TABLE I. Calcium Lesions in Mice of Related Strains and in Swiss Mice.

Strain	Hearts with calcium	No. of animals examined	% with calcium
DBA/2	60	74	81
C/St	39	56	70
C3H/St	19	56	34
DBA/St	6	26	22
CHI/St	4	29	14
Swiss ICR/Ha	10	112	9

The diffusion of calcium along the epicardium and outer layers of myocardium of Swiss mice (Fig. 3) presented a distribution pattern different from that of the mice previously described. In Swiss mice calcium deposits were less evident and pericardium seemed less involved than in the other strains.

In experiments on the effect of tobacco diets on rodents(3), DBA mice given commercially available tobacco products mixed with ground Purina diet gained less weight than control animals and 9 of 34 mice so treated had calcareous pericarditis as opposed to 1 of 20 control animals which received the Purina chow without the tobacco products. The difference in final weight of the DBA mice was statistically significant at the 0.05% level of probability. Results obtained in the tobacco experiments are consistent with the hypothesis that the lesion seen in DBA mice may represent a response to malnutrition. The presence of calcareous deposits in the hearts of all of these related strains of mice suggests the possibility that the development of this lesion is influenced by genetic susceptibility. Calcareous pericarditis is increased by dietary restrictions as indicated by a lower final weight of tobacco fed mice than the control DBA mice.

Summary. Calcium was found in the hearts of mice of related strains, including DBA and C strains. The distribution of calcium foci in the pericardium and myocardium of the related strains differs from the distribution in Swiss mice. It is proposed that these related mice have a genetic susceptibility to this lesion.

1. Hare, W. V., Stewart, H. L., *J. Nat. Cancer Inst.*, 1956, v16, 889.
2. Strong, L. C., *ibid.*, 1955, v15, 1417.
3. DiPaolo, J. A., *Nature*, 1962, v195, 1316.

Received October 17, 1963. P.S.E.B.M., 1964, v115.

Effect of Certain Compounds on Solubility of Cholesterol in Coconut Oil.*‡ (28951)

LEMUEL D. WRIGHT AND JUDITH A. PRESBERG
Graduate School of Nutrition, Cornell University, Ithaca, N. Y.

Several investigators have reported that cholesterol is absorbed from the gastrointestinal tract only when dissolved in fat(2,3,4). Recently Wilkens *et al*(5) have reported a correlation coefficient of 0.982 between the solubility of cholesterol in a series of natural fats and the hypercholesterolemic effect of these fats in man. Wilkens *et al* have suggested as a working hypothesis ". . . that the solubility of cholesterol in different fats may be the characteristic property which determines their effect on the serum cholesterol concentration." Thus, they would suggest that the continued ingestion of corn oil is associated with relatively low levels of blood cholesterol because the solubility of cholesterol in corn oil is relatively low. Conversely, they would suggest that continued ingestion of coconut oil is associated with relatively high levels of blood cholesterol because the solubility of cholesterol in coconut oil is relatively high.

If the hypothesis of Wilkens *et al* is the explanation for the effect of different fats on serum cholesterol concentration, it is appar-

* Supported by research grants from Nat. Science Foundation, Nat. Inst. Health, and by funds made available through State University of New York.

‡ A preliminary report of this work has been presented(1).

ent that fats being absorbed from the intestinal tract must be saturated with cholesterol at time of absorption, or that cholesterol is better absorbed from fats in which it is more soluble. In either case, it would appear that if the solubility of cholesterol in fats could be reduced by even a small amount as a consequence of the presence of some extraneous compound, then this compound might be useful in preventing absorption of cholesterol and the associated hypercholesterolemia. In addition, a knowledge of factors that influence the solubility of cholesterol in fats could lead to a better understanding of why cholesterol precipitates in various parts of the circulatory system as, for example, in atherosclerosis or xanthomatosis.

This paper describes the development of a simple assay procedure for determining the effect of compounds on the solubility of cholesterol in various oils. In these studies, in addition to confirming the fact that the solubility of cholesterol in coconut oil is reduced by the presence of β-sitosterol, it has been shown that the solubility of cholesterol in coconut oil is reduced by the presence of certain dicarboxylic acids or by imidazole. Some studies of the specificity and mechanism of the effect have been made and other information accumulated.

