Possible Roles of Bile Lipids and Colipase in Lipase Adsorption†

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ABSTRACT: The adsorption isotherms of bile salts, phospholipids, and cholesterol were determined with siliconized glass beads. It was observed that the molar fractions of cholesterol, phospholipid, and bile polypeptide fractions increased simultaneously and considerably on the surface of the beads in comparison to the corresponding fractions found in bile. The composition of the adsorbed film is approximately 1 cholesterol: 2 phospholipid: 3 bile salt molecules. The preformed complex of lipase, colipase, and bile lipids behaves as an entity which determines lipase adsorption. The modification of the interface quality of a lipid substrate by a detergent is not per se the reason for the lack of lipase adsorption. A model is proposed according to which lipolysis under physiological conditions would occur in two steps requiring two cofactors. Colipase would be necessary for the formation of the lipase–bile lipoprotein complex, and bile lipids would be required to direct the adsorption of this lipolytic entity toward the emulsified substrate.

During the last several years, many studies in vitro have been concerned with the interfacial adsorption of pancreatic lipase and colipase in the presence of lipids and detergents (Vandermeers et al., 1975; Borgström, 1975; Chapus et al., 1975; Momsen and Brockman, 1976a). It is generally agreed that interfacial adsorption of lipase is an important preliminary step which does not occur in the presence of bile salts but which is fully restored by colipase. Although this scheme is generally accepted, there, nevertheless, exist contradictory and occasionally confusing reports concerning the molecular mechanisms of bile salt inhibition of lipolysis and the associations of lipase and colipase (Donner et al., 1976), lipase and bile salts (Borgström and Donner, 1976), and colipase and bile salts (Charles et al., 1975; Donner et al., 1976). Incited by our previous observations in vivo (Nalbone et al., 1974), the present study was undertaken to clarify the physiological significance of the above-mentioned studies, which did not take into account the existence of amphipaths other than bile salts.

There are several minor constituents in bile secretion, particularly a well-defined lipoprotein complex (Lairon et al., 1972; Lafont et al., 1977), which has an equivalent molecular weight of 100 000 and contains nearly all bile phospholipids, cholesterol, and a well-characterized polypeptide. When lipase and colipase are incubated with bile they are found tightly associated with the lipoprotein complex (Lairon et al., 1975) and are resistant to inactivation by trypsin and heat (Lairon et al., 1978a). In most mammals, biliary and pancreatic secretions are delivered in a topographically limited region of the duodenum. In rats, these secretions are mixed before entering the intestine. In addition, it is now well established that biliary and pancreatic secretions are simultaneously triggered by the same hormonal stimulus (Jorpes and Mutt, 1966).

These physiological situations indicated the possible importance of preformed associations among lipase, colipase, and biliary constituents during lipolysis. The purpose of the present report was to qualitatively determine the influence of biliary lipids, i.e., bile salts, phospholipids, and cholesterol, on lipase and colipase adsorption on a model interface composed of siliconized glass beads.

Materials and Methods

Enzymes. Lipase was purified (Verger et al., 1969) from delipidated porcine pancreas powder (Organon). Traces of cofactor were removed as previously described (Rietch et al., 1977). The specific activity of the mixture of lipase LA and LB was 3170 units/mg of protein.

Pancreatic colipase was a mixture of porcine colipase I and II, prepared as previously described (Maylié et al., 1973). The specific activity of the preparation was 5480 units/mg of protein.

Lipase activity was potentiometrically measured in the presence of 6 mM bile salts on triolein stabilized with gum arabic at pH 9.0 and 25 °C (Desnuelle et al., 1955).

Sufficient amounts of colipase (bovine colipase, a generous gift of L. Sarda, Marseille) were added to the assay medium, since colipase-free lipase is strongly inhibited by micellar solutions of bile salts. Cofactor activation of lipase was also used to determine the amount of colipase present under the same conditions.

Lipids. Sodium glycodeoxycholate and sodium taurocholate (Calbiochem, A grade) were used without further purification. [^{14}C]Deoxycholate and [^{14}C]taurocholate were obtained from the Radiochemical Centre, Amersham. The quantitative determination of 3α-OH bile salts was performed with an automated enzymatic method (Domingo et al., 1972). Radioactivity was determined by liquid scintillation counting after the addition of aliquots to 10 mL of Unisolve (Kock Light).

Bile was obtained from the gall bladder of pigs, excised in the slaughterhouse immediately after death, and stored at +4 °C. Insoluble material was removed by centrifugation at 15 000g for 10 min. Supernatants were used within 1 week of bile collection. Bile phosphatidylcholines were purified from rat bile by column chromatography on silicic acid.

