Effect of an aqueous phase on the solubility of cholesterol in an oil phase

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Abstract In the absence of water, the solubility of cholesterol in triolein at 21°C was 2.8%. When water was added to the system, the solubility of cholesterol in the oil phase decreased to 1.9%, and cholesterol monohydrate precipitated. The precipitation of the sterol evidently resulted from the excess concentration of the surface-active cholesterol at the interface, allowing the rapid interaction of water with cholesterol and the resulting formation of cholesterol monohydrate with its attendant lower energy and less soluble crystalline lattice. The ternary phase diagram for the system cholesterol–triolein–water, modified to include cholesterol monohydrate formation with the consequent decrease in sterol solubility, differs from the previously reported phase diagram. Other cholesterol–oil–aqueous systems related to biologically important lipids were studied. Cholesteryl oleate was more soluble than cholesterol in triolein (23% at 21°C), and this value did not decrease when water was present. Water caused cholesterol to precipitate from cholesteryl linoleate at 37°C. Thus crystalline cholesterol may be present in lipids found in atherosclerotic plaques at a lower concentration of free cholesterol than had been predicted previously. In another experiment, a micellar taurocholate solution precipitated cholesterol from triolein and from corn oil. These effects of aqueous systems suggest the possibility of cholesterol precipitation from dietary fat when it becomes mixed with water in the diet or stomach, or with the micellar phase in the intestine. Plant sterols were precipitated also from oil solutions by an aqueous phase. Water-induced sterol precipitation is a phenomenon that could occur in a variety of biological systems, and may be applicable to sterols in general.

Supplementary key words cholesterol–oil–water phase diagram · cholesterol monohydrate · cholesterol precipitation · atherosclerosis · cholesterol absorption

The solubility of cholesterol in a variety of edible fats and oils has been reported by Wright (1) and Kritchevsky (2). Small and Shipley (3) have investigated the solubility of this sterol in the lipids that are components of atherosclerotic plaques. These reports either did not consider the effect of water on the solubility of cholesterol in oil or dismissed it as negligible. Stauffer and Bischoff (4) reported that the presence of an aqueous phase reduced cholesterol solubility in a variety of organic phases. This effect was attributed to the formation of cholesterol monohydrate. Because both aqueous and oil phases occur in tissues and in the lumen of the intestine, as well as in many diets, this altered solubility can have significant consequences. In the studies described here, we have further investigated cholesterol–oil–water interactions.

MATERIALS AND METHODS

Materials

[4-^{14}C]Cholesterol was obtained from New England Nuclear Company, Boston, MA. Thin-layer chromatography with both benzene–ethyl acetate 60:40 and ethyl ether–petroleum ether 60:40 showed this material to be essentially homogeneous; more than 95% of the ^{14}C had an R_f equal to that of a cholesterol standard. Cholesterol was obtained from MCB Manufacturing Chemists, Norwood, OH; it was recrystallized from ethanol and found to be homogeneous by thin-layer chromatography. This material was cocrystallized with [4-^{14}C]cholesterol under conditions that gave the two crystalline forms of cholesterol; the anhydrous form was prepared by crystallization from acetone, while the monohydrate was crystallized from aqueous ethanol. The X-ray diffraction patterns of the two crystalline forms corresponded to those previously reported (5). Titration with Karl Fischer reagent showed a 1:1 molar ratio of cholesterol to water in the cholesterol monohydrate crystals.

Cholesteryl oleate was synthesized by base-catalyzed interaction of cholesterol with methyl oleate. Crystallization of the product yielded a material that was greater than 95% sterol ester as determined by thin-layer chromatography (heptane–ethyl

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ether–acetic acid 80:20:1). Cholesteryl linoleate was obtained from Nuchek Prep, Elysian, MN; the 99% purity claimed for this material was confirmed by thin-layer chromatography. Triolein and glyceryl-1-monooleate were synthesized in our laboratories and purified by Florisil column chromatography (6). Sodium taurocholate from Maybridge Chemical Company, North Cornwall, U.K., was estimated to be at least 95% pure by thin-layer chromatography (isopentyl acetate–propionic acid–propanol–water 40:30:20:10).