Materials and methods. Cholesterol-4-C^{14} of relatively low radioactivity was prepared by evaporating to dryness on the steam bath a solution prepared from 100 g cholesterol (Distillation Products Industries, Rochester, N. Y.) and 100 μg (10 μc) cholesterol-4-C^{14} (benzene solution, New England Nuclear Corp., Boston, Mass.) in 500 ml of chloroform. The product was pulverized in a laboratory mortar before use. Two different lots were prepared. One lot had approximately 187 cpm (374 dpm)/mg; the other lot had approximately 145 cpm (290 dpm)/mg.

The coconut oil used was N.F. IX grade obtained from Magnus, Mabee and Reynard, Inc., N.Y.C. The corn oil used was a commercial preparation of 'Mazola' from Best Foods, N.Y.C. The mineral oil used was a white paraffin oil from the General Chemical Division, Allied Chemical Corp., N.Y.C.

Most of the compounds studied were commercial products obtained primarily from Distillation Products Industries, Rochester, N. Y.; Aldrich Chemical Co., Inc., Milwaukee, Wisc.; Nutritional Biochemicals Corp., Cleveland, Ohio; or General Biochemicals, Inc., Chagrin Falls, Ohio. In a few instances, because of obvious impurities, it was necessary to recrystallize compounds prior to use.

We are indebted to the following individuals at Cornell University, Ithaca, N. Y., for compounds studied that are not commercially available: Dr. James L. Gaylor for a purified sample of β-sitosterol and for a number of cholesterol esters; Dr. A. T. Blomquist for samples of 3,3,3′,3′-tetramethyladipic acid, 2,2,2′,2′-tetramethylpimelic acid, and 5,5-dimethylazelaic acid; Dr. Yvonne C. Meinwald for 2,2-dimethylpimelic acid; and to Mr. John C. Tsibris for 2-methylpimelic acid.

The solubility studies were carried out according to the following general procedure: 100 mg amounts of cholesterol-4-C^{14} were weighed out into 100 mm $\times$ 13 mm stoppered pyrex test tubes. Various amounts of the compounds to be studied with respect to influence on the solubility of cholesterol were added to the tubes containing the dry cholesterol. Two ml of freshly melted coconut oil or, in some instances, corn oil, mineral oil, or oil mixtures were added to each tube. The tubes were then stoppered tightly and placed in a specially constructed apparatus that rotated the tubes at a rate of 60 rotations per minute. Rotation with incubation at 37° was continued for approximately 18 hours. Following this equilibration period the tubes were cooled at 5° for 6-8 hours to obviate instances of supersaturation. The tubes were then equilibrated at 37° for an additional 18 hours. After this second equilibration, the mixtures were centrifuged for 15 minutes in a clinical centrifuge maintained at 37°. Approximately 1 ml aliquots of the supernatant solution from each tube were transferred by disposable pipettes to tared counting vials. After weighing, 10 ml of scintillation solution (4 g PPO (2,5-diphenyloxazole) and 30 mg POPOP (1,4-di-2(5-phenyloxazoyl)-benzene per liter of toluene) were added to each vial and the solutions were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer.

Calculations were made of the solubility of cholesterol in the oil making the assumption that the weighed aliquot taken was entirely coconut oil rather than a mixture of coconut oil, cholesterol, and, in most instances, a compound studied with respect to influence on solubility. Since the solubility of cholesterol in coconut oil is in the order of 4% and, in most instances, compounds were tested at a level of 2% or less, it is apparent that the results are not in error by more than 6% as a consequence of this assumption.

In the random screening of compounds for activity, the compounds were examined at a level of 40 mg of compound per tube (20 mg per ml of coconut oil). In the evaluation of acids having structural relationships to the dicarboxylic acids found in the present work to have an influence on the solubility of cholesterol, the acids were examined at a level of 0.25 mmoles per tube (for pimelic acid 40 mg equals 0.25 mmoles). It was established that quantities up to at least 2 ml of coconut oil, of the grade used, do not influence the counting rate of cholesterol-4-C^{14} in the scintillation solution used. Some of the compounds studied showed quenching effects and it was necessary in these cases to make appropriate corrections with the use of an internal standard. None of the compounds described as "active" in the present work, however, showed a quenching effect.

