The Effect of Various Compounds on the Adsorption of Cholesterol in an *in Vitro* System[1] (36153)

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In an attempt to devise *in vitro* adsorption and/or precipitation systems that conceivably might have some analogy to the deposition of cholesterol *in vivo*, the adsorption of cholesterol from solution in triglycerides by various adsorbing agents has been studied (1). Cholesterol is not readily adsorbed from triglyceride solution by Norit. Cholesterol is, however, readily adsorbed from triglyceride solution by Permutit, yielding adsorption isotherms that are "concave upwards," described by $y = ax^n$ where $a < 1$ and $n > 1$. Such adsorption curves are typical of those encountered in "multiple-layer" adsorption. In multiple-layer adsorption, one layer of adsorbate is first attracted with relatively low affinity to an adsorbing surface. The presence of this first adsorbed layer renders the surface more attractive to a second layer than to the first layer, and the presence of this second layer renders the surface more attractive to a third layer than to the second layer, and so on. The accumulation of layer on top of layer at a surface has some of the features of crystallization.

The effect of various compounds on this adsorption of cholesterol by Permutit from triglyceride solution has now been studied. Compounds appear to fall into three classes: (a) those compounds that have no effect on the adsorption of cholesterol; (b) those compounds that are associated with an inhibition in the adsorption of cholesterol, presumably by preempting the adsorption surfaces; and (c) those compounds that enhance the adsorption of cholesterol, possibly by increasing in some way the attraction between cholesterol and Permutit.

Materials and Methods. Procedures that have been described in detail were used in these adsorption studies (1–7). In brief, various amounts (usually 30 mg) of 4-^{14}C-cholesterol and 500 mg of Permutit were weighed out into 13 × 150 mm screw-cap test tubes. Where indicated, 30 mg amounts of various test compounds were added to some of the tubes. Each tube then received 2 ml of safflower oil. The tubes were then rotated at 37° for 18 to 24 hr. Following equilibration, the tubes were centrifuged in a clinical centrifuge at 37°. Aliquots of the supernatant solutions were removed, by disposable pipettes, to tared counting vials and, after weighing, 10 ml of scintillation solution was added to each vial; the aliquots were then counted in a liquid scintillation spectrometer. Results shown in Table I are calculated in terms of 4-^{14}C-cholesterol (cpm/g) in the supernatant solutions. Results shown in Fig. 1 are presented in the conventional manner, with amounts of material in solution at equilibrium (mg/tube) on the abscissa and the amount of material adsorbed (mg/tube) on the ordinate.

The 4-^{14}C-cholesterol was material of relatively low activity, about 145 cpm/mg (290 dpm/mg), as determined with the scintillation solution and scintillation spectrometer used. The miscellaneous compounds tested were commercial material, presumably of good quality. The Permutit was prepared as described by Folin and was obtained from the Eimer and Amend Co. The safflower oil

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TABLE I. Summary of Effect of Various Compounds on the Adsorption of Cholesterol from Triglyceride Solution.

Expt.	Test material	Amounts used (mg/tube)			Supernatant solution (cpm/g)
		Material	Cholesterol	Permutit	
1	—	—	10	500	522
	—	—	20	500	770
	—	—	30	500	933
	—	—	40	500	1060
	—	—	50	500	1210
	—	—	60	500	1360
2	—	—	30	—	2180
	—	—	30	500	925
	Unlabeled cholesterol	30	30	500	712
3	—	—	30	500	925
	β-Sitosterol	30	30	500	651
	Stigmasterol	30	30	500	645
	Lanosterol	30	30	500	878
	Cortisone	30	30	500	1210
	Testosterone	30	30	500	1120
	Pregnenolone	30	30	500	860
	Pregnanedione	30	30	500	945
	Cholestenone	30	30	500	765
	Cholesterol acetate	30	30	500	870
	Cholesterol benzoate	30	30	500	840
4	—	—	30	500	895
	Desmosterol	30	30	500	656
	Cholestane	30	30	500	855
	Δ^7-Cholestenol	30	30	500	575
	Lauric acid	30	30	500	815
	Myristic acid	30	30	500	738
	Palmitic acid	30	30	500	698
	Nicotinic acid	30	30	500	1050
	Isonicotinic acid	30	30	500	975
	Glutamic acid	30	30	500	830
	Caffeine	30	30	500	1130
	Indole	30	30	500	831
5	—	—	30	500	885
	Cortisone acetate	30	30	500	1110
	Deoxycorticosterone	30	30	500	1270
	Hydrocortisone acetate	30	30	500	968
	Progesterone	30	30	500	1020
	Stearic acid	30	30	500	767
6	—	—	30	500	940
	Lauric acid	30	30	500	870
	Myristic acid	30	30	500	835
	Palmitic acid	30	30	500	785
	Stearic acid	30	30	500	785

