Lipolysis and Lipid Movement in a Membrane Model

ACTION OF LIPOPROTEIN LIPLASE

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The action of purified bovine milk lipoprotein lipase on tri[14C]oleoylglycerol and the effect of albumin on movement of lipolytic products at an argon-water interface were studied in a specially designed tricompartmental trough. The amount of trioleoylglycerol applied was 14 times that needed to cover the surface of the aqueous subphase (0.1 mM Tris-HCl, pH 7.4) with a monolayer.

It is concluded that trioleoylglycerol was present in lenses on the surface of the aqueous subphase, that hydrolysis by lipoprotein lipase occurred in or near the lipid/argon-water interface, and that lipolytic products immediately located and spread throughout the interface, displacing substances with lower spreading pressures from the interface. Addition of albumin to the aqueous subphase accelerated markedly the desorption of oleic acid and monooleoylglycerol from the interface and thereby enhanced lipolysis. When albumin was not contiguous with the site of hydrolysis, oleic acid and monooleoylglycerol readily moved in the interface to the area of contact with albumin where they were desorbed from the interface. These findings support the hypothesis of transport of lipolytic products by lateral movement in cell membranes.

Chylomicrons consist of a core of triacylglycerol and traces of cholesteryl ester surrounded by a surface film of polar lipid and protein (1, 2). Triacylglycerol in chylomicrons is hydrolyzed to fatty acid and glycerol by lipoprotein lipase in medium containing sufficient serum albumin to bind all the fatty acids formed (3). When a 9-fold excess of albumin is present, triacylglycerol is hydrolyzed mostly to monoacylglycerol and fatty acid (3). This effect has been attributed to binding of monoacylglycerol to albumin (4), thereby removing it from the interface, the site of enzyme action (5). When albumin is limiting, lipolysis slows down as soon as fatty acid binding sites on albumin are filled, and fatty acid, monoacylglycerol, and diacylglycerol accumulate in the chylomicrons (3, 5). Morphological studies showed that under these conditions lipolytic products locate at the interface between triacylglycerol and the aqueous medium and extend the chylomicron surface as a monolayer lining and spiralling within aqueous spaces that develop in the triacylglycerol core (5). These findings suggested that lipolytic products can transfer from the site of enzyme action by lateral movement in the interface.

Uptake of triacylglycerol from chylomicrons by extrahepatic tissue in vivo involves hydrolysis of the triacylglycerol to di- or monoacylglycerol and fatty acid by lipoprotein lipase bound to the luminal surface of capillary endothelium (2, 6, 7). Some of the fatty acid formed bind immediately to albumin in the blood stream, while the rest transfer with di- or monoacylglycerol to the tissue (6–9). Scow et al. (2, 9) recently proposed that transfer of lipolytic products from chylomicrons occurs by lateral movement in a continuous lipid-water interface composed of the chylomicron surface film and the external leaflet of plasma and intracellular membranes of endothelial, interstitial, and parenchymal cells. They also proposed that transfer is enhanced by removal of lipolytic products from the interface at points distal to the enzyme where lipolytic products are re-esterified to triacylglycerol and separate from the interface.

This paper describes experiments designed to test the hypothesis that lipolytic products are transported by lateral movement in membranes. Trioleoylglycerol applied to the surface of aqueous medium in a tricompartmental trough was hydrolyzed to oleic acid and monooleoylglycerol when lipoprotein lipase was added to the aqueous subphase. Desorption of oleic acid and monooleoylglycerol from the interface was accelerated by addition of albumin as a trapping agent to the underlying aqueous phase. Lateral movement of lipolytic product in the interface was observed when albumin and enzyme were added to separate compartments.

EXPERIMENTAL PROCEDURES

Lipids—Oleic acid (96% pure, Catalog No. 57092, Fluka, Chi 9470, Buchs, Switzerland), 1(3)-monooleolyglycerol (90% 1(3)-isomer and 10% 2-isomer, Catalog No. M-1378, Sigma Chemical Co., St. Louis, Mo.), and 1,2-dioleoylglycerol (Applied Science Laboratories, Inc., State College, Pa.) were used without further purification. Trioleoylglycerol was purified from commercial olive oil (La Fleur d’Olivier, extra vierge) by chromatography on Florisil (10). Tri[9,10-3H]oleoylglycerol (713 mCi/mmol, Catalog No. TRA 191, Batch 32, Radiochemical Centre, Amersham, Buckinghamshire, England) was diluted 1:2.3 with unlabeled trioleoylglycerol. Thin layer chromatography (11) showed that 98.4% of the H was in trioleoylglycerol, 0.3% in dioleoylglycerol, 0.5% in 1,2-monoooleoylglycerol, and 0.8% in oleic acid.