Siliconized Glass Beads. Most experiments were performed with spherical siliconized glass beads obtained from Serva (Fein-biochemica, Heidelberg, Germany). Their mean diameter (Chapus et al., 1975) was 167 μm, corresponding to a surface of 164 cm^2/g of beads.

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Very similar lipase and colipase adsorption values (±3%) were obtained with spherical siliconized beads obtained from Applied Science Laboratories (170–230 mesh). Their mean specific surface, determined with a Nikon Model 5CT2 comparator, was 352 cm^2/g of beads. Beads were rinsed thoroughly with distilled water and dried in an air stream at 40 °C. All glassware was soaked in chromic acid cleaning solution for 15 min, rinsed with distilled water, and dried.

Determination of Adsorbed Lipids and/or Proteins. A known amount of beads was added to several solutions of different concentrations of proteins and/or lipids prepared in buffer in 32-mL glass test tubes. Beads were omitted in control tubes. The contents of the tubes were thoroughly mixed for 1 min in a Cenco prestomix vortex mixer, beads were allowed to sediment for 1 min, and the protein and/or lipid content was determined in aliquots of the supernatant. The maximal adsorption capacity was estimated by extrapolating to zero concentration in the supernatant as described (Chapus et al., 1975; Brockman et al., 1973). The buffer used was 100 mM Tris-HCl[1] (pH 7.0), 150 mM NaCl, 20 mM $CaCl_2$.

Adsorption Measurements of Purified Lipids. Three different solutions of purified lipids were used successively.

(1) An equimolar solution (20 mM) of ^{14}C-labeled taurocholate and deoxycholate, each with specific radioactivities of 2×10^3 dpm/μmol. This mixture was chosen in order to maintain the dihydroxyl/trihydroxyl balance of bile salts normally found in bile. Bile salt concentrations in the range of 25 μM to 2 mM were determined in aliquots of the supernatant in the presence or absence of 5 g of beads in 4 mL of solution.

(2) An optically clear mixed phosphatidylcholine–bile salt solution, prepared by adding an equimolar solution of glycodeoxy cholate and taurocholate (final bile salt concentration 20 mM) to a known amount of purified bile phosphatidylcholine.

(3) Unilamellar liposomes, prepared (Huang, 1969) by filtering the sonicated (M.S.E. Sonicator, 20 kHz, 20 min, 4 °C) suspension of purified bile phosphatidylcholines (final lipid concentration 1 mg/mL) on Sepharose 4B. Reconstituted phosphatidylcholine vesicles containing 5% (w/w) biliary polypeptide were obtained by sonicating the unilamellar liposome preparation for 3 min with the biliary peptide fraction, prepared as described (Lafont et al., 1977). Experiments were performed in the presence or absence of 5 g of glass beads in 3 mL of solution. Phosphatidylcholine concentrations in the range of 5 to 53 μM were determined by phosphorus analysis (Amic et al., 1972) in 0.8-mL aliquots of the supernatant extracted with $CHCl_3$/MeOH (2:1, v/v).

Measurements of Pig Bile Adsorption. The freshly collected and centrifuged pig bile was diluted 100 times with an equimolar solution of taurocholate and glycodeoxycholate (final bile salt concentration 0.1 or 20 mM). Identical quantities of ^{14}C-labeled taurocholate and deoxycholate were added to obtain a final specific radioactivity of 1.7×10^3 dpm/μmol. Experiments were performed in the presence or absence of 5 g of beads in 3 mL of solution. Bile salt concentrations in the range of 0.6–2.5 mM were determined by scintillation counting of aliquots of the supernatant.

Cholesterol concentrations in the range of 16–100 μm were determined in aliquots of the supernatant by a standard colorimetric method (Boehringer-Mannheim, Germany). Phospholipid concentrations in the range of 5–33 μM were estimated by phosphorus analyses of 0.8-mL aliquots of supernatants after extraction with $CHCl_3$/MeOH (2:1, v/v).

Measurements of Biliary Protein Adsorption. The freshly collected and centrifuged pig bile was diluted 470 times with an equimolar solution of taurocholate and glycodeoxycholate (final bile salt concentration 20 mM). The quantity of adsorbed polypeptide was determined after filtering the bile sample through Agarose as described (Lafont et al., 1974). The polypeptide-containing lipoprotein complex was collected in the void volume and dispersed in the same bile salt solution. Assays involved adding 40 mL of the diluted bile solution or of the purified lipoprotein complex to 70 g of beads. The final biliary protein concentrations were 11 and 4 μg/mL, respectively. After mixing for 15 min, the beads were allowed to settle for 2 min. The protein concentration in aliquots of the supernatant was determined after acid hydrolysis (Spackman et al., 1958).

