Results and discussion. The results obtained with a large series of natural fats and oils are presented in Table I. There is a rough correlation with iodine number, but in our hands the levels of solubility are relatively close and the differences do not lend themselves to easy correlation with cholesteremia.

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TABLE I. Solubility of Cholesterol (%) in Various Fats at 37°C.

Fat	Iodine No.	Solubility: Present study	Wilkens et al (1)	Wright and Presberg (6, 7, 9)	Lin *et al* (8)
MCT[a]	.2	5.04		4.69	
Coconut oil[a]	10	4.97	4.33	4.40	
Beef fat	48	4.45	3.71		4.6
Butter oil[b]	40	4.12	3.97		
Chicken fat	65	4.11			
Cocoa butter[c]	38	4.07			
Hydrogenated corn oil[a]	79	4.00			
Goose fat	70	3.94			
Castor oil[d]	88	3.82			
Olive oil[e]	86	3.72	3.59		
Lard[e]	66	3.63			
Corn oil	120	3.59	3.13	3.14	2.7
Rapeseed oil[g]	101	3.57			
Peanut oil[h]	104	3.43	3.29		
Cottonseed oil[c]	110	3.26	3.22		
Soybean oil[c]	134	3.13	3.14		
Safflower oil[j]	140	2.95			
Codliver oil[k]	160	2.70	3.12		

Source of fats and oils: a) Drew Chemical Co., Boonton, N. J.; b) National Dairy Products, Glenview, Ill.; c) Proctor and Gamble Co., Cincinnati, Ohio; d) Jan Laboratories, Philadelphia, Pa.; e) Armour and Co., Chicago, Ill.; f) Corn Products Co., Argo, Ill.; g) Nopco Chem. Canada, Ltd.; London, Ont.; h) Planters Co., Wilkes-Barre, Pa.; j) Pacific Vegetable Oil Co., San Francisco, Calif.; k) Hull and Grimsby Ltd. (British Cod Liver Oils), Hull, England.

The results in Table I are also comparable with solubilities reported by Wilkens(1) and Lin *et al*(8) who used colorimetric assay for cholesterol, and by Wright(6,7,9) who used radioactive assay. In general, the order of solubilities is comparable in all of the studies.

The two fats which show the greatest solubility for cholesterol, MCT and coconut oil, are also the fats which contain the fatty acids of shortest chain length. We conducted another experiment using pure triglycerides. This experiment was limited in scope because only the shorter chain triglycerides (up to C_{10}) are fluid at 37°C. From trilaurin and increasing chain length, all of the available triglycerides are solids until the unsaturated C_{18} triglycerides are reached. Our experimental results with the available glycerides are tabulated in Table II. It is evident that from the peak solubility observed with tricaprin, there is a fall to triolein and trilinolein. Lin *et al*(8) reported that cholesterol was soluble to the extent of 2.7% in triolein at 39°C.

TABLE II. Solubility of Cholesterol (%) in Pure Triglycerides at 37°C.

Triglyceride	Fatty acid chain length	Solubility
Triacetin[a]	2:0	.33
Tripropionin[a]	3:0	1.38
Tributyrin[b]	4:0	2.63
Tricaproin[c]	6:0	3.72
Tricaprylin[c]	8:0	4.38
Tricaprin[c]	10:0	5.47
Triolein[d]	18:1	3.77
Trilinolein[d]	18:2	3.41

Source: a) Calbiochem., Los Angeles, Calif.; b) Fisher Scientific Co., Pittsburgh, Pa.; c) Drew Chemical Co., Boonton, N. J.; d) Proctor and Gamble Co., Cincinnati, Ohio.

The inability to use C_{12} to C_{18} triglycerides in this experiment still left the question of the solubilizing effect of C_{12}-C_{16} fatty acids open. To this end we investigated the effects of fatty acids from capric (C_6) through arachidic (C_{20}) when added (at the 2% level) to either corn or coconut oil. In a preliminary experiment, we had measured the effect of 9% of fatty acids derived from MCT, coconut, corn and hydrogenated corn oils, on the solubility of cholesterol in each of these oils. The 9% figure was chosen because this is the level at which the fatty acids are incorporated in the atherogenic rabbit diets(5). This amount of fatty acid increased solubility of cholesterol in MCT from 5.04 to 8.24%, in coconut oil from 4.97 to 8.83%, in corn

TABLE III. Influence of Fatty Acids (2% on Solubility of Cholesterol (%) in Corn Oil and in Coconut Oil at 37°C.