Cholesterol solubility in triglycerides

Cholesterol solubility in triolein was measured in thermostatically controlled constant temperature rooms at 21°C and 37°C, or with an ambient temperature of 22–24°C as noted in Table 1. Triolein and corn oil were each saturated with [4-^{14}C]cholesterol by continuous shaking of the oil with an excess of crystalline anhydrous cholesterol or cholesterol monohydrate for 24 hr followed by removal of the remaining crystals by centrifugation and withdrawal of the supernatant oil. The absence of crystals was established by examination of the oil between crossed Nicol prisms at 100×. Preliminary experiments had shown that shaking for 24 hr was sufficient to saturate the oils with either crystalline form of cholesterol. The solubility of the sterol was determined by radiochemical assay of the supernatant oil.

The effect of the presence of an aqueous phase on the solubility of cholesterol in an oil phase was determined by adding 1 ml of the supernatant oil that was saturated with cholesterol to 2 ml of an aqueous phase consisting of either distilled water or a micellar solution comprising 0.01 M sodium taurocholate, 3 mM glycerol-1-monooleate, 0.05 M NaH_2PO_4, 0.0125 M Na_2HPO_4, and 0.065 M NaCl (pH 6.3). The phases were combined in small vials and were mixed by mechanically inverting the vials 40 times per min for 24 hr. The resulting emulsion was separated by centrifugation for 15 min at 2000 *g* when the aqueous phase was water, and for 30 min at 200,000 *g* when the aqueous phase was the micellar solution. Samples of the clear supernatant oil and the aqueous phase were assayed for ^{14}C concentration.

As will be described later, this exposure of oil saturated with cholesterol to an aqueous phase resulted in the appearance of crystalline material at the oil/water interfacial region. A quantity of this material, sufficient for characterization by X-ray diffraction and TLC, was obtained by shaking 7 ml of triolein saturated with cholesterol with 7 ml of distilled water. The resulting crystalline precipitate was isolated by filtration through an 8 μ Millipore filter. X-Ray crystallographic powder diffraction with a cylindrical camera and Cu$K\alpha$ radiation was used to identify the compound and crystalline form of this precipitate. Thin-layer chromatography (benzene–ethyl acetate 60:40) was used to test for the presence of cholesterol autoxidation products in the isolated precipitate.

Water solubility in triolein

The solubility of water in cholesterol–triolein solutions was measured at 21°C. To 10-ml portions of triolein, in which 0.0 and 1.9% cholesterol had been dissolved, 5 ml of distilled water was added and the mixture was inverted 40 times per min for 24 hr. Preliminary studies had shown that equilibrium was reached in this period. The emulsion was separated by centrifugation at 2000 *g*, and the clear supernatant oil was removed and analyzed for water content by Karl Fischer titration.

Cholesterol solubility in cholesteryl linoleate with water present

The effect of water on cholesterol dissolved in cholesteryl linoleate was measured by techniques similar to those described above. A mixture of anhydrous crystalline [4-^{14}C]cholesterol and cholesteryl linoleate was heated on a steam bath under a stream of nitrogen to form the isotropic phase of the cholesteryl linoleate. Crystals of cholesterol (m.p. 150°C) persisted during this step and a subsequent period of shaking for 24 hr at 37°C. At this temperature the cholesteryl linoleate remained as an isotropic liquid; the transition temperature to the cholesteric phase is 36.5°C (7). No attempt was made to ensure saturation of the cholesteryl linoleate, but the presence of the crystalline cholesterol prevented supersaturation. A clear oil phase of cholesteryl linoleate and dissolved cholesterol was separated from the crystals by centrifugation (37°C, 2000 *g*, 15 min). As described above, this oil phase was mixed with water at 37°C for 24 hr and then centrifuged (37°C, 2000 *g*, 15 min). The concentration of [4-^{14}C]-cholesterol in portions of the clear, supernatant cholesteryl linoleate was measured before and after the addition of water.