Measurements of Pure Lipase and Colipase Adsorptions. All experiments were performed in 100 mM Tris-HCl (pH 7.0), 150 mM NaCl, 20 mM $CaCl_2$. Adsorptions on siliconized glass beads were determined by adding 500 mg of beads to 4 mL of various protein solutions containing from 75 to 750 nM protein, in buffer or lipid solutions contained in 32-mL test tubes. Lipase and colipase activities were determined by measuring enzyme activity in aliquots of supernatants. Bound protein was estimated by extrapolating to zero concentration in the supernatant (Chapus et al., 1975; Brockman et al., 1973). It was not possible to extrapolate when 500 mg of beads was added to only 2 mL of solution, perhaps due to the sensitivity of pancreatic lipase to interfacial inactivation.

Measurements of Lipase and Colipase Adsorptions When Previously Combined with Biliary Lipids. Bile phosphatidylcholines were labeled in vivo by intravenously injecting [^{14}C]oleic acid to fasting rats, as described by Ulrich et al. (1974). Bile was recovered by canulating the main bile duct for 6 h following injection. The phospholipid fraction of the collected bile contained 95% of the [^{14}C]oleic acid, of which 90% was phosphatidylcholine. The specific radioactivity of this phospholipid was 1.75×10^6 dpm/μmol. Equimolar mixtures of lipase and colipase were added to varying quantities of rat bile containing between 4 and 200 μmol of [^{14}C]phosphatidylcholine. The mixture was stirred for 2 h at 4 °C and was then passed through a column of Agarose A-5m previously equilibrated with buffer containing 3 mM each of taurocholate and glycodeoxycholate. The second void volume contained a bile–lipoprotein complex with which almost all lipase and colipase activities were associated (Lairon et al., 1975) and which contains fixed proportions of phosphatidylcholine, cholesterol, and biliary peptide (Lairon et al., 1972; Lafont et al., 1974). Adsorptions of lipase, colipase, and phosphatidylcholines from this complex to siliconized glass beads were determined by adding 500 mg of beads to 4 mL of solutions of the complex containing from 80 to 550 nM lipase and colipase in 32-mL glass test tubes. Lipase and colipase adsorption was determined as described above for the pure proteins. Phospholipid content was determined by the radioactivity contained in 1 mL of the supernatant.

Results

The adsorption of an equimolar mixture of taurocholate and deoxycholate to siliconized beads as a function of bile salt concentration is shown in Figure 1a. It was calculated that the quantity of bile salts adsorbed was negligible compared to the nonadsorbed fraction.

The formation of premicelles and micelles in the presence of beads was simultaneously followed by recording the ab-

[1] Abbreviation used: Tris-HCl, 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride.

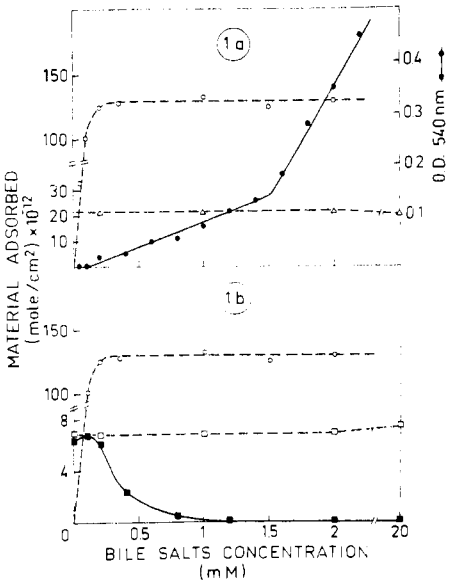

FIGURE 1: Adsorptions of bile salts, lipase, and colipase on siliconized glass beads as a function of bile salt concentration. See Materials and Methods for experimental details: (a) (●) absorbance increase at 540 nm due to Rhodamine 6G, (○) quantities of adsorbed bile salts, (△) quantities of adsorbed colipase; (b) (○) quantities of bile salts adsorbed, lipase adsorption in the presence (□) or absence (■) of colipase.

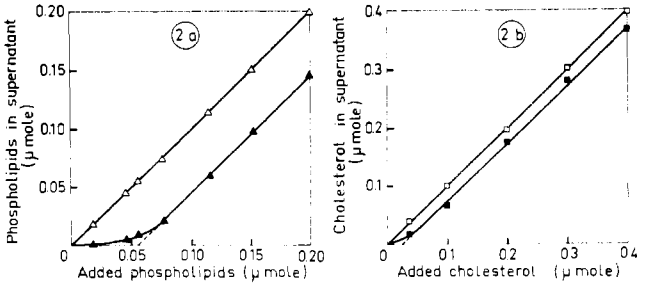

FIGURE 2: Adsorption of diluted pig bile on siliconized glass beads. See Materials and Methods for experimental details. Filled and clear symbols represent the presence or absence, respectively, of beads: (△, ▲) phospholipids, (□, ■) cholesterol.