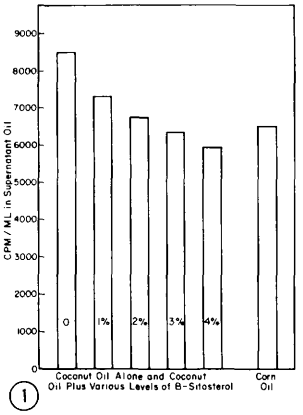
The procedure described was designed primarily for detection of compounds that might be associated with a *reduction* in the solubility of cholesterol in coconut oil. It became apparent during the studies that certain compounds were associated with an *increase* in solubility of cholesterol in coconut oil. Where it was necessary to determine the extent of this effect, 120 mg of cholesterol were used per tube instead of 100 mg, since 100 mg frequently did not provide an excess of cholesterol that is essential for determination of the solubility. In certain of the studies to be described, an amount of cholesterol just sufficient to saturate the coconut oil was used. This amount was found to be 90 mg per tube or 4.5%.

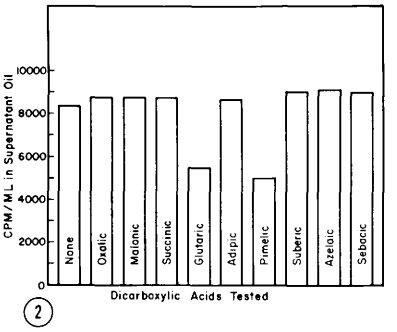
Results. Effect of β-sitosterol on solubility of cholesterol in coconut oil. As shown in Fig. 1, the solubility of cholesterol in coconut oil is reduced by the presence of small amounts of β-sitosterol. In fact, in the presence of 4% β-sitosterol the solubility of cholesterol in coconut oil is reduced to a level below that of cholesterol in corn oil. In the present studies, a considerably smaller effect with β-sitosterol was observed than that previously described by Wilkens *et al*(5) who reported that in the presence of β-sitosterol cholesterol is virtually insoluble in coconut oil. However, in the studies of Wilkens *et al* the coconut oil was saturated with β-sitosterol before the addition of cholesterol and, furthermore, cholesterol was equilibrated with the mixture for only 2 hours before assay of the supernatant solution for cholesterol content. Quite possibly 2 hours is too short an equilibration period for an accurate determination of solubility, particularly when the oil was previously saturated with a compound (β-sitosterol) that presumably competes with cholesterol for solubility sites.

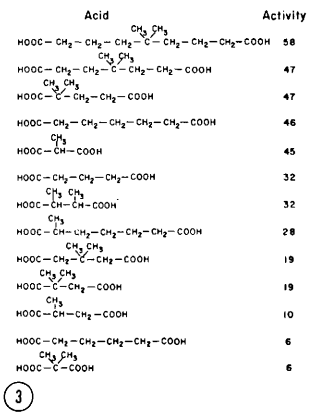
Many other sterols were examined (see subsequent section) for effect on the solubility of cholesterol. The effect of β-sitosterol is apparently specific since even a compound as closely related to β-sitosterol as stigmasterol, for example, is without effect on the solubility of cholesterol.

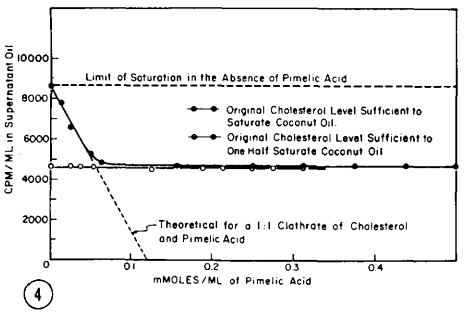
Effect of various dicarboxylic acids on solubility of cholesterol in coconut oil. During the random screening of compounds for capacity to reduce the solubility of cholesterol in coconut oil, it was observed that the equilibration of coconut oil, cholesterol, and certain dicarboxylic acids resulted in the formation of crystalline precipitates and considerable reductions in cholesterol content of the supernatant oil. Fig. 2 shows the influence of dicarboxylic acids from oxalic through sebacic. Subsequently, dicarboxylic acids through 1,12-dodecanedicarboxylic acid, with the exception of 1,9-nonanedicarboxylic acid, have been studied. As far as the unsubstituted dicarboxylic acids are concerned, the effect is essentially limited to glutaric and pimelic acids.

