Inhibition of lipolysis by hydrocarbons and fatty alcohols

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Abstract The hydrolysis of long-chain triglyceride by pancreatic lipase (EC 3.1.1.3) is inhibited by hydrophobic solutes that are dissolved in the fat. Solutes tested included n-alkanes (C_{10}-C_{16}), aromatic and chlorinated aromatic hydrocarbons (including a PCB and DDT), n-alcohols (C_{10}-C_{16}), and cholesterol. Except for cholesterol, which stimulated lipolysis at low concentrations, all compounds produced roughly similar inhibition curves that followed the pattern of a typical Langmuir adsorption isotherm (Mattson, F. H., R. A. Volpenhein, and L. Benjamin, 1970. *J. Biol. Chem.* **245:** 5335–5340). According to this interpretation, hydrophobic solutes dissolved within fat droplets partition between the interior oil phase and the surface monolayer where lipolysis occurs. Although the aromatic and chlorinated aromatic hydrocarbons were ~25% more inhibitory than the long-chain aliphatic hydrocarbons, as a single class, hydrocarbons were 7–10 times weaker inhibitors of lipolysis than fatty alcohols. In contrast to the alcohols whose inhibitory action may involve several mechanisms, the hydrocarbons behaved like simple dilution inhibitors; i.e., at 50% inhibition the mass ratio of hexadecane to triglyceride was 0.42. The lack of a chain length effect indicates that the hydrocarbons are not adsorbed at the interface but interdigitate the triglyceride molecules and align parallel to the lipid acyl chains. Inhibition by hydrophobic solutes was not reversed by the presence of 4 mM taurodeoxycholate and pancreatic procolipase or colipase. —**Ferreira, G. C., and J. S. Patton.** Inhibition of lipolysis by hydrocarbons and fatty alcohols. *J. Lipid Res.* 1990. **31:** 889–897.

Supplementary key words chlorinated aromatic hydrocarbons • DDT • drug delivery • fatty alcohols • polychlorinated biphenyls • pancreatic lipase

Under physiological conditions, pancreatic lipase (EC 3.1.1.3) catalyzes the hydrolysis of water-insoluble long-chain triglyceride. The reaction occurs at the surface of oil droplets, and the end products, fatty acids and monoglycerides, separate from the undigested oil and form transitory liquid crystalline product phases that are eventually dispersed and solubilized by bile salts prior to cellular absorption (1–3). Experiments with group-specific reagents that bind to lipase suggest that the initiation of lipolysis involves three sequential steps: interfacial adsorption, interfacial activation, and finally catalysis proper (4, 5). Interfacial activation refers to the ability of lipase to hydrolyze aggregated or emulsified substrates much faster than monomeric substrates (4, 5). Each of the three steps can be selectively inhibited by the formation of specific lipase-inhibitor derivatives, which suggests that there are at least three separate substrate interaction sites of the lipase molecule (6–8).

Inhibition of interfacial adsorption of lipase occurs in the presence of micellar concentrations of bile salts (9, 10) and phospholipids (11,12). Although pancreatic procolipase can reverse the inhibition caused by bile salts, tryptic removal of a pentapeptide from procolipase is required before colipase can reverse phospholipid inhibition of lipolysis (9, 13, 14). Presumably these amphiphiles inhibit lipolysis by covering the substrate or enzyme surface and preventing the enzyme from "seeing" its substrate (9, 15–17). It has been proposed that interfacial activation of lipolysis involves a conformational change in lipase (18). However, the effect somehow arises from the dense aggregation of substrate molecules that occurs at the surface of a fat droplet (4, 5), and any hydrophobic molecules that dilute the interfacial substrate concentration should inhibit interfacial activation. Fatty acids, fatty alcohols, and halogenated phenylamines all appear to adsorb to the substrate interface and inhibit lipolysis (19–21). Fatty acids and fatty alcohols, however, are also potential substrates for the reversible lipase reaction (22); thus, their inhibition of lipolysis could be a combined effect of substrate dilution (i.e., inhibition of interfacial activation) and competition with triglyceride for the active site region (i.e., inhibition of catalysis proper).

Many compounds are highly soluble in fat droplets (23). Dietary fat can carry a wide variety of hydrophobic food additives, plasticizers, vitamins, xenobiotics, and carcinogens (24), and the use of triglceride as a drug excipient and drug delivery vehicle is increasing in importance (23). Despite the large literature on lipase, with the

Abbreviations: LCT, long-chain triglyceride; PMSF, phenylmethylsulfonyl fluoride.

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exception of fatty alcohols (20), little is known about how hydrophobic solutes that are dissolved in fat affect lipolysis. In this study we compared the effects of hydrocarbons and fatty alcohols on the rate of triglyceride hydrolysis by pancreatic lipase.