TABLE I: Adsorption of Purified Lipids on Siliconized Glass Beads.

lipids adsorbed[a]	physical state	lipid density on the beads ($\times 10^{-12}$ mol/cm^2)	molecular area (Å^2/molecule)
PC	liposome suspension	178	92
PC	liposome suspension with biliary peptide	146	113
equimolar tCh + dCh	micellar soln	126	131
PC	mixed micellar soln in bile salts	83	200

[a] Abbreviations used: PC, phosphatidylcholines; tCh, taurocholate; dCh, deoxycholate.

sorption at the maximum wavelength (540 nm), resulting from the incorporation of Rhodamine G into the aggregates, as described by Maylie et al. (1973) who published a similar study concerning taurodeoxycholate micelle formation. The interesting finding emerging from Figure 1a is that bead surface is nearly saturated with bile salts when "primary" micelles begin to appear (0.2 mM). The formation of "secondary" micelles (after 1.5 mM) apparently does not change the amount of adsorbed bile salt. This agrees with results obtained for the *n*-hexyl laurate-water interface (Nano and Savary, 1976).

The adsorptions of pure lipase, colipase, and their mixtures were also studied as a function of bile salt concentration. In the range of 0–20 mM bile salts, colipase adsorption is identical (Figure 1a). The surface occupied per colipase molecule was calculated as 790 Å^2, which is quite similar to the value reported by Borgström (1976) in the absence of bile salts. Chapus et al. (1975), however, found that colipase did not adsorb to siliconized glass beads in the absence of bile salts. In disagreement with the findings of Borgström, we found that colipase binding is not affected by bile salts. We do not presently have an explanation for these differences, but our observations do not support the belief that colipase specifically recognizes one type of interface.

The adsorption of pure pancreatic lipase is optimal until 0.2 mM bile salts (Figure 1b), when "primary" micelles begin to appear. Bead surface is nearly saturated with bile salt molecules at this point. At concentrations of bile salts greater than 0.2 mM, lipase rapidly loses its adsorption capacity, which falls to zero well before 1.5 mM, representing the "second critical micellar concentration" under our experimental conditions.

We observed that colipase facilitates lipase adsorption in the presence of bile salt concentrations as high as 20 mM, which is in agreement with previous findings (Vandermeers et al., 1975; Borgström, 1975; Chapus et al., 1975; Momsen and Brockman, 1976a). The physiological significance of this observation will be discussed below.

The adsorption isotherm measurements of purified lipids on siliconized glass beads are shown in Table I. The surface occupied by a phosphatidylcholine molecule, 92 Å^2, roughly corresponds to a tightly packed lipid monolayer (Van Deenen, 1971). This figure increases to 113 Å^2/molecule when reconstituted phosphatidylcholine vesicles containing 5% (w/w) biliary peptide are used; this difference probably corresponds to the surface occupied by the polypeptide. The surface occupied by phosphatidylcholine increases to 200 Å^2/molecule in a solution of optically clear mixed phosphatidylcholine–bile salt micelles containing 1 phospholipid per 1000 bile salt molecules; the remainder of the surface is occupied by bile salts. Considering the areas of 131 and 92 Å^2 occupied by a bile salt and a phosphatidylcholine molecule, respectively, we may state that the surface of siliconized glass beads is occupied by a mixed monolayer composed of an approximately equimolar mixture of bile salts and phosphlipids. The significance of this result should be stressed: the phospholipid molar fraction undergoes a 1000-fold relative increase in the presence of a hydrophobic interface. This means that a relatively minor component, e.g., phospholipid in a detergent solution, can undergo a large concentration at a hydrophobic interface, where lipolytic reactions occur, as a result of its particular tensioactivity.

The simultaneous adsorptions of bile lipids on siliconized glass beads are shown in Figure 2 when diluted bile is used as the source of lipids. The data indicate that adsorption involves a mixed lipid film, probably a condensed monolayer. From column 3 of Table II we may deduce that each lipid molecule occupies an average of 95 Å^2 of bead surface. The cholesterol/phospholipid/bile salt composition of this film is approximately 1:2:3, which represents about a 1000-fold enrichment of cholesterol, and phospholipid compared to the

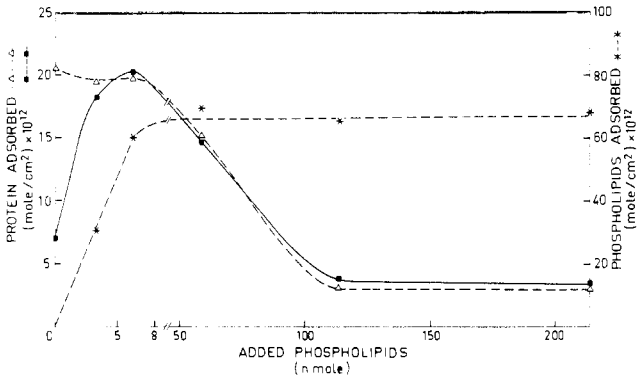

FIGURE 3: Adsorptions of lipase and colipase preassociated with variable amounts of the bile-lipoprotein complex. See Materials and Methods for experimental details: (✱) quantities of phospholipids adsorbed, (■) quantities of lipase adsorbed, (△) quantities of colipase adsorbed.