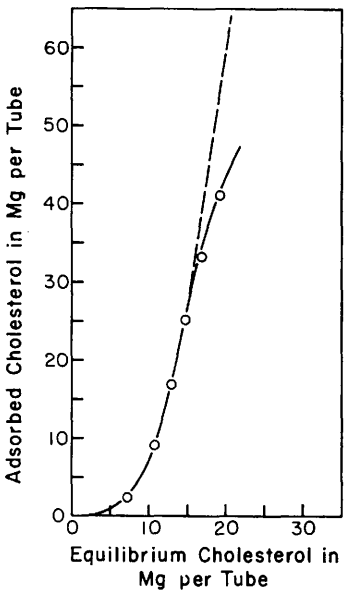

FIG. 1. Adsorption isotherm depicting the adsorption of cholesterol from solution in safflower oil by Permutit: values determined experimentally (—); values calculated (- - -) using the equation $y = 0.0101x^{2.9}$.

was commercial material from a local supermarket.

Results. A typical isotherm for the adsorption of cholesterol on Permutit from solution in safflower oil, calculated from the data of Expt. 1, Table I, is shown in Fig. 1. The equation for a close-fitting curve is given by $y = 0.0101x^{2.9}$ and is shown by the broken line in Fig. 1. Obviously, the experimental curve departs from the calculated curve at the higher equilibrium levels of cholesterol as the limit in the capacity of the Permutit for adsorption is approached.

The effect of a number of compounds on the adsorption of cholesterol by Permutit is summarized in Table I under Expts. 3, 4, 5, and 6. In carrying out experiments of this type, it is important that a level of cholesterol that will yield an equilibrium concentration of cholesterol well below the inflection point in the adsorption isotherm be used. At much higher equilibrium levels of cholesterol the capacity of the Permutit to adsorb is being approached and the adsorption and solubility phenomena become complicated.

A number of compounds, for example pregnenolone, pregnanedione, and cholestane, appear to have essentially no effect on the adsorption of cholesterol, at least at the levels studied, since the equilibrium level of cholesterol found in the presence of both cholesterol and the compound under investigation is really no different than that found in the presence of cholesterol alone. A number of other compounds, for example cortisone, cortisone acetate, deoxycorticosterone, testosterone, nicotinic acid, and caffeine, appear to be readily adsorbed by the Permutit, independent of any interaction with cholesterol, leaving fewer adsorption sites for cholesterol since the equilibrium level of cholesterol found in the presence of both cholesterol and the compound under investigation is higher in the presence of both cholesterol and the compound under investigation than that found in the presence of cholesterol alone.

The compounds that appear to be of greatest interest are those which, when present along with cholesterol, yield equilibrium levels of cholesterol lower than those found in the presence of cholesterol alone. Stated in another way, these compounds appear to increase the adsorption of cholesterol by Permutit. Such compounds include β-sitosterol, stigmasterol, cholestenone, desmosterol, Δ^7-cholestenol, fatty acids as exemplified by lauric acid, myristic acid, palmitic acid, and stearic acid, and also, to a lesser extent, by cholesterol acetate and cholesterol benzoate. It might be argued that these compounds increase the adsorption of cholesterol by decreasing the solubility of cholesterol in the triglyceride. However, it has been shown that fatty acids increase rather than decrease the solubility of cholesterol in triglycerides (2). It has also been found that cholesterol esters have no effect on the solubility of cholesterol in triglycerides (8). For the present, it would seem that possible explanations for the phenomenon are that cholesterol and the active compounds are associated by some loose bonding so that cholesterol is "dragged along" or that the active compounds have enough structural similarity to cholesterol that they fill the adsorption

spaces nearly as well or perhaps better than cholesterol itself, keeping in mind that, at equilibrium levels of cholesterol well below the limit of adsorption of Permutit, the more cholesterol adsorbed the greater is the tendency for more to be taken up in sort of a "bootstrap" or vicious circle manner.

While the number and diversity of the compounds examined in this study is not large, a number of compounds having interesting effects on the adsorption of cholesterol have been found. These compounds should be studied in greater detail using these and more sophisticated techniques.

While there may be no analogy whatsoever between these *in vitro* studies and the deposition of cholesterol *in vivo*, it may be significant to point out that deposits of cholesterol, other than chololiths, are associated with deposits of other lipid material. Conceivably, as in these studies, cholesterol "goes along (or is dragged along) for the ride."

Summary. A number of compounds have been studied with respect to influence on the adsorption of cholesterol from solution in safflower oil by Permutit. While some compounds have no effect on the adsorption and other compounds appear to be preferentially adsorbed over cholesterol, a number of compounds, including some fatty acids, when present with cholesterol appear to be associated with an increase in the adsorption of the sterol by Permutit in this *in vitro* system.

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