Solubility Corn oil	Fatty acid added		Solubility, Coconut oil
3.59	None		4.97
4.47	Caproic,	6:0	5.83
4.50	Caprylic,	8:0	5.86
4.52	Capric,	10:0	5.70
4.26	Lauric,	12:0	5.62
4.05	Myristic,	14:0	5.59
4.03	Palmitic,	16:0	5.59
4.01	Stearic,	18:0	5.43
3.79	Arachidic,	20:0	5.32
3.99	Oleic,	18:1	5.44
4.06	Linoleic,	18:2	5.43
4.05	Linolenic,	18:3	5.34
3.97	Erucic,	22:1	5.25

oil from 3.59 to 5.20% and in hydrogenated corn oil from 4.00 to 5.99%. Wright and Presberg(6) had reported that 10 to 50 mg amounts of stearic, linoleic and linolenic acids increased the solubility of cholesterol in corn and coconut oils. We chose 2% incorporation of fatty acid in the various oils as an arbitrary, workable level. Table III details the effects of fatty acids of varying chain length on cholesterol solubility in corn and coconut oils. The short chain (C_6-C_{10}) fatty acids have the greatest cholesterol solubilizing effect in corn oil, lauric acid (C_{12}) has an intermediate effect and from myristic to stearic acids the solubilizing effect is equal, with an additional drop of efficacy observed with arachidic acid. The unsaturated fatty acids follow a similar pattern. With coconut oil, the fatty acid pattern is similar, but not as sharply defined. This may be due to the composition of coconut oil.

The data indicate that the solubility of cholesterol in various fats and oils is a function of the composition of the fat. Solubility is greatest in fats containing relatively short chain fatty acids (C_6-C_{12}). This has been shown in experiments with both pure and naturally occurring triglycerides. The solubility of cholesterol in coconut oil is decreased by addition of sitosterol(1,6), which is not surprising in view of the relatively low solubility of cholesterol and its structural similarity to sitosterol. Since many of the vegetable oils used by ourselves and the other workers contain between 0.2 and 1.5% sitosterol(10), the effect of sitosterol may be reflected to a slight extent in the lower solubility of cholesterol in these oils. In colorimetric analyses, the lower extinction shown by sitosterol(10) might also affect the results.

It is doubtful whether the solubility of cholesterol in any of these fats exerts a great influence upon their effects on serum cholesterol levels. In their experiments on factors affecting cholesterol absorption, Ivy and his co-workers(8,11) found no correlation between absorption of cholesterol in rats when fed with certain fats and its solubility in those fats at 38-39°C. Thus, the apparent cholesterol absorption in rats fed cholesterol plus corn oil was 64.5% while it was only 46.5% when triolein was the fat. In Ivy's hands, cholesterol was soluble in both of these fats to the same extent (2.7%). We find that cholesterol is slightly more soluble in triolein (3.77%) than it is in corn oil (3.59%). More striking was Ivy's finding that when beef tallow was the dietary fat (solubility—4.6%, ref. 8; 4.45%, this study) apparent cholesterol absorption was 59.6% or roughly what it was with corn oil. When oleic acid was the source of fat, apparent cholesterol absorption was significantly higher than with corn oil, 74.2% compared with 64.5%. The increased absorption of cholesterol under the influence of fatty acids has been observed by others as well(12). The solubility of cholesterol in oleic acid is 22%, almost 10 times its solubility in corn oil. The difference in percentage absorption is not nearly as great as the solubilities. All the data suggest that solubility measurements *in vitro* cannot be used to explain the effects of various fats on serum cholesterol levels.

Summary. The solubility of cholesterol in various fats and oils at 37°C has been studied using radioactive cholesterol. The amount of radioactivity present in the supernatant obtained after an excess of cholesterol-4-C^{14} and the fat have been shaken for 18 hours is used to assay the amount of cholesterol dissolved. Using both pure and natural triglycerides, it has been shown that the fats and oils composed largely of short chain fatty acids (C_6-C_{12}) have the greatest solubilizing effect. Addition of 2% of pure fatty acids (C_6

through C_{20}) to corn oil or coconut oil shows that while all the fatty acids used enhance cholesterol solubility, highest solubility is obtained when C_6, C_8, C_{10}, or C_{12} fatty acids are used.

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Changes in Pituitary LH Concentration during Pseudopregnancy in the Rat.* (29173)

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Accompanying ovulation in the cyclic rat, pituitary LH content falls from maximal values at proestrus to minimal values at estrus(1,2). During pseudopregnancy, when ovulation is suppressed, pituitary folliculotrophin† (FTH) content, as measured by the mouse uterine weight assay, rises above levels found in mixed cyclic pituitaries(3). Since this assay method does not distinguish between FSH and LH, the question of what happens to pituitary LH content during pseudopregnancy has remained unanswered. In the present study direct measurements of pituitary LH concentration were made daily during pseudopregnancy. The ovarian ascorbic acid depletion method was used to assay LH(4); this assay method is not sensitive to FSH or other pituitary hormones(1,4, 5). Pituitary LH concentration was also measured in cyclic rats on the days of proestrus, estrus and the first day of diestrus (metestrus) to provide control data.

Materials and methods. (1) *Donor rats and pituitary collection.* All donor rats were of the Sprague-Dawley strain (obtained from Holtzman Rat Co., Madison, Wis.). They were housed 10 to 20/cage, in a room lighted with natural daylight, and allowed *ad libitum* access to Purina rat chow and water. Vaginal smears were taken daily, except Sunday, until each rat became part of an experimental group, after which smears were taken daily. Pituitaries of cyclic rats were removed on

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