MATERIALS AND METHODS

Materials

Tri[9,10(n)-^{3}H]oleoylglycerol (1 Ci/mmol) was purchased from Amersham (Arlington Heights, IL) and exhibited a radiopurity of greater than 99% as judged by thin-layer chromatography. The long-chain alkanes, alcohols, and cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO) and were judged to be 99% pure or better by thin-layer chromatography. The aromatics and chlorinated aromatics were purchased from Aldrich Chemical Co. (Milwaukee, WI) with the following purities: naphthalene 99 + %; *p*-dichlorobenzene, 97 + %; biphenyl, 99%; 2,6-dimethyl naphthalane, 100%; 2,3-dimethyl naphthalane, 98%; fluorene, 98%; phenanthrene, 98%; fluoranthrene, 98%; 4,4-dichlorobiphenyl, 98%; perylene, 99 + %; and *p*, *p*'DDT, 99 + %. Vacuum desiccation over anhydrous calcium sulfate at room temperature for 3 days was used to remove any traces of water from the above chemicals. Gravimetric analyses before and after desiccation suggested that the stock chemicals contained less than 0.1% water. Highly refined olive oil, the protease inhibitors, phenylmethylsulfonyl fluoride (PMSF) and benzamidine, bovine serum albumin, porcine pancreatic trypsin type IX, porcine pancreas powder (Grade VI), and gum arabic were purchased from Sigma Chemical Co.

Purification of porcine pancreatic lipase

Porcine pancreas powder was delipidated by the method of Verger et al. (25), and pancreatic lipase was purified according to the method of Rovery, Boudonard, and Bianchetta (26) with the following modifications. Fibrous DE23 (Whatman, Maidstone, U.K.) was used instead of DE-11, and the final CM cellulose step which separates lipase isozymes was omitted. Protein was measured by the method of Lowry et al. (27). The purest preparation had a final specific activity of 1810 tributyrin units/mg protein per min (and 205 long chain triglyceride (LCT) units/mg protein per min) as determined by continuous titration of liberated fatty acids at pH 8.0 and 23°C (9). The purified enzyme was free of colipase activity and showed no significant loss of activity after storage at −20°C for 6 months.

In addition to purified lipase, crude enzyme was also used in some experiments with aromatic and chlorinated aromatic solutes. Enzyme preparations were made up each day in the following manner: 0.2 g of delipidated pancreas powder was added to 5.0 ml of a pH 9.0 solution (100 mM Tris-base, 0.5 mM PMSF, 1 mM benzamidine, and 0.02% sodium azide) and stirred for 20 min at 4°C. The mixture was then centrifuged at 15,600 *g* for 4 min, and the supernatant containing the lipase was poured off the stored on ice. This lipase had an activity of approximately 158 LCT units/ml at pH 8.0. Both crude and purified enzyme gave similar results in the absence of bile salts and colipase. In the presence of bile salts, crude lipase would be expected to contain bile salt-stimulated lipases as well as pancreatic lipase (24).

Preparation of procolipase and colipase

Porcine procolipase I (101 residues) was a generous gift from Bengt Borgström and Anita Larsson (University of Lund, Sweden) and was purified by them as previously described (28). Final specific activity was 27,000 tributyrin units/mg protein as determined with 10 μg lipase in the presence of 4 mM taurodeoxycholate at pH 8.0 and 23°C (28).

Activated colipase was prepared by incubating procolipase with porcine trypsin for 10 min at 22°C according to Erlansson-Albertsson (29). The lag phase against 500 μl bovine milk cream went from 18.0 min with procolipase to 1.8 min with trypsin-treated colipase in an assay system containing 205 LCT units lipase/ml.

Preparation of emulsions

The olive oil substrate was prepared by emulsifying 3 ml of olive oil in 6 ml of a 10% gum arabic solution (40 mM Tris base, 150 mM NaCL, 1 mM $CaCl_2$, 0.02% NaN_3, pH 7.5) and 3 ml of distilled water. To emulsify the solution, it was sonicated for three periods of 20 sec each, with a Heat Systems Ultrasonic Cell Disruptor (Model W1751, Brandson Sonic Power Co.) with a microtip at setting 7. The molecular weight of triolein (885 g/mol) was used to calculate the substrate concentration.

Emulsions that contained triglyceride plus hydrophobic solute were prepared by dissolving the solute in the oil then adding the aqueous components. All emulsions with solutes were made with 1.0 ml of olive oil (containing the predissolved solute), 2 ml of 10% gum arabic solution, and then enough distilled water to give a final total volume of 4 ml. Emulsification was accomplished as described above. Unless stated otherwise, the substrate concentration was kept constant while increasing amounts of solute were added.

Droplet size determination of emulsions

Emulsion droplet sizes were determined according to the method of Schott and Royce (30). Droplet diameters were determined in control emulsions, in emulsions with the highest hexadecane concentration, and in emulsions with the highest decanol concentrations. To inhibit Brownian motion, emulsions were thickened with 5% gelatin (Eastman Kodak, Rochester, NY).