Cholesteryl oleate solubility in triolein

Clear, isotropic solutions were prepared by heating a mixture of crystalline cholesteryl oleate and triolein under a stream of nitrogen on a steam bath. The solution was allowed to stand for 24 hr at 21°C. Only a few crystals were visible in the tri-

olein to which 23% of cholesteryl oleate had been added; many crystals were evident where 25% had been added. The sample to which 23% of cholesteryl oleate had been added was allowed to stand for 3 additional days at 21°C. The crystals then were removed by centrifugation (2000 *g*, 15 min). The concentration of cholesteryl oleate in the triolein, measured as described below, was 23 ± 1%, a value consistent with the removal of a negligible mass of crystals. The greater solubility of the esterified form of cholesterol has been reported by Small (7), although this value of 23% was realized in his studies at 37°C.

An 0.8 ml portion of the supernatant oil was removed and combined in a vial with 1 ml of distilled water. The contents were mixed for 24 hr. Unlike the studies with free cholesterol, crystalline material could not be seen at the oil–water interface, nor was any detected on microscopic examination with crossed Nicol prisms (100×). The separation of the oil and water phases was assured by centrifugation (2000 *g*, 15 min). The composition of the supernatant oil was determined before and after the addition of water by measurement of the refractive index at 21°C. This was based on the observation that the refractive indexes of solutions of known compositions of cholesteryl oleate and triolein, including a 25% cholesteryl oleate supercooled isotropic phase, are a linear function of the cholesteryl oleate concentration. A similar relationship has been observed for other two-component systems of pure lipids (8). Cholesteryl oleate concentration was determined with an estimated accuracy of ±1 wt. % by the following equation:

$$\text{Wt. \% cholesteryl oleate} = 3460\ [n_D - 1.4689]$$

where n_D is the refractive index at 21°C.

Interfacial tension

Interfacial tensions were determined by the pendant drop technique (9), with reproducibility of ±1 dyne/cm. Drop shapes and dimensions were converted to interfacial tensions with density measurements from these laboratories, and with published tables (9).

RESULTS AND DISCUSSION

Water-induced precipitation of cholesterol from triolein

As shown in **Table 1**, System 1, the solubility of cholesterol in triolein at 21°C was 2.8%. When this solution was shaken with water and the resulting emulsion separated by centrifugation, an apparently crystalline material appeared at the oil/water interface. Analysis of the clear, supernatant oil phase showed that it now contained only 1.9% cholesterol (Table 1, System 2). Similar results were obtained at 37°C with a decrease in cholesterol concentration from 4.3% to 3.2% (Table 1, Systems 3 and 4). Radioactive cholesterol could not be detected in the aqueous phase; this observation conforms with the reported solubility of cholesterol in water as 1.8 μg/ml (10), an amount below the level of detection in our studies. The decrease in the concentration of cholesterol in the oil phase thus was the result of precipitation from the oil phase rather than a movement of cholesterol from triolein to water.

The precipitation effect was studied microscopically as well. Crystal growth at the oil/water interface was observed when a drop of triolein saturated with cholesterol was added to distilled water. These discrete, plate-like crystals are shown in **Fig. 1**; this photograph was taken 15 min after the oil had contacted the water surface.

The crystals that formed after water had been added to the cholesterol–triolein solution were isolated by filtration and characterized by X-ray diffraction and thin-layer chromatography. The powder pattern of the crystals was identical to that reported for cholesterol monohydrate, although the material that had been dissolved in the triolein was anhydrous cholesterol.

Since cholesterol forms significant quantities of oxidation products in some aqueous systems (11), the water-induced precipitate was examined for the presence of these. Thin-layer chromatography of the precipitated sterol did not disclose the presence of such autoxidation products. The X-ray diffraction pattern and thin-layer chromatography thus established the material precipitated from the triolein to be cholesterol monohydrate. The formation of cholesterol monohydrate under somewhat similar conditions is suggested by the work of Gilbert, Tanford, and Reynolds (12). They observed that water accompanies the transfer of cholesterol from an aqueous phase to a hydrocarbon phase.