Certain methyl and particularly *gem*-di-









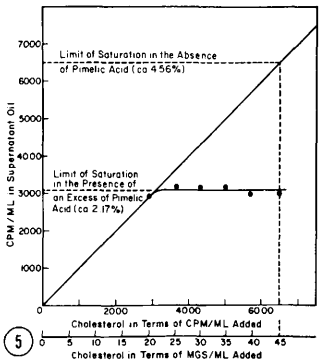


FIG. 1. Solubility of cholesterol in coconut oil and corn oil and effect of β-sitosterol on solubility of cholesterol in coconut oil. Mixtures equilibrated contained 100 mg cholesterol-4-C^{14}, the indicated amounts of β-sitosterol, and 2 ml coconut oil or corn oil. Solubility of cholesterol in coconut oil in absence of β-sitosterol is approximately 4.5%. Solubility of cholesterol in corn oil is approximately 3.4%.

FIG. 2. Effect of various dicarboxylic acids on solubility of cholesterol in coconut oil. Mixtures equilibrated contained 100 mg cholesterol-4-C^{14}, 0.25 mmoles of dicarboxylic acid, and 2 ml coconut oil. Solubility of cholesterol in coconut oil is approximately 4.5%.

FIG. 3. Relative activity of various dicarboxylic acids in reducing solubility of cholesterol in coconut oil. Activity is expressed as per cent reduction in cholesterol content of supernatant oil following equilibration of 100 mg cholesterol-4-C^{14}, 0.25 mmoles of dicarboxylic acid, and 2 ml coconut oil.

FIG. 4. Effect of pimelic acid on solubility of cholesterol in coconut oil. Mixtures equilibrated contained either 90 mg cholesterol-4-C^{14}, indicated amounts of pimelic acid, and 2 ml coconut oil (series involving cholesterol just sufficient to saturate coconut oil) or 90 mg cholesterol-4-C^{14}, indicated amounts of pimelic acid, and 4 ml coconut oil (series involving cholesterol sufficient to one-half saturate coconut oil).

FIG. 5. Effect of an excess of pimelic acid on solubility of cholesterol in coconut oil. Mixtures equilibrated contained indicated amounts of cholesterol-4-C^{14}, 0.25 mmoles pimelic acid, and 2 ml coconut oil.

methyl† substituted dicarboxylic acids yield precipitates with cholesterol relatively insoluble in coconut oil. It was observed that a quantitative method for evaluation of acids was available if the per cent of cholesterol removed from solution following the equilibration of cholesterol (100 mg), acid (0.25 mmoles, 40 mg in the case of pimelic acid), and coconut oil (2 ml) was calculated. Fig. 3 summarizes the active acids that have been discovered in decreasing order of activity starting with the most active acid found, 5,5-dimethylazelaic acid. Despite the fact that 5,5-dimethylazelaic acid is quite active, azelaic acid itself is completely inactive. Similarly, methylmalonic acid is active, yet malonic acid is completely inactive. A summary of inactive acids is given in a subsequent sec-

† The prefix *gem*-(*L. geminus* twin) refers to 2 like atoms or groups attached to the same carbon atom.

tion. Structural requirements for activity cannot easily be summarized. In general, however, *gem*-dimethyl substitution usually leads to increased activity, whereas monomethyl substitution may lead to either increased or decreased activity. The presence of either ethyl, propyl, phenyl, cyclotetramethylene, amino, keto, or additional carboxyl group in an otherwise active acid (glutaric acid) is associated with loss of activity. The crystal structures of the precipitates obtained have varied. For example, the crystals obtained with 5,5-dimethylazelaic acid are particularly granular, whereas with dimethylmalonic acid, an acid not particularly active, a gelatinous mass is obtained. Similarly, the time required for the formation of crystals varies. With methylmalonic acid crystals are obtained in a matter of minutes. Crystals with pimelic acid form in 4 to 6 hours while crystals with 5,5-dimethylazelaic acid were not encountered prior to the period of refrigeration.