TABLE II: Adsorption of Diluted Pig Bile on Siliconized Glass Beads.[a]

bile constituent	molar fract in dil bile (per 1000)	molar fract on the surf of the beads (per 1000)	surf dens on the beads ($\times 10^{-12}$ mol/cm^2)	surf dens on the beads after wash. ($\times 10^{-12}$ mol/cm^2)
cholesterol	from 0.11 to 1.10	170	30	28
phospho-lipid	from 0.25 to 2.50	380	66	63
bile salt	from 999.64 to 996.40	450	78	16

[a]The native vesicular bile was diluted 800- to 8000-fold with a solution of 10 mM each of taurocholate and glycodeoxycholate.

TABLE III: Adsorption of an Equimolar Mixture of Lipase and Colipase on Siliconized Glass Beads.[a]

siliconized glass beads	incubat condit prior to bead addit	colipase dens on beads (10^{-12} mol/cm^2)	lipase dens on beads (10^{-12} mol/cm^2)
untreated	pure lipase + colipase	22.0	6.6
untreated	pure lipase + colipase	20.0	7.3
precoated with biliary lipids	pure lipase + colipase	23.0	6.1
untreated	lipase and colipase preassoc with bile lipids	20.0	21.0
precoated with biliary lipids	lipase and colipase preassoc with bile lipids	0.5	0.8

[a]An equimolar mixture of lipase and colipase was incubated for 15 min in the absence or presence of diluted bile (2.5 μM phospholipid and 20 mM bile salts). Adsorptions of lipase and colipase were measured by adding 500 mg of precoated or untreated beads to 4 mL of several solutions of variable concentrations. Beads were precoated by incubating for 15 min with 4 mL of diluted bile (2.5 μM phospholipid and 20 mM bile salts). Beads were then washed three times with 4 mL of buffer. Bile salts concentration was 20 mM in all experiments except the first one which was performed in absence of bile salt.

molar fraction found in diluted bile (column 1 of Table II). The molar fraction of adsorbed bile salts is thus reduced, and this reduction was even more pronounced after washing the beads with three aliquots of 4 mL of buffer (column 4 of Table II): 80% of the adsorbed bile salts could be washed off under these conditions. Adsorbed phospholipid and cholesterol, however, could not be washed off, probably as a result of strong interactions between these lipids and the hydrophobic surface. These results with bile confirm those obtained with purified lipids (Table I), and their possible physiological significance will be discussed below.

Proteins constitute another minor bile component. Albumin, the major bile protein, is not significantly adsorbed to the beads under our conditions. A biliary polypeptide of 6000 daltons, however, was found to be stoichiometrically associated after gel filtration as a lipoprotein complex (Lafont et al., 1974). This polypeptide, free of albumin and bile salts (see Materials and Methods), was adsorbed to beads, and the polypeptide/phospholipid weight ratio at the interface (3.7%) was similar to that in the lipoprotein complex (4.3%).

Bile salts are not the only biliary lipids present in the intestinal tract; cholesterol and phospholipids could thus participate in lipase and colipase adsorptions. The results of adsorption isotherm studies of biliary lipids to a hydrophobic surface (Table II) support this contention. This was directly verified by determining the adsorption of an equimolar mixture of lipase and colipase previously combined with varying quantities of bile lipids, as described under Materials and Methods. Lipase and colipase adsorptions to siliconized glass beads in the presence of 10 mM each of taurocholate and glycodeoxycholate are shown in Figure 3 as a function of biliary lipid concentration, detected by phospholipid assays. It can be seen that phosphatidylcholine adsorption is linear until a plateau, corresponding to the value obtained with diluted bile (Table II). During the linear phase of adsorption, lipase adsorption underwent a threefold increase, while the quantity of colipase was essentially unchanged. At maximal adsorption on the surface of the beads, the ratio of lipase/colipase/phosphatidylcholine is 1:1:3. After saturation of the surface with bile lipids, further addition to the medium results in an increased phospholipid concentration in the micellar phase. Thus, a continuous and parallel decrease of the quantities of lipase and colipase adsorbed is observed.