Solubility of cholesterol monohydrate in triolein

The identification of the precipitated cholesterol as the monohydrate, and the earlier observations of Stauffer and Bischoll (4), indicated the cause of the precipitation to be a difference in the solubility of the anhydrous and the monohydrate forms. The observation that the monohydrate dissolves more slowly than the anhydrous form (13) shows the monohydrate to be the low energy form of cholesterol. The solubility

TABLE 1. Solubility of cholesterol in oil and aqueous/oil systems

System No.	Solvent Oil	Solute[a]	Aqueous Phase	Temp.	Solubility in Oil[b]
				°C	wt%
1	Triolein	Cholesterol	None	21	2.8 ± 0.1
2	Triolein	Cholesterol	Water	21	1.9 ± 0.1
3	Triolein	Cholesterol	None	37	4.3 ± 0.1
4	Triolein	Cholesterol	Water	37	3.2 ± 0.1
5	Triolein isolated from triolein/water emulsion	Cholesterol	None	21	2.8 ± 0.1
6	Triolein isolated from triolein/water emulsion	Cholesterol	Water	21	1.8 ± 0.1
7	Triolein	Cholesterol monohydrate	None	21	2.1 ± 0.1
8	Triolein	Cholesterol monohydrate	Water	21	1.9 ± 0.1
9	Triolein	Cholesteryl oleate	None	21	23
10	Triolein	Cholesteryl oleate	Water	21	23
11	Cholesteryl linoleate	Cholesterol	None	37	5.0 ± 0.1[c]
12	Cholesteryl linoleate	Cholesterol	Water	37	3.8 ± 0.3
13	Triolein	Cholesterol	None	22–24	3.0 ± 0.1
14	Triolein	Cholesterol	Micellar[d]	22–24	1.8 ± 0.1
15	Corn Oil	Cholesterol	None	22–24	2.7 ± 0.1
16	Corn Oil	Cholesterol	Water	22–24	1.9 ± 0.1
17	Corn Oil	Cholesterol	Micellar[d]	22–24	1.9 ± 0.1

[a] Unless otherwise indicated, anhydrous cholesterol was the solute.
[b] Average of $n \geq 3$ determinations, except $n = 1$ for Systems 9 and 10.
[c] Saturation not established.
[d] 10 mM sodium taurocholate, 3 mM 1-monoolein, 0.06 M sodium phosphate buffer, 0.15 M total Na^+, all at pH 6.3.

of cholesterol monohydrate in triolein was found to be 2.1% (Table 1, System 7), compared with 2.8% for the anhydrous form (Table 1, System 1) and with 1.9% (Table 1, System 2) for the concentration of cholesterol in triolein after the water-induced precipitation effect. These data indicate that most of the precipitation from the anhydrous cholesterol–triolein system can be explained by the formation of the lower-energy, less soluble cholesterol monohydrate.

The addition of water to triolein saturated with cholesterol monohydrate produced visible crystals. This was accompanied by a decrease in cholesterol concentration from 2.1 to 1.9% in the oil (Table 1, System 8). The latter value is the same as that obtained in the studies on anhydrous cholesterol. The precipitation from triolein saturated with sterol monohydrate by the addition of water is discussed further in relation to the cholesterol–triolein–water phase diagram.

Interactions of water with triolein and cholesterol

Although the solubility of water in triolein is negligible **(Table 2),** we tested the hypothesis that cholesterol precipitated from triolein as a result of an alteration of the composition of the solvent from one consisting solely of triolein to one of triolein saturated with water. The solubility of cholesterol in triolein that had been shaken with water for 24 hr prior to use in cholesterol solubility studies was the same as that in anhydrous triolein (Table 1, Systems

5 and 1). Moreover, the subsequent exposure of these oils to an aqueous phase resulted in the same decrease in cholesterol solubility (Systems 6 and 2). These observations are discussed further in relation to the cholesterol–triolein–water phase diagram.