In an attempt to learn more of the nature of the precipitate involved, the amount of dicarboxylic acid was varied in the presence of an amount of cholesterol just sufficient to saturate the coconut oil. Fig. 4 shows results obtained with pimelic acid. At levels of added pimelic acid up to about 0.05 mmoles/ml for every increment in pimelic acid a similar increment in cholesterol is precipitated. These results strongly suggest the formation of an intermolecular complex involving equimolar amounts of cholesterol and dicarboxylic acid. The precipitates disappear on extraction with water which would remove free dicarboxylic acid suggesting that the complex is a clathrate of cholesterol and dicarboxylic acid rather than a covalent compound. A precipitate does not form with any level of pimelic acid if the original concentration of cholesterol is one-half of saturation.

Subsequent experiments have been carried out in which the amount of dicarboxylic acid added to tubes has been maintained constant at 0.25 mmoles/tube and the amount of cholesterol varied. Fig. 5 shows results obtained in the presence of pimelic acid in excess (0.25 mmoles/tube). Under these conditions, it is impossible to attain a concentration of cholesterol in coconut oil greater than about 2.17% although in the absence of pimelic acid a concentration of about 4.56% is readily obtained. Similarly, in the presence of methylmalonic acid in excess, it is impossible to attain a concentration of cholesterol in coconut oil greater than about 2.51%.

Mention should be made of the fact that many of the dicarboxylic acids studied are relatively insoluble in coconut oil. For example, the solubility of pimelic acid in coconut oil is only approximately 1.5 mg/ml (0.01 mmoles/ml), the solubility of methylmalonic acid is approximately 5.6 mg/ml (0.046 mmoles/ml), while the solubility of 3,3-dimethylglutaric acid is approximately 7.0 mg/ml (0.044 mmoles/ml). On the basis of only a few determinations, no simple relationship was apparent between the solubility of an acid in coconut oil and the effect of the acid on the solubility of cholesterol in coconut oil.

Effect of imidozole and related compounds on solubility of cholesterol in coconut oil. The only compound thus far discovered that is associated with a decrease in the solubility of cholesterol in coconut oil other than β-sitosterol or a carboxylic acid is imidazole. As with the dicarboxylic acids that have been studied, no level of imidazole is associated with a reduction in the solubility of cholesterol greater than about 50%.

Compounds related in structure to imidazole such as benzimidazole, 5,6-dimethylbenzimidazole, histidine, urocanic acid, or adenine are completely inactive. Imidazole and dicarboxylic acids are not synergistic with respect to influence on the solubility of cholesterol in coconut oil. In fact, cholesterol is a little more soluble in the presence of both imidazole and pimelic acid than it is in the presence of either compound alone.

Effect of free fatty acids on solubility of cholesterol in coconut oil and in corn oil. During the screening of compounds that might be useful in reducing the solubility of cholesterol in hypercholesterolemic oils it was observed that cholesterol is more soluble in coconut oil or corn oil supplemented with small amounts of free fatty acids than it

TABLE I. Effect of Certain Acids on Solubility of Cholesterol in Coconut and Corn Oils.