Lipoprotein Lipase and Albumin—Lipoprotein lipase used in these studies was purified from bovine skim milk (12, 13). The preparation, kindly supplied by Dr. Thomas Oltvivecz of the University of Umeå, Sweden, contained 976 units of lipolytic activity/mg of protein (1 unit = 1 μmol of fatty acid released/min at 24°C) and was dissolved in 1.5 M NaCl/0.05 mM sodium veronal, pH 7.4, at a protein concentration of 0.17 mg/ml. The enzyme preparation was stored at −70°C and small aliquots were thawed immediately prior to use. A preparation containing apo lipoproteins C-I, C-II, and C-III, isolated as one fraction by column chromatography from human high density lipoproteins,
was kindly supplied by Dr. Bryan Brewer of the National Heart, Lung and Blood Institute, Bethesda, Md. This preparation, designated below as C-peptide, was used to activate lipoprotein lipase (14).

A stock solution of 2.4 nmol albumin was prepared by dissolving 16 mg of bovine serum albumin powder (Fraction V, Lot G-36006, Armour Pharmaceutical Co., Chicago, Ill.) in 100 ml of 100 mM Tris-HCl/1 mM CaCl₂/0.1 mM EDTA solution (pH 7.4), filtering the solution through a 0.45-μm Millipore filter, and adjusting the pH to 7.4.

**Measurement of Lipolysis and Movement of Lipolytic Products—**The effect of lipoprotein lipase on trioleoylglycerol and the effect of albumin on movement of lipolytic products at the argon-water interface were studied in a specially designed tricompartment Teflon trough (Fig. 1). The three compartments, A, B, and C, were interconnected by narrow shallow channels, 0.4 mm deep. The channels were connected in series by narrow shallow surface channels. The reservoir and third compartment (III) were used for measuring the relation between surface area and pressure and the effect of surface pressure on desorption, whereas the second compartment (II) was also used in the studies with albumin. In all assays, the film area of a compartment III was reduced to 30 cm² by placing two Teflon bars, 4 cm apart, lengthwise across the compartment. Albumin was added to only compartment II; when desorption was expected to be fast, the area of contact between the film and albumin solution was decreased beforehand by placing a Teflon bar widthwise across compartment II.

The aqueous subphase in compartment II was agitated by two magnetic stirrers turning at 250 rpm (15). Surface pressure was measured in compartment III, between the parallel bars, by the Wilhelmy method with a thin platinum plate (perimeter, 3.94 cm) attached to a Beckman model LM-600 electromicrobalance (16). The trough, 13.5 ml and 12.5 cm²; and C, 7.4 ml and 7.1 cm². The enzyme was added to compartment A, and albumin was added to compartment B. 

**RESULTS**

**Compression Isotherms for Oleoyl Lipids—**Compression isotherms for trioleoylglycerol and its hydrolytic derivatives,

**TRI-COMPARTED TROUGH**

![Fig. 1. Schematic diagram of tricompartment trough used to study lipolysis and movement of lipolytic products at an argon-water interface. Lipoprotein lipase was used to hydrolyze trioleoylglycerol at the interface and was put into compartment A, and albumin used as a trapping agent for oleic acid and monooleoylglycerol was added either to compartment A for immediate desorption of lipolytic product or to compartment B for lateral movement and then desorption of product from the interface.](http://www.jbc.org/)

<table>
<thead>
<tr>
<th>component</th>
<th>film area</th>
<th>reservoir</th>
<th>compartment A</th>
<th>compartment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>39 ml</td>
<td>38.5 cm²</td>
<td>39 ml</td>
<td>38.5 cm²</td>
</tr>
<tr>
<td>B</td>
<td>38 ml</td>
<td>37.0 cm²</td>
<td>38 ml</td>
<td>37.0 cm²</td>
</tr>
<tr>
<td>C</td>
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**RESULTS**