Cholesterol monohydrate is formed by hydrogen bonding of water with the hydroxyl group of cholesterol (14). In the studies reported here the water-induced precipitation of cholesterol from triolein occurred in spite of the virtual immiscibility, <0.1% (Table 2), of water and triolein. However, as shown in **Fig. 2**, the interfacial tension of a triolein/water system was reduced by the dissolution of free cholesterol in the triolein, indicating an interfacial excess concentration of cholesterol. The hydrophilic hydroxyl group of the cholesterol would be oriented toward the aqueous phase with a consequent opportunity for hydrogen bonding with water. Thus, multilayer accumulation of cholesterol monohydrate and subsequent crystal formation could occur rapidly in the vicinity of the interface. Other surface-active compounds, such as 1-acetyl-3-monostearin and 1-propylene glycol monostearate, have been reported to form solid films at the oil/water interface (15,16). The excess interfacial concentration of these compounds was proposed to explain the formation of a solid film at the interface. A similar phenomenon is suggested in a study of the kinetics of absorption of sterols at the paraffin oil/water interface (17). Although precipitation was not observed, the formation of a solid interfacial phase of sterol was predicted.

Cholesteryl oleate–triolein–water system

The mechanism proposed to explain these solubility characteristics was tested by comparing the solubility of cholesterol and cholesteryl oleate. The solubility of cholesteryl oleate in triolein (Table 1, System 9) was more than eight times that of anhydrous cholesterol (System 1). Unlike cholesterol (System 2), no precipitation of cholesteryl oleate was caused by the addition of water (System 10). Also unlike cholesterol, cholesteryl oleate neither crystallized in a hydrated form nor did it exhibit surface activity (Fig. 2). Thus the esterification of the hydroxyl group of cholesterol results in a compound that neither accumulates at the interface nor forms the less soluble crystalline lattice. As a consequence, the solubility in an oil phase of cholesterol as the ester is markedly increased and crystallization at the oil/water interface does not occur.

Cholesterol–triolein–water phase diagram

Table 2 lists the pertinent solubility data and other measurements that were used in the construction of the triolein–cholesterol–water isothermal phase diagram that is shown in **Fig. 3.** Five regions exist in this ternary system: (I) a triolein-rich single phase with composition of less than 0.1% dissolved water and less than 2.8% cholesterol; (II) a region with three phases—anhydrous cholesterol, cholesterol monohydrate, and the phase at the boundary of region I with composition S; (III) two phases—cholesterol monohydrate and a conjugate phase in region I; (IV) three phases—cholesterol monohydrate, water, and the phase at the boundary of region I with composition B; and (V) two phases—water and a conjugate phase in region I.

Fig. 1. Cholesterol monohydrate crystals present 15 min following the addition of water to triolein that was saturated with anhydrous cholesterol (crossed Nicols, 75×).

The water-induced precipitation of cholesterol from triolein can be described with the phase diagram of Fig. 3 by moving from an anhydrous composition to a water-rich composition. Thus, the addition of water to triolein saturated with anhydrous cholesterol is equivalent to moving from point S toward point W. The composition changes from that of the single phase at point S to two conjugate phases of region III where cholesterol monohydrate exists in equilibrium with a phase of region I. The further addition of water results in the invariant compositions of the three-phase region IV where the oil phase has the composition given at point B.

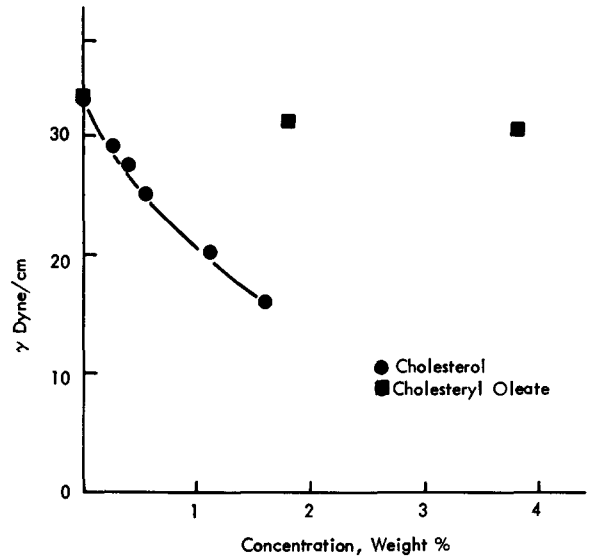

Fig. 2. Interfacial tension of triolein/water systems containing various amounts of cholesterol or cholesteryl oleate in the triolein.