Measurement of lipolytic activity

Lipase activity was measured both by titration and with a radioisotopic assay. Most activity measurements were made by titration of the released fatty acids with 0.025 N NaOH using a recording pH-stat (Radiometer, Copenhagen, Denmark) with a combined electrode (GK 2421C). The reaction was carried out in 10 ml of reaction medium on 500 μl of emulsion of pH 8.0 at 37°C with a constant rate of stirring. The reaction medium contained 2 mM Tris-maleate, 150 mM NaCl, 1 mM $CaCl_2$, and 0.02% NaN_3. Typically, ca. 8 units of lipase were added per reaction. Lipase activity was determined from the slope of the linear portion of the curve. One unit of lipase is defined as 1 μmol of long chain fatty acid released per min.

To determine the effect of hydrophobic solute on ionization of released fatty acids, the titration assay was compared with a radioisotopic assay. Radioactive triolein was added to the olive oil emulsions in the presence or absence of hexadecane (ca. 2.25 × 10^4 dpm/μl oil) and the reaction was carried out as before in the pH stat. At 0, 2, 5, 7, and 10 min of reaction time, 50 μl aliquots were withdrawn. The subsamples were acidified with 3 N HCl, and extracted with ethyl ether (500 μl). The resulting lipids were concentrated to dryness with a stream of N_2 and then separated on silica gel plates in a solvent system of ethyl ether–ammonium hydroxide 100:1 (v/v). This system leaves free fatty acids at the origin and separates triglycerides (R_f, 0.8), diglycerides (R_f, 0.7), and monoglycerides (R_f, 0.15). Individual classes were visualized by brief exposure to iodine vapors, and after sublimation of the iodine, the spots were scraped into scintillation vials and counted in a Beckman liquid scintillation counter.

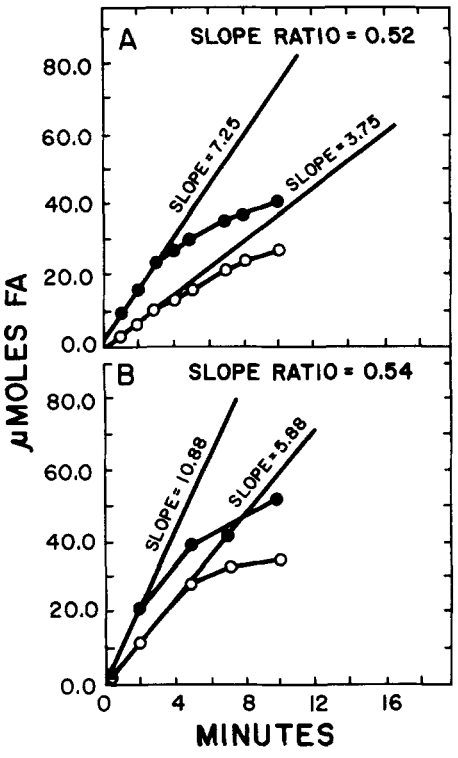

Fig. 1. A comparison of lipolytic reaction as determined by titration (A) or by radioisotopic assay (B); (●—●) pure triglyceride substrate; (○—○) triglyceride substrate plus hexadecane (1.65 μmol hexadecane per μmol olive oil substrate). Reactions were carried out in 10.8 ml total volume at 37°C, pH 8.0, with 8 LCT units of purified lipase on 128 μmol of substrate. Enzyme activity is calculated from tangents drawn to the initial reaction rates.

RESULTS

Validity of titrimetric assay

Titration of long chain fatty acids released by lipase at pH 8.0 was the assay method of choice for this study because of its reproducibility, speed, and continuous rate output. The titration assay was compared with a tracer assay (**Fig. 1**). Although only two out of every three fatty acids released were measured by titration at pH 8.0, this ratio remained constant even in the presence of the highest concentrations of hydrophobic solute (0.42 mg hexadecane/mg of triglyceride). At pH 9.0, titration measures a greater percentage of the total fatty acids released, but total lipase activity is less than 50% of that at pH 8.0 (12). There was a linear relationship between enzyme activity against 12 mM substrate (128 μmol) and enzyme protein (0.4–4.0 μg pure enzyme) (data not shown). The reaction was well saturated with substrate at 12 mM triglyceride and 8 LCT units of lipase (data not shown). Unless stated, all subsequent assays were carried out on 128 μmol substrate with 8 LCT units (1.6 μg) pancreatic lipase.

Solute effect on emulsion particle dimensions

The addition of decanol or hexadecane to the triglyceride substrate only slightly affected the frequency-size distribution of the subsequent emulsion particles (**Fig. 2**). Since the concentrations of these solutes in Fig. 2 represented the highest levels that were used in the inhibition experiments, it is assumed that lower concentrations also did not greatly effect the frequency-size distribution. In the absence of solute the average surface area of the substrate (128 μmol) was ca. 270 mm^2. Addition of 0.25 μmol decanol or 1.75 μmol hexadecane per μmol substrate increased the total substrate surface area to 370 mm^2 and 400 mm^2, respectively.