Exp No.	Oil	Supplement	Cholesterol: Cpm in supernatant solution	Cholesterol: % solubility
1	Coconut	None	8565*	4.54
	"	10 mg Stearic acid	8890	4.71
	"	20 " " "	9155	4.85
	"	30 " " "	9335	4.95
	"	40 " " "	9500	5.09
	"	50 " " "	9640	5.11
2	Corn	None	4720†	3.26
	"	10 mg Stearic acid	4800	3.31
	"	20 " " "	4920	3.42
	"	30 " " "	5120	3.53
	"	40 " " "	5360	3.70
	"	50 " " "	5450	3.76
3	Coconut	None	6300†	4.35
	"	10 mg Stearic acid	6820	4.71
	"	20 " " "	7350	5.07
	"	30 " " "	7600	5.24
	"	40 " " "	8050	5.55
	"	50 " " "	8160	5.63
4	Coconut	None	6510†	4.49
	"	50 mg Linoleic acid	7300	5.04
	"	50 " Linolenic "	7330	5.06
	Corn	None	4290	2.96
	"	50 mg Linoleic acid	4840	3.34
	"	50 " Linolenic "	4770	3.29

* Cholesterol used had approximately 187 cpm/mg.
† Cholesterol used had approximately 145 cpm/mg.

Mixtures equilbrated contained 120 mg of cholesterol-4-C^{14}, indicated increments of the acids studied, and 2 ml oil.

is in the unsupplemented oils alone. The effect of some fatty acids on the solubility of cholesterol in coconut or corn oil is summarized in Table I. It is apparent that the solubility of cholesterol in coconut or corn oil is increased by about 10-15% by the presence of as little as 2.5% (50 mg/tube) of either stearic, linoleic, or linolenic acid. The effect is not limited to naturally-occurring fatty acids since the solubility of cholesterol in coconut oil is increased, for example, by phenylacetic acid. The solubility of cholesterol in coconut oil is increased by the presence of a number of other compounds as evidenced by visual inspection, but precise data were not obtained.

Effect of mineral oil on solubility of cholesterol in coconut oil. It has been well known to nutritionists that ingestion of mineral oil is associated with a decrease in absorption of carotene. Presumably the partition coefficient of carotene between mineral oil and other components of the gastrointestinal tract is so high in favor of the mineral oil that mineral oil serves to extract carotene from the tract. Carotene dissolved in mineral oil is then excreted in the feces. The possibility was entertained that cholesterol might be sufficiently soluble in mineral oil that ingestion of mineral oil might lead to a similar extraction of cholesterol from the intestinal tract. The data in Table II show, however, that cholesterol is relatively insoluble in mineral oil. It is apparent that the ingestion of very large amounts of the material would be essential for any significant amount of cholesterol to be excreted by the body as a consequence of solution in mineral oil.

Summary of compounds inactive with respect to a decrease in solubility of cholesterol in coconut oil. Under the experimental conditions described, none of the following compounds is active with respect to *decreasing* the solubility of cholesterol in coconut oil: Acetic acid, acetyl choline, adenine, allantoin, alloxan, *p*-aminobenzoic acid, 1-amino-2-naphthol-4-sulfonic acid, 2-aminopimelic acid, aspirin, azelaic acid, benzimidazole, 2-benzylhydrocinnamic acid, 2-biphenylbutyric acid, 2-bromo-3-methylbutyric acid, *iso*-butyric acid, n-butyric acid, caffeine, *n*-capric acid, *n*-caproic acid, cholestan-3β-ol, Δ^5-cholesten-

TABLE II. Effect of Mineral Oil on Solubility of Cholesterol in Coconut Oil. Comparison of solubility of cholesterol in coconut oil, mineral oil, corn oil.

Oil or composition of oil mixture	Cpm in supernatant solution	% solubility
Coconut oil alone	8355*	4.42
" " 95%, mineral oil 5%	8335	4.41
" " 90%, " " 10%	8320	4.41
" " 80%, " " 20%	8285	4.39
" " 70%, " " 30%	8110	4.30
" " 50%, " " 50%	7445	3.95
Mineral oil alone	2631	1.39
Corn oil alone	6463	3.43

* Cholesterol used had approximately 187 cpm/mg.

Mixtures equilbrated contained 100 mg cholesterol-4-C^{14} and 2 ml coconut oil, mineral oil, corn oil, or coconut oil-mineral oil mixture of the composition indicated.