In the presence of bile lipids, it is interesting to note that the ratio of adsorbed colipase/lipase is nearly equal to unity, as it was in the incubation medium; this stoichiometry was investigated further. Two molar ratios of colipase/lipase were compared in the incubation medium (3:1 and 1:1) and on the surface of the beads in the presence of buffer only, 20 mM bile salts or 2.5 μM biliary phospholipids in 20 mM bile salts. In the absence of biliary phospholipids, the molar ratio of adsorbed colipase/lipase was always 3:1. In the presence of biliary phospholipids in the medium, however, the colipase/lipase ratios were the same in solution and on the bead surface. We attempted to clarify the possible influence of preformed lipase-colipase-lipoprotein complexes before lipase adsorption. Experiments were performed (Table III) involving an equimolar mixture of lipase and colipase preassociated with the quantity of bile lipids resulting in maximal adsorptions of lipase and colipase (see Figure 3). Before addition of the beads, they were coated with a mixed bile lipid film, mentioned in Table II. Adsorbed lipase and colipase under these conditions corresponded to 2 and 4% of the maximal values in the case of untreated beads. In the presence of 20 mM bile salts, however, lipase and colipase adsorptions were identical, regardless of whether or not the beads were coated with bile lipids. In ad-

dition, this adsorption behavior is not significantly changed by the omission of bile salts.

Discussion

Siliconized glass beads were recently introduced as a model interface for the study of catalysis by adsorbed enzyme (Brockman et al., 1973). The experimental simplicity of this system was presently utilized to study the inhibition of pancreatic lipase adsorption by bile salts and its reversal by colipase. In our hands, the respective behaviors of lipase and colipase were qualitatively similar when using siliconized glass beads as a model interface in place of a triglyceride emulsion (Vandermeers et al., 1975; Borgström, 1976). Namely, lipase and colipase adsorbed readily to the beads surface.

Although the adsorption of lipase is prevented by concentrations of bile salts greater than 0.2 mM, that of colipase still occurs; lipase adsorption is thus facilitated (Figure 1). It is noteworthy that lipase desorption from beads occurs in a range of bile salt concentration corresponding to the formation of "primary" micelles. This suggests that the lipase-"primary" micelle complex cannot be adsorbed on an interface which is covered with a bile salt monolayer. This is consistent with the conclusions reached by Momsen and Brockman (1976b). Recently, the inhibitory effect of bile salts on lipase activity was reported to supposedly result from the building of a detergent monolayer at the oil-water interface, thus preventing lipase from reaching its substrate (Morgan et al., 1969; Borgström and Erlanson, 1973; Chapus et al., 1975). Subsequent research has shown that the surface charge of a glyceride emulsion is not significantly changed by the presence of bile salts (Brown et al., 1977). The present findings demonstrate that lipase alone can be adsorbed on an interface covered with pure bile salts (Figure 1). This was previously observed after affinity chromatography on a hydrophobic support coated with bile salts (Lairon et al., 1978b). Thus, detergent-provoked changes of the interface quality of a lipid substrate, probably leading to a decrease of the interfacial substrate packing, do not per se explain the lack of lipase adsorption. This conclusion is compatible with previous monolayer studies in which lipase could penetrate a lipid film only under conditions of a given critical lipid packing (Verger et al., 1977).

We also determined the adsorptions of lipid to siliconized glass beads (Tables I and II) and observed that the molar fractions of cholesterol and phospholipid were simultaneously and considerably increased on the surface of the beads in comparison to the bile solution (Table II). The interface concentrations of cholesterol and phospholipid were increased by about 1000 times. This may be understood by considering that bile salts, unlike phospholipid and cholesterol, are highly water-soluble detergents. In addition, the latter lipids spontaneously form insoluble monolayers at an oil-water interface. In contrast to the phospholipid-bound polypeptide, soluble bile proteins such as albumin were not adsorbed to the beads (Table II), probably for the same physicochemical reasons.

The concentration increase of the bile polypeptide was identical to that of phospholipid and cholesterol during bile adsorption to beads. This suggests that the bile-lipoprotein complex behaves as an entity during adsorption. The formation of such a biliary lipid monolayer around an oil droplet could be expected to be physiologically important for several reasons. Linthorst et al. (1977) studied triglyceride emulsification by amphipaths present in the intestinal lumen during fat digestion and showed that bile salts alone emulsified small quantities of triolein, but that, as expected, fatty acid soaps and phosphatidylcholine alone produced highly stable concentrated emulsions after gentle mechanical rotation. Decreased emulsification was observed when the taurocholate concentration was greater than 1 mM. It can be deduced from these data that only bile phospholipids can assure a stable fine emulsification of dietary triglycerides. These emulsification properties are certainly of prime importance in the light of the fact that the rate of lipolysis is directly proportional to the emulsion area (Schönheyder and Volquartz, 1945; Sarda and Desnuelle, 1958).