Effect of hydrophobic solutes on lipolysis

The effect of four alkanes (C_{10}, C_{12}, C_{14}, and C_{16}) on lipase activity in the absence of bile salts and colipase is shown in **Fig. 3**. All four compounds inhibited the reaction in a similar manner, and no significant chain length

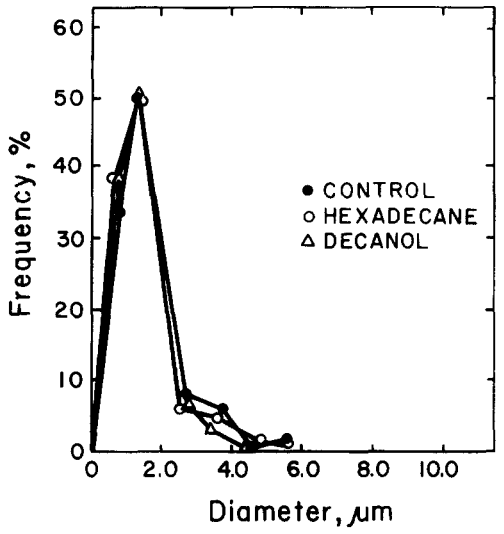

Fig. 2. The effect of the addition of decanol (0.25 μmol decanol/μmol substrate) or hexadecane (1.65 μmol hexadecane/μmol substrate) to triglyceride (128 μmol) on the frequency size distribution of the subsequent emulsion.

effect was seen. Inhibition by hexadecane in the presence of bile salts (4 mM taurodeoxycholate) and excess amounts of procolipase or trypsin-activated colipase was similar to that shown in Fig. 3 (data not shown). The shapes of the curves were hyperbolic and similar to the Langmuir adsorption isotherm (31).

The effect of cholesterol and four long-chain fatty alcohols on lipase activity in the absence of bile salts and colipase is shown in **Fig. 4**. As a class on a molar basis, hydrophobic alcohols were about 7–10 times more inhibitory than the n-alkanes. Although the inhibitory effects of the four n-alcohols were not as uniform as the n-alkanes, they also exhibited an eventual decrease in inhibitory effect at higher inhibitor concentrations (except for dodecanol). On a molar basis, a moderate chain length effect was apparent, with the shorter chain C_{10} decanol inhibiting more than C_{14} tetradecanol, which inhibited more than C_{16} hexadecanol (again dodecanol did not fit this pattern). Cholesterol was unique and stimulated lipolysis up to 13% at low molar ratios (i.e., 0.02:1 cholesterol:triglyceride). This stimulation disappeared at higher cholesterol concentrations, and inhibition of lipolysis occurred at cholesterol:triglyceride mole ratios above 0.04. No similar activation of lipolysis by the long-chain fatty alcohols at low mole ratios (i.e., >0.04) was observed (data not shown). In the presence of bile salts (4 mM taurodeoxycholate) or excess amounts of procolipase or trypsin-activated colipase, the inhibition of purified lipase by decanol was similar to that shown in Fig. 4.

A variety of aromatic and chlorinated aromatic compounds were dissolved in the triglyceride substrate, and the subsequent emulsions were subjected to lipolysis by

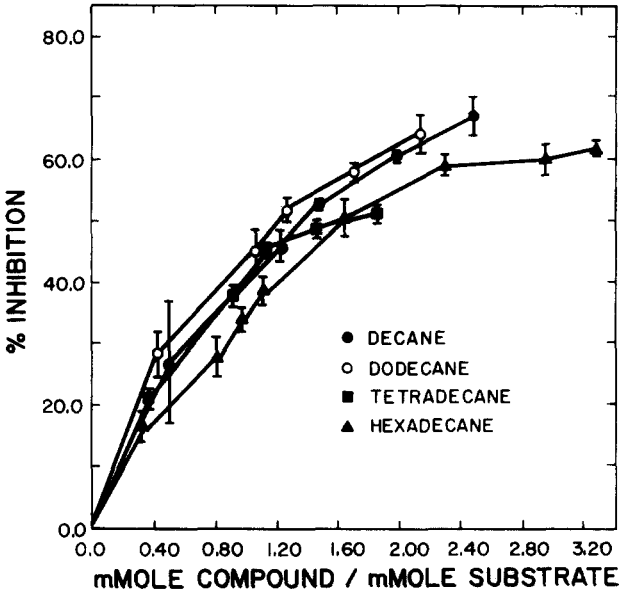

Fig. 3. The effect of n-alkanes on the hydrolysis of long chain triglyceride (128 μmol) by purified pancreatic lipase (8 LCT units) at pH 8.0 and 37°C as measured by the titrimetric assay in the absence of bile salts and colipase. Each point represents the mean ± SE of three determinations.