The addition of water to triolein that had been saturated with cholesterol monohydrate is shown by moving from the composition at X toward W. Again, region IV is reached and the resulting cholesterol concentration in the oil is given by the composition at point B.

The measurement of the solubility of cholesterol in triolein that had been mixed with water (Table 1, System 5), resulted in the same solubility (2.8%) as that of cholesterol in "anhydrous" triolein. In Fig. 3 this corresponds to moving from point A through regions I and III to region II. The cholesterol concentration in the oil phase in region II is 2.8% (point S).

Cholesterol–cholesteryl linoleate–water system

Because of the importance of cholesteryl esters in biological systems, the effect of water on cholesterol dissolved in cholesteryl linoleate at 37°C was measured. The dissolution procedure resulted in a 5.0% concentration of cholesterol in cholesteryl linoleate (Table 1, System 11). It is possible that saturation of the cholesteryl linoleate was not reached, since a solubility of 8% has been reported (3). However, the procedure employed here assured that supersaturation did not take place. Regardless of the true solubility value, the presence of water reduced the cholesterol concentration to 3.8% (Table 1, System 12), and a crystalline precipitate formed. Thus measurement of the solubility of cholesterol in anhydrous cholesteryl linoleate overestimates the solubility if an aqueous phase is present as well. Presumably this observation applies also to mixtures of cholesteryl esters that are in the liquid state.

Small and Shipley (3) recently discussed the phase diagrams of lipids found in atherosclerotic plaques and proposed that dissolution or removal of crystalline cholesterol is a critical step in reversal of the lesion. Their measurements of cholesterol solubility in cholesteryl ester apparently are those for an anhydrous system. As shown here, this would give higher solubility values than those realized when water is present. The effect of water on cholesterol solubility in cholesteryl esters would alter quantitatively the cholesterol–cholesteryl ester–phospholipid–

TABLE 2. Compositions at the indicated points in the cholesterol–triolein–water phase diagram

Triolein	Cholesterol	Water	Point Label in Fig. 3	Description
	w%			
0	95.6	4.4	M	Stoichiometry of cholesterol monohydrate
97.2	2.8	0.0	S	Solubility of anyhydrous cholesterol in triolein
>99.9	0	<0.1[a]	A	Solubility of water in triolein
98.0	1.9	0.1	B	Solubility of cholesterol monohydrate in triolein, water present (System 8)
0	100	0	C	Pure cholesterol
100	0	0	T	Pure triolein
0	0	100	W	Pure water
97.9	2.0	0.08	X	Solubility of cholesterol monohydrate in triolein, water absent—determined from 2.1% solubility of cholesterol monohydrate in triolein and the stoichiometry of cholesterol monohydrate (System 7).

[a] Not significantly different from the blank used in the Karl Fischer reagent titration.

water phase diagrams presented by them. The concentration of cholesterol necessary to saturate the oil phase when water is present would be less than that predicted if the solubility of cholesterol in cholesteryl ester was determined in an anhydrous system. Thus, the determination of the phases of lipids in arterial intima from their composition and from a cholesterol–cholesteryl ester–phospholipid–water phase diagram that takes water-induced cholesterol precipitation into account would predict the existence of crystalline cholesterol monohydrate in compositions that had been thought to consist only of an oil phase and a liquid crystalline phase. These observations do not conflict with Small and Shipley's prediction of the phases of lipids in atherosclerotic placque, but only indicate the existence of crystalline cholesterol at a lower concentration of free cholesterol than that proposed by them.