The accretion of a mixed lipid monolayer around emulsified droplets in the intestinal lumen could also influence the adsorptions of lipase and colipase. In the absence of phospholipid in solution, however, the existence per se of a mixed biliary lipid film on the surface of the beads does not significantly change the adsorption patterns of lipase or of a lipase-colipase mixture (Table III). This is to be compared with Figure 1, where lipase could be adsorbed to an interface covered with pure bile salts. The data of Table III show that optimal lipase adsorption occurred when untreated beads were added to lipase and colipase preassociated with bile lipids: lipase adsorption was three times greater than that observed in the absence of bile lipids. If under these later conditions one considers that the beads surface was totaly covered by lipase molecules, it is difficult to understand how the presence of a bile-lipoprotein complex can further increase this amount of enzyme adsorbed by a factor of 3. The explanation for the above observations is not straightforward, since it can be explained by many mechanisms including a change in the protein conformation, an increase in the total surface area available for adsorption, etc.

It is also noteworthy that lipase and phospholipid adsorptions both increase until the point corresponding to phospholipids saturation of the interface (Figure 3); i.e., the quantity of bile lipids required for maximal lipase adsorption corresponds to the quantity covering the surface of the beads with a monolayer. The excess of bile lipids in micellar solution produces a simultaneous decrease of the quantities of adsorbed lipase and colipase. It is probable in vivo, however, that bile lipids increase the emulsion surface, as a result of their emulsification properties, to a point where almost all the bile lipids coat the emulsion, thus enabling maximal lipase adsorption to occur. This hypothesis is consistent with our previous results (Nalbone et al., 1974), which showed that all the lipase, colipase, and bile lipids were associated with the glyceride emulsion in the contents of the rat intestines after a fatty meal. In addition, the molar ratio of adsorbed colipase/lipase in the absence of bile phospholipids is always 3, regardless of the proportions of these proteins in solution. This value also corresponds to the cross-section ratio of lipase/colipase. In the presence of bile phospholipids in the medium, however, the colipase/lipase ratios in solution and on the beads are identical. These experiments strongly suggest that the preformed lipase-colipase-bile lipids association behaves as an entity during adsorption. In contrast, the association of three soluble components such as lipase, colipase, and bile salts (Maylie et al., 1973; Donner et al., 1976) would not be the form in which lipase is adsorbed on emulsions. As already postulated (Vandermeers et al., 1975; Chapus et al., 1975; Borgström, 1976), it is probable that in the presence of pure bile salts colipase is adsorbed first, serving as an anchor for lipase. Such a sequential mechanism apparently does not operate when bile lipids are present. Under these conditions, which are close to physiological reality, the bile lipoprotein complex would provide an interfacial vehicle for lipase and colipase for their simultaneous adsorption on the emulsion.

Based on the preceding observations in vitro, as well as on the anatomical considerations presented in the introduction, we propose that under physiological conditions lipolysis would

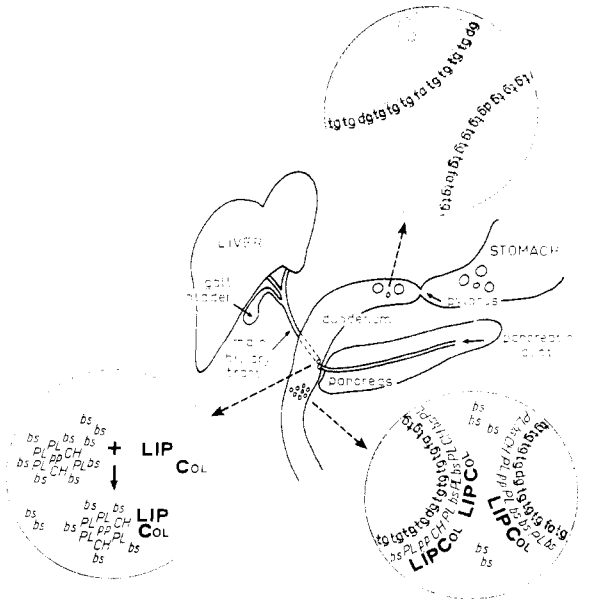

FIGURE 4: Model for the interaction in vivo between pancreatic lipase, colipase, bile-lipoprotein complex, and dietary glycerides: LIP, lipase; COL, colipase; PL, phospholipids; CH, cholesterol; pp, polypeptide; bs, bile salts; tg, triglycerides; dg, diglycerides; fa, free fatty acids.

occur in two successive steps, schematically shown in Figure 4, requiring colipase and bile lipids. The first step involves the formation of a lipase-colipase-bile lipoprotein complex association, with bile salts as micellar solvent. Colipase is required for the formation of this association but not for the adsorption of lipase to the glyceride emulsion. This preformed complex could be the functional lipolytic entity, as previously suggested by Lairon et al. (1978a). This preformed complex can be efficiently adsorbed as a single unit on the oil emulsion flowing from the pylorus into the duodenum. As a result of their high interface adsorption capacity, bile lipids could serve as a carrier for lipase and colipase toward the glyceride droplets. According to this scheme, under physiological conditions colipase would be needed for the formation of a lipase-bile lipoprotein complex, and bile lipids would be required to direct the adsorption of the lipolytic entity toward the emulsified substrate.