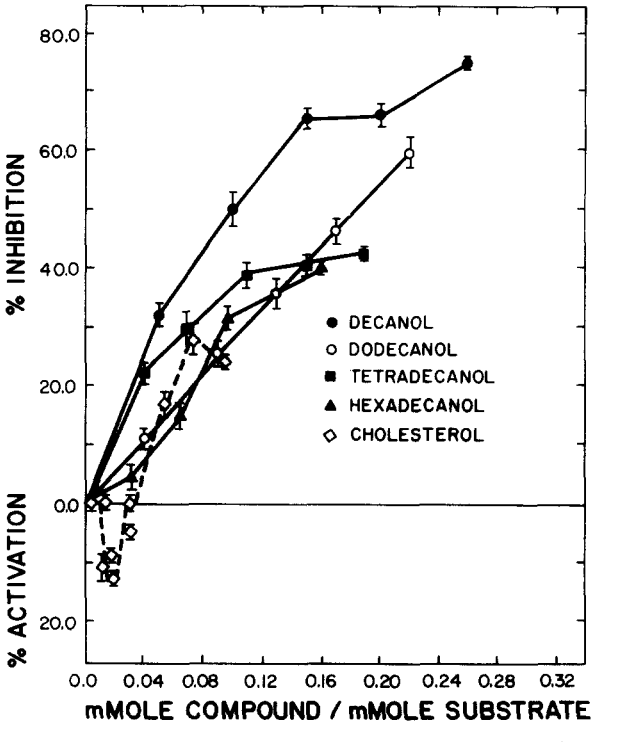

Fig. 4. The effect of n-alcohols and cholesterol on the hydrolysis of long chain triglyceride (128 μmol) by purified pancreatic lipase (8 LCT units) at pH 8.0 and 37°C as measured by the titrimetric assay in the absence of bile salts and colipase. Each point represents the mean ± SE of three determinations.

crude lipase. Crossover experiments with selected solutes showed that both crude and purified lipase gave similar results. Inhibition curves were similar to those produced by the n-alkanes although, because of the solubility limits of the solute, maximum inhibition was often considerably less than 50%. **Table 1** summarizes the inhibition results with these solutes and also the data for the n-alkanes, n-alcohols, and cholesterol. Mole ratios at 20% inhibition were presented since many compounds were not soluble enough in triglyceride at 37°C to cause 50% inhibition. The aromatic and chlorinated aromatic hydrocarbons, taken as a single class, were roughly 25% more inhibitory on a molar basis than the long chain aliphatic hydrocarbons. Compared to the alcohols, however, the polycyclic hydrocarbons, like the n-alkanes, were relatively weak inhibitors of lipolysis. There was no obvious correlation between octanol–water partition coefficients or molecular weights of the solutes and their capacity to inhibit lipolysis. The strongest correlate of inhibitory capacity was the presence or absence of an hydroxyl group in the inhibitor.

Hexadecane and decanol were chosen as representative solutes for a kinetic analysis of the mechanism of solute inhibition. Dixon plots (1/Vi vs [I]) of hexadecane and decanol inhibition of lipase are shown in **Fig. 5A and B**. These plots and plots of s/Vi against i at several s values (substrate concentrations) (not shown) suggest that the inhibition is of the competitive type.

DISCUSSION

The results of this study suggest that a wide variety of structurally diverse hydrocarbons (and probably halogenated hydrocarbons), when dissolved in fat, inhibit lipolysis by a similar mechanism. The results (Fig. 3) show that fat droplets can contain remarkable concentrations of hydrocarbon solute and still undergo digestion by pancreatic lipase. The introduction of a single hydroxyl group into a hydrocarbon increases its ability in inhibit lipolysis nearly tenfold (Table 1). This study confirms and expands the earlier report of Mattson, Volpenheim, and Benjamin (20) in which n-alcohols were shown to inhibit the hydrolysis of methyl oleate by pancreatic lipase. In their study, as in this study, no discernible effect of alcohol chain length between C_{10} and C_{16} on inhibition was seen. They found that their data could be successfully treated by assuming that the alcohols adsorbed onto the substrate

TABLE 1. Physical and chemical properties of the hydrophobic solutes used in this study and their effects on lipase activity