3-one, Δ^4-cholesten-3-one, cholesteryl acetate, cholesteryl benzoate, cholesteryl butyrate, cholesteryl stearate, cholic acid, *trans*-cinnamic acid, cortisone, cytosine, 1,10-decanedicarboxylic acid, deoxycholic acid, 2,2′-diaminopimelic acid, 3,3-diethylglutaric acid, 3,3-dimethylacrylic acid, 5,6-dimethylbenzimidazole, 2,2′-dimethylglutaric acid, 2,3-dimethylglutaric acid, diphenylacetic acid, 1,12-dodecanedicarboxylic acid, 17*a*-estradiol, estriol, estrone, 3-ethyl-3-phenylglutaric acid, glutamic acid, guanine, heptadecanoic acid, heptanoic acid, histidine, hydrocinnamic acid, hydrocortisone, 8-hydroxyquinoline, isonicotinic acid, 2-ketoglutaric acid, 4-ketopimelic acid, kynurenic acid, lanosterol, lauric acid, malonic acid, menadione, menthol, 2-methylbutyric acid, 3-methyl-3-ethylglutaric acid, 3-methyl-3-hydroxyglutaric acid, 2-methylglutaric acid, 3-methylglutaric acid, 3-methyl-3-phenylglutaric acid, methylsuccinic acid, muconic acid, myristic acid, nicotinamide, nicotinic acid, nonanoic acid, norvaline, octanoic acid, oleic acid, oxalic acid, palmitic acid, pentadecanoic acid, pimelonitrile, pivalic acid, phenylacetic acid, 4-phenylbutyric acid, 6-phenylhexanoic acid, 3-phenyl-3-propylglutaric acid, 4-phenylvaleric acid, 5-phenylvaleric acid, 17*a*-hydroxy pregnenolone, pregnenolone, 1,2,3-propanetricarboxylic acid, progesterone, 17*a*-hydroxy progesterone, quinic acid, stearic acid, stigmasterol, sorbic acid, suberic acid, succinic acid, sulfanilamide, 3,3-tetramethyleneglutaric acid, tridecanoic acid, trimesic acid, 1,11-undecanedicarboxylic acid, undecanoic acid, uracil, uric acid, urocanic acid, *iso*-valeric acid, *n*-valeric acid.

Discussion. The studies described here were originally undertaken in an attempt to find a compound other than β-sitosterol that would decrease the solubility of cholesterol in oils. Although compounds with this property have been found, at least as far as *in vitro* assay is concerned, none would appear to warrant *in vivo* studies since the compounds found appear to reduce the solubility of cholesterol in oils by formation of oil-insoluble clathrates that are decomposed with regeneration of cholesterol on contact with an aqueous phase. β-Sitosterol appears to be unique in that it is both oil soluble and competes with cholesterol for solubility in oils. Whether or not the hypocholesterolemic effect of β-sitosterol(6) can be attributed solely to its effect on cholesterol solubility has not been determined.

The possibilities appear remote of finding dicarboxylic acids that are highly soluble in oils that also form insoluble clathrates with cholesterol. A systematic study of dicarboxylic acids has not been possible because of the general unavailability of such compounds. As far as unsubstituted dicarboxylic acids are concerned, only 2 acids between oxalic acid and 1,12-dodecanedicarboxylic acid (14 carbons), namely glutaric and pimelic acids, are active. Many of the methyl substituted acids that would be desirable to study have never been synthesized. Where it has been possible to study acids containing substitutions other than methyl groups, namely in the glutaric acid series, such substituted acids have been inactive.

Similarly, the compounds described here do not appear to have general utility in removing cholesterol from oils since precipitates with cholesterol are not obtained at cholesterol concentrations that are encountered in natural oils.

On the basis of present knowledge it would appear that, despite the fact that at least 2 of the acids found active in reducing the solubility of cholesterol in oils occur naturally, namely methylmalonic acid and pimelic acid, the precipitation of cholesterol in certain areas of the body cannot be attributed to the presence of free dicarboxylic acids since concentrations that are essential to yield precipitates with cholesterol would not be expected to occur naturally.