This model of intestinal lipolysis may be compared to the conditions of lipoprotein lipase action (Chung et al., 1973; Elgerud and Olivecrona, 1973; Scow and Olivecrona, 1977). This enzyme, as pancreatic lipase, requires a low-molecular-weight cofactor and the chylomicrons are one of its physiological substrates (Higgins and Fielding, 1975). Chylomicrons are composed of a triacylglycerol core surrounded by a monolayer surface film of phospholipids, cholesterol, and protein (Zilversmit, 1967). It may be imagined that the mode of action of both lipases present some similarities and research in progress is exploring this possibility.

Note Added in Proof

Since this paper was submitted for publication, Patton et al. (1978) reported that no significant lipase-colipase binding occurs in vivo at the duodenal protein concentrations measured. This conclusion was drawn from data performed mainly in the presence of bile salts only. However, based on the results shown in the present paper, the bile lipoprotein complex when present enables the lipase-colipase interaction.

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Relationships between Membrane Cholesterol, α-Adrenergic Receptors, and Platelet Function†

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ABSTRACT: Platelets incubated with cholesterol-rich phospholipid dispersions acquire membrane cholesterol, and this is associated with decreased membrane fluidity and increased sensitivity of these platelets to the aggregating agent epinephrine. Conversely, platelets incubated with cholesterol-free phospholipid dispersions lose membrane cholesterol, have increased membrane fluidity, and exhibit a decreased sensitivity to epinephrine. Epinephrine-stimulated platelet aggregation is initiated by the interaction of the amine with α-adrenergic receptors. Therefore, we tested whether the altered responsiveness of cholesterol-enriched or -depleted platelets to epinephrine results from a change in the number or affinity of α-adrenergic receptors or in the activity of these receptors in inhibiting adenylate cyclase. Platelet α-adrenergic receptors in intact platelets or platelet particulates were studied using [^{3}H]dihydroergocryptine ([^{3}H]DHE), an α-adrenergic antagonist. Although cholesterol-rich platelets required an 18-fold lower concentration of epinephrine for aggregation, the α-adrenergic receptors of these platelets were similar to those of normal control platelets in several respects: kinetics of association and dissociation of [^{3}H]DHE, the number of [^{3}H]-DHE binding sites, and the affinity of the receptors for [^{3}H]DHE and epinephrine. Moreover, cholesterol incorporation had little effect on basal adenylate cyclase activity or on activity stimulated by NaF or prostaglandin E_1. The maximal inhibition of adenylate cyclase activity by epinephrine was also unchanged by cholesterol incorporation, and the potency of epinephrine in producing this inhibition was only slightly (less than twofold) increased. Cholesterol-depleted platelets required eightfold more epinephrine than normal for aggregation, but, as with cholesterol-rich platelets, binding of [^{3}H]DHE resembled binding to controls. These data indicate that the increase or decrease in platelet responsiveness to epinephrine caused by cholesterol incorporation or depletion, respectively, must result from effects of cholesterol distal to the binding of epinephrine to its receptor. Furthermore, the increased platelet responsiveness caused by cholesterol incorporation is probably independent of changes in adenylate cyclase activity. Since binding to the α-adrenergic receptor appears to be unaffected by cholesterol incorporation or depletion, these results suggest that α-adrenergic receptors may exist in a lipid domain in the membrane that is inaccessible to cholesterol.

Cholesterol is a major structural component of plasma membranes and, as such, it functions to maintain membrane phospholipids in an intermediate fluid state (Chapman, 1968). In this action, cholesterol appears to be closely interposed with the phospholipids so that certain acyl carbon atoms near the membrane surface are more constrained while those deep within the hydrophobic core of the bilayer have greater freedom of motion (Rothman & Engelman, 1972). Cholesterol has been shown to affect fluidity of the lipid bilayer as assessed by rotational diffusion of hydrophobic fluorescent probes (Copper et al., 1978; Vanderkooi et al., 1974; Shinitzky & Inbar, 1976). In addition, cholesterol influences membrane transport and permeability (Wiley & Copper, 1975; Deuticke & Ruska, 1976) and the activity of certain membrane-bound enzymes (Alirisatos et al., 1977; Farras et al., 1975; Kimelberg, 1975).

Human platelets have been useful in examining the effects of increasing or decreasing membrane cholesterol content. Incubation of platelets in vitro with lipid dispersions containing 2.2 mol of cholesterol/mol of phospholipid (C/PL = 2.2) re-

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