Inhibitor	Mol Wt	Melting Point	$LogK_{ow}$[a]	Inhibitor Solubility in Olive Oil at 37°C[b] Moles I/Moles S	Inhibitor/Substrate Mole Ratio at 20% Inhibition
		°C			
Alkanes					
Decane	142.29	−30	5.64	miscible	0.37
Dodecane	170.34	−9.6	6.70	miscible	0.30
Tetradecane	198.40	−5.5	7.76	miscible	0.37
Hexadecane	226.45	18	8.82	miscible	0.49
Aromatics and chlorinated aromatics					
p-Dichlorobenzene	147.01	53.1	3.38	2.25	0.37
Biphenyl	154.21	71	4.09	0.84	0.44
Naphthalene	128.19	80.2	3.30	0.59	0.24
Phenanthrene	178.24	101	4.46	0.30	0.29
2,3-Dimethyl naphthalene	156.23	105	4.40	0.26	0.25
2,6-Dimethyl naphthalene	156.23	108	4.31	0.24	0.24
p,p'-DDT	354.49	109	6.19	0.24	(11%)[c]
Fluoranthrene	202.36	111	5.22	0.21	0.21
Fluorene	166.23	116	4.18	0.19	(6.6%)[c]
4,4-Dichlorobiphenyl	223.11	149	5.58	0.08	(6.4%)[c]
Perylene	252.32	277	6.50	0.004	(8.3%)[c]
					0.29 ± 0.08
Aliphatic alcohols					
Decanol	158.28	6.4	4.01	miscible	0.031
Dodecanol	186.33	24	5.06	miscible	0.072
Tetradecanol	214.38	38	6.11	miscible	0.036
Hexadecanol	242.43	49.9	7.17	0.73	
					0.053 ± 0.019
Sterols					
Cholesterol	386.64	148.5	10.27	0.08	0.060

[a]Calculated according to (44).
[b]Ideal solubilities calculated according to (23).
[c]Inhibition in parentheses at solubility limit of solute. These values are not included in the mean.

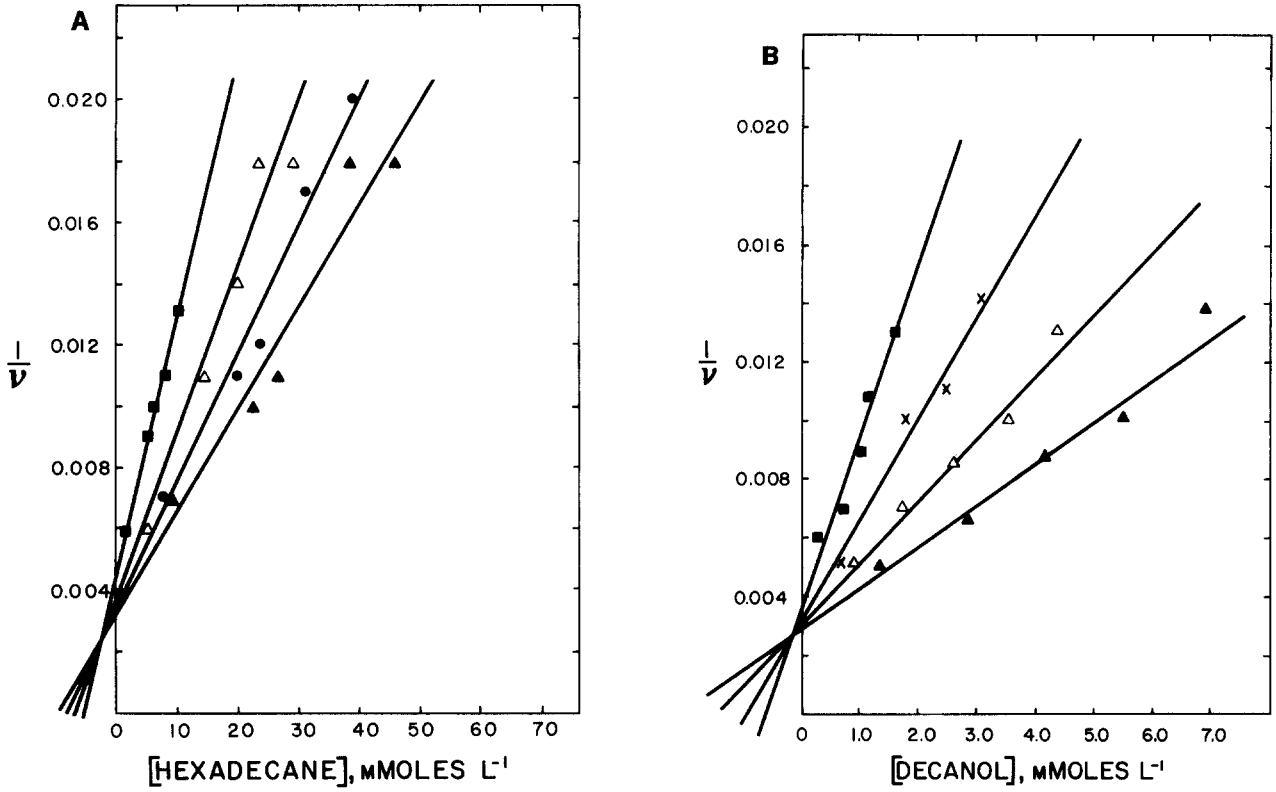

Fig. 5. Dixon plots of the inhibition of purified pancreatic lipase activity (8 LCT units) by (A) hexadecane and (B) decanol at 37°C, pH 8.0, in the absence of bile salts. Inhibition of lipolysis by various amounts of triglyceride by (A) hexadecane and (B) decanol.

surface from the aqueous phase in a manner typical of a Langmuir adsorption isotherm.