Cholesterol precipitation in corn oil and taurocholate systems

Another area of biological importance that may be affected by water-induced cholesterol precipitation is the absorption of dietary cholesterol. Cholesterol that is fed in the crystalline state is absorbed less completely than that dissolved in triglycerides (18). The solubility values in Table 1 show that 2.8% of cholesterol can be dissolved in triolein (System 1) or in corn oil (System 15). This amount would probably remain dissolved in the fat if it were incorporated into a dry diet. However, the addition of water to the diet, either directly or in the stomach, would decrease the solubility to 1.9% with a portion of the cholesterol now present in the crystalline state (Systems 2 and 16). The observed decrease in the absorbability of cholesterol when it is ingested at higher dietary levels (19) may in part be a reflection of this water-induced precipitation. As shown in Table 1, Systems 14 and 17, an aqueous micellar phase similar to that found in the intestinal lumen was as effective as water in reducing the concentration of cholesterol in triolein (Systems 13 and 14) and corn oil (Systems 15 and 17). Analysis of the micellar phase showed that only 0.3% of the cholesterol that had disappeared from the triolein was now dissolved in this phase. The remainder was present as a crystalline material at the interface. Centrifugation of the triolein–micellar system at 200,000 *g* yielded a pellet that was identified as cholesterol monohydrate. Thus in the intestinal tract the micellar phase, which as part of the absorptive process transports cholesterol from the oil phase, could result also in the conversion of a portion of the cholesterol that initially was dissolved in the oil phase

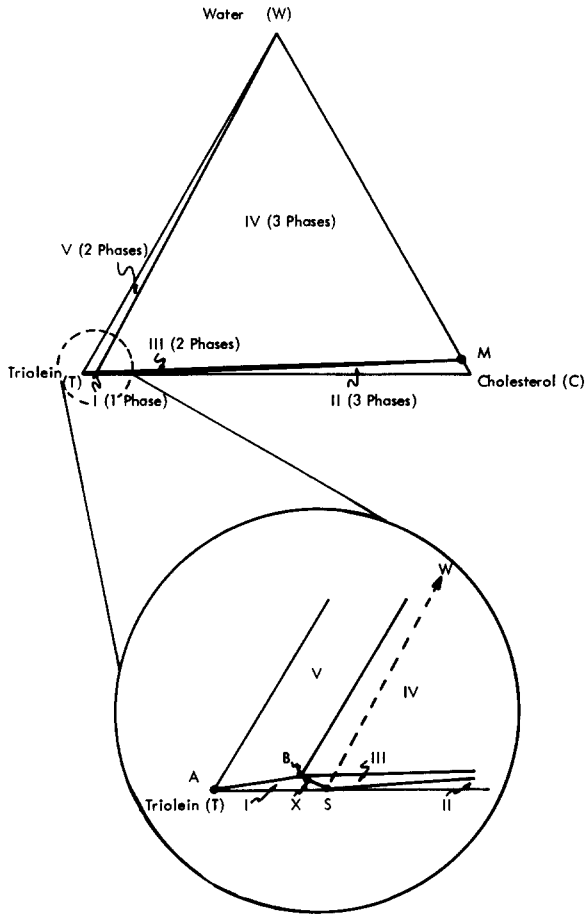

Fig. 3. The cholesterol–triolein–water phase diagram at 21°C. The letters denote compositions given in Table 2. Regions I–V are discussed in the text.

into the less absorbable crystalline state. The occurrence of this phenomenon in vivo is indicated by the finding of precipitated cholesterol in the intestinal lumen of man (20) and rats (21). These observations of the precipitation of cholesterol from thermodynamically stable solutions in fat as a result of the introduction of an aqueous phase have particular implications when diets are prepared in which the concentration of anhydrous cholesterol in the fat exceeds 2%; the presence of water in such a diet could precipitate a portion of the cholesterol as the monohydrate crystal.

These observations of a decreased solubility of cholesterol in an oil phase, if there is the simultaneous presence of an aqueous phase, are probably applicable to all biological systems that contain discrete aqueous and oil phases. Moreover, the, phenomenon is not limited to cholesterol. We have observed that β-sitosterol and mixed β-sitosterol–cholesterol crystals (but not β-sitosteryl oleate) similarly are precipitated from oil by water. It is probable that the

phenomenon can occur with all sterols that form a hydrated crystal of lower energy than the anhydrous lattice. The quantitative relationships shown in Fig. 3 are for the simplest system, that is, cholesterol, oil, and water. If a surface-active material, such as lecithin, is present also, cholesterol can exist in the liquid crystalline state as well as in the solid and soluble states.

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