Summary. A simple assay method has been developed for determining the effect of various compounds on the solubility of cholesterol in oils. The method depends on the counting of an aliquot of supernatant solution following the equilibration of oil, an excess of cholesterol-4-C^{14}, and compound studied with respect to influence on the solubility of cholesterol. It was confirmed that the solubility of cholesterol in coconut oil is decreased by the presence of β-sitosterol. The effect is specific as far as a large number of

sterols is concerned. The solubility of cholesterol in coconut oil is decreased by the presence of certain dicarboxylic acids and methyl substituted dicarboxylic acids, as well as by imidazole. Active acids thus far discovered in order of decreasing activity are the following: 5,5-dimethylazelaic acid; 4,4-dimethylpimelic acid; 2,2-dimethylglutaric acid; pimelic acid; methylmalonic acid; glutaric acid; 2,2'-dimethylsuccinic acid; 2-methylpimelic acid; 3,3-dimethylglutaric acid; 2,2-dimethylsuccinic acid; and 2-methylsuccinic acid. Approximately 100 miscellaneous compounds, including many other dicarboxylic acids and imidazole derivatives, are not associated with any decrease in the solubility of cholesterol in coconut oil. Free fatty acids in relatively small amounts increase the solubility of cholesterol in coconut oil.

The effect of the active dicarboxylic acids on the solubility of cholesterol is accomplished by the formation of an insoluble crystalline clathrate of cholesterol and dicarboxylic acid. In the case of pimelic acid this clathrate involves cholesterol and pimelic acid in equimolar amounts.

1. Wright, L. D., Presberg, J., *Fed. Proc.*, 1963, v22, 269.
2. Cook, R. P., *Biochem. J.*, 1936, v30, 1630.
3. Pihl, A., *Acta Physiol. Scand.*, 1955, v34, 183.
4. Wilkens, J. A., deWitt, H., *Canad. J. Biochem. and Physiol.*, 1962, v40, 1079.
5. Wilkens, J. A., deWitt, H., Bronte-Stewart, B., *ibid.*, 1962, v40, 1091.
6. *Nutrition Rev.*, 1956, v14, 39.

Received October 17, 1963. P.S.E.B.M., 1964, v115.

Lipid Composition and Synthesis in Rat Liver During Pregnancy and the Puerperium.* (28952)

WARREN N. DANNENBURG, RICHARD L. BURT AND NORMAN H. LEAKE
Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, Wake Forest College and North Carolina Baptist Hospital, Winston-Salem

The composition(1-4) and synthesis(5,6) of liver lipids has been reported for male and female rats, but a paucity of data exists for pregnant and puerperal animals. Some reports(7-9) compare the total liver lipids in non-pregnant and pregnant rats, but they do not relate the concentration of cholesterol, triglycerides, or phospholipids present, or describe these lipids in the puerperium. Most investigations in lipid synthesis during pregnancy are concerned with the origin of fetal lipids(5,10) and tend to neglect the metabolism of the maternal liver. Though data do exist showing hepatic lipogenesis in pregnancy(11-13) they are not in agreement.

This report describes the composition and synthesis of rat liver lipids during pregnancy and the early puerperium, and presents further evidence of the metabolic changes that influence the hyperlipidemia of pregnancy.

Materials and methods. Pregnant albino rats of the Sprague-Dawley strain (Holtzman Co., Madison, Wis.) were obtained 2 days after being sperm positive. Non-pregnant control and sperm positive rats were shipped at the same time so both groups would be subjected to the same conditions during transportation. When the animals were received, they were randomized, placed in cages, and subjected to a 15 hour day and 9 hour night period by use of artificial lighting in order to reduce the differences in the amount of food consumed. Rats were fed a rat diet†

* This investigation was supported by U. S. Public Health Service Grant from Nat. Inst. of Arthritis and Metab. Dis.

† Ingredients of the diet were as follows: non-fat powdered milk, 11.1%; dried brewer's yeast, 4.5%; salt, 1.5%; soy bean oil meal, 15%; ground yellow corn, 10%; 2nd clear wheat flour, 37.5%; wheat bran, 1.5%; alfalfa meal, 2%; fish meal, 10%; calcium carbonate, 0.5%; animal fat containing antioxidant, 5.9%; and vit. A and D supplement.