The results obtained in our experiments with the hydrocarbons are also consistent with a Langmuir effect. However, instead of a partitioning of the inhibitor between aqueous phase and substrate surface as proposed by Mattson et al. (20), the partitioning is more likely between the core of the oil droplet and its surface monolayer. To test this hypothesis we followed the approach of Mattson et al. (20). The linear form of the Langmuir solution adsorption isotherm is given by the equation:

$$\frac{Ce}{y} = \frac{a}{Y_m} + \frac{Ce}{Y_m}$$

where Ce is the equilibrium bulk concentration of solute, y is the amount of solute adsorbed, Y_m is the total possible amount of solute that is adsorbed (i.e., a pure complete monolayer of solute), and a is K_1/K_2 where K_1 and K_2 are proportionality constants related to the desorption and adsorption characteristic of each hydrophobic solute. The rate of hydrolysis is proportional to the fraction of the surface not occupied by hydrophobic solute. Substituting in the Langmuir equation yields

$$\frac{I}{V_o - V_i} = \frac{K}{V_o} + \frac{I}{V_o}$$

where I is the total concentration of solute in the reaction mixture, V_o is the rate of lipolysis in the absence of solute, V_i is the rate of lipolysis in the presence of solute, and $K = K_d/K_a$ where K_d and K_a are proportionality constants related to desorption and adsorption. According to this interpretation $V_o - V_i$ is proportional to the amount of oil surface that is occupied by hydrophobic solute. A plot of $I/(V_o - V_i)$ against I should yield a straight line if treatment of the data is in accord with the Langmuir theory, with the intercept K/V_o and the slope $1/V_o$. The plot for hexadecane is shown in **Fig. 6**. The slopes, as predicted, are the same since the rates of hydrolysis of the uninhibited reaction does not change with the levels of substrate used. Thus we have modified the interpretation of Mattson et al. (20) for a large number of structurally diverse hydrophobic compounds that are highly soluble in fat and we propose that they partition into the substrate monolayer from the interior of the droplet.

Fat droplets are thought to be composed to two distinct phases, an oil core and a surface monolayer of ca. 20 Å thickness (i.e., the length of an 18-carbon fatty acid) (32).

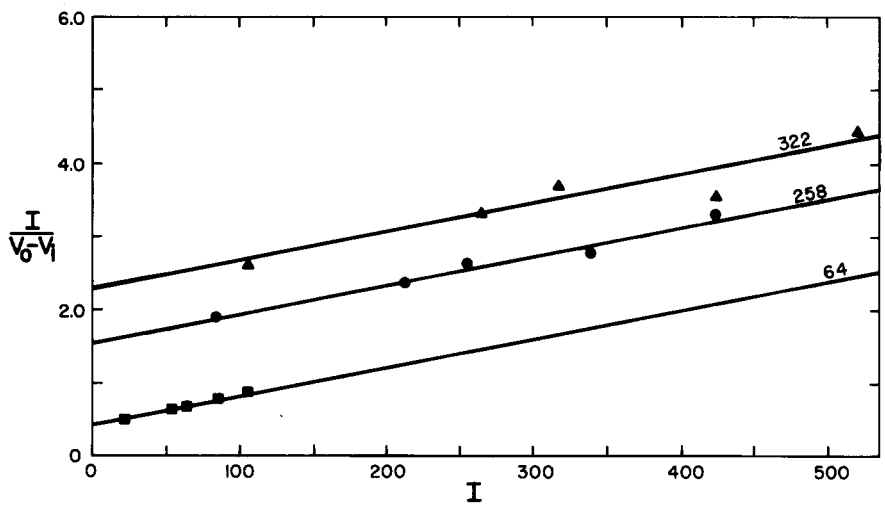

Fig. 6. Plot of I/V_o-V_i versus I for hexadecane. Values above lines represent substrate concentrations in μmol. V_o and V_i are the rates of hydrolysis in the absence and presence of hydropobic solute; I, hexadecane concentration in μmol.

Triglyceride molecules that exist in tuning fork configurations in the oil core take a different configuration in phospholipid bilayers in which all three carbonyl groups of the triglyceride molecule are exposed to the aqueous interface (33). Presumably this more ordered configuration also occurs at the surface of a fat droplet. Although the surface monolayer is the only part of the droplet in direct contact with water, the ordered arrangement of molecules in this zone is thought to orient those lipid molecules immediately below them so that there may be one or more layers of partially ordered lipids below the surface monolayer (34). Despite this order, the movement of the lipid molecules should still be very fast in the surface layers with molecules changing lateral position relative to each other at rates of 10^8–10^9 times/sec (35). The emulsifier used in this study, gum arabic, is a high molecular weight (M_r 340,000–560,000) polymer whose backbone consists of D-galactose units and its side chains of D-glucuronic acid units with L-arabinose as end units (36). The extremely stable triglyceride emulsions made from gum arabic are thought to be stabilized by an interlocking noncovalent network of long polysaccharide filaments (37). The dimensions of the filament network (i.e., mesh size) are not known although the triglyceride surface monolayer is sufficiently exposed to allow free adsorption of lipase, colipase, bile salts, and phospholipids (12).

The air–water interface has been extensively used as a model of lipid–water interfaces. At this surface lipids can form tightly packed monolayers with their hydrophobic tails projecting into the gas phase. According to Small (38), the hydrocarbons (nonpolar lipids) used in this study do not form monolayers at the air–water interface but instead rest on the surface as oil drops or crystals. The long-chain triglyceride, cholesterol, and n-alcohols used in this study (Class 1 nonswelling amphiphiles), because of their ability to form hydrogen bonds with water, can spontaneously spread at the air–water interface and form a monolayer (38). Hydrocarbons, like hexadecane, may occur in a monolayer if there are already spreading lipids such as triglyceride or phospholipid present in which the hydrocarbons can dissolve (where they align parallel to the lipid acyl chains) (39, 40). Thus, if the air/water and oil/water monolayers are comparable, there will be a basic difference in the interaction of hydrophobic alcohol and hydrocarbons with the fat droplet monolayer. While the alcohols can compete with the triglyceride molecules for hydrogen bonding in the aqueous phase, the hydrocarbons cannot. The parallel alignment of n-alcohols and triglycerides in the surface monolayer is partially induced by the interaction of parts of their molecules with water. The parallel alignment of the hydrocarbons in the substrate surface monolayer would be expected to be induced by the ordering effect of the aligned acyl chains of triglyceride molecules (39, 40). The absence of a chain length effect with the hydrocarbons (Fig. 3) suggests that the hydrocarbons are not adsorbed to the surface but that they interdigitate the triglyceride molecules and align parallel to the lipid acyl chains.

The greater inhibition of lipolysis by the alcohols, as compared to the hydrocarbons, may be due to the greater solubility of the alcohols in the surface monolayer and/or to a specific alcohol-hydroxyl group interaction with the active site of lipase. Mattson et al. (20) found that there was competition between water and fatty alcohol as the fatty acid acceptor during lipolysis which led to fatty acid-fatty alcohol ester (wax ester) formation. Although we did

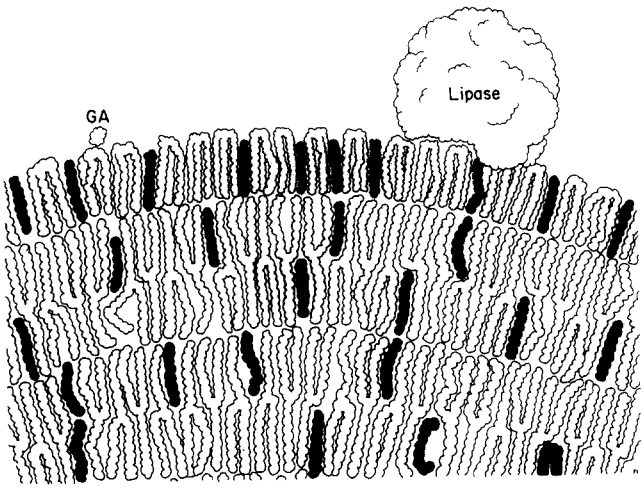

Fig. 7. Hypothetical scale drawing of pancreatic lipase at the oil-water interphase of a fat droplet containing hexadecane (in black) and long chain triglyceride (in white) in a molar ratio of 1:3. Note the different folding of the triglyceride molecule in the surface monolayer (33). A cross section of a strand of the polysaccharide emulsifier (equivalent to the cross section of sugar molecule) is shown as GA (gum arabic).

not measure wax esters in this study, they are known to be slowly hydrolyzed by lipases (41) and could have contributed to the observed inhibition. The activation of lipolysis by low cholesterol concentrations may reflect cholesterol's ability to condense monolayers of acyl lipids (42). This would effectively increase the surface concentration of substrate molecules and thereby stimulate lipolysis. With higher concentrations, presumably substrate dilution and/or ester formation occurs, and lipolysis is inhibited.

The results of this study suggest that the parts per million and parts per billion levels of hydrocarbon pollutants in dietary fat (24) should not significantly affect fat digestion. From a practical standpoint, alkanes and particularly fatty alcohols could be used to control the biodegradation of triglyceride and the release of drugs dissolved in triglyceride vehicles.

In summary, hydrocarbons that are dissolved in fat droplets appear to inhibit lipolysis by partitioning (from within) into the surface monolayer of the droplet and diluting the substrate concentration that is available to lipase (5, 43). **Fig. 7** shows a schematic scale drawing of how the surface of a triglyceride:hexadecane fat droplet (mole ratio 3:1) might look at the instant that lipase binds to it. A cross section of a strand of gum arabic polysaccharide equivalent to the cross section of a sugar molecule is shown at GA. It is hypothesized that hydrocarbons inhibit the interfacial activation of pancreatic lipase by diluting the high surface concentration of substrate.

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