**Compression Isotherms for Oleoyl Lipids—**Compression isotherms for trioleoylglycerol and its hydrolytic derivatives,
dioleoylglycerol, monooleoylglycerol, and oleic acid, are presented in Fig. 2. The surface pressure of trioleoylglycerol increased linearly from 1 to 12 dynes/cm when concentration of lipid in the surface film was increased from 0.31 to 0.40 nmol of oleoyl moiety/cm² by compressing the film. Further compression to an apparent surface concentration of 2.5 nmol of oleoyl moiety/cm² had little effect on surface pressure, indicating collapse of the film. The isotherm for dioleoylglycerol was also biphasic: surface pressure increased from 14 to 26 dynes/cm as surface concentration was increased from 0.4 to 0.53 nmol of oleoyl moiety/cm² and then increased slowly to 31 dynes/cm as surface concentration was increased to 2.5 nmol/cm². The latter, the small increase in pressure with a large increase in apparent surface concentration, indicates collapse of the surface film. Surface pressure of monooleoylglycerol, in contrast, increased linearly from 15 to 35 dynes/cm when surface concentration was increased from 0.4 to 0.51 nmol/cm². Similar results were obtained with oleic acid.

It is evident from these studies that lipid products resulting from hydrolysis of trioleoylglycerol can withstand, because of their strong amphipathic character, much higher surface pressures before collapsing than can trioleoylglycerol. The consequence of this important property is discussed below (see Fig. 4).

**Effect of Albumin on Desorption of Oleoyl Lipids from Monolayers at Various Surface Pressures**—Monolayers of monooleoylglycerol and oleic acid both decreased in surface area when maintained at constant surface pressure between 20 and 36 dynes/cm (Fig. 3). The rate of decrease, however, was much lower for monooleoylglycerol than for oleic acid. Monooleoylglycerol decreased in surface area 0.3%/min at 36 dynes/cm, whereas oleic acid, at pH 7.4, decreased 1%/min at 20 dynes/cm, 2.5%/min at 30 dynes/cm, and 22%/min at 36 dynes/cm. The rates of decrease in film area for both lipids were markedly accelerated by adding albumin to the aqueous subphase, and these effects were proportional to the amount of albumin added. The effect was less on monooleoylglycerol, however, than on oleic acid. The data summarized in Table I indicate that desorption of oleic acid in the presence of 4.4 μM albumin is 2 to 3 times faster than that of monooleoylglycerol in the presence of a 3-fold higher concentration of albumin. Although albumin preferentially binds oleic acid, at high concentrations it can also serve as a trapping agent for monooleoylglycerol.

Sustained compression of dioleoylglycerol at 31 dynes/cm and trioleoylglycerol at 13 dynes/cm had no significant effect on the surface area occupied by these lipids. The presence of

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**Figure 3.** Effect of albumin at different surface pressures on the rate of desorption of oleic acid (OA) and monooleoylglycerol (MOG) from surface film to aqueous subphase. Pure films composed of either oleic acid or monooleoylglycerol were spread at the interface between argon and Tris-HCl buffer solution, pH 7.4. Desorption rates were calculated from the reduction in film area at given pressures. Surface pressure was maintained constant with use of a surface barostat (15). Values are averages of two to four experiments. AA, change in film area; A₀, initial film area.

**Figure 2.** Compression isotherms for oleoyl lipids spread at the interface between argon and a Tris-HCl buffer solution, pH 7.4, containing EDTA and CaCl₂. Values for oleic acid are corrected for desorption during the experiment (Fig. 3). Surface concentration of the lipids was increased by compressing the surface film.

**Table I.** Effect of albumin on rate of desorption of oleic acid and monooleoylglycerol from surface film to aqueous subphase at various surface pressures.

<table>
<thead>
<tr>
<th>Surface pressure (dyne/cm)</th>
<th>Oleic acid</th>
<th>Monooleoylglycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without albumin</td>
<td>With albumin (μM)</td>
</tr>
<tr>
<td>22</td>
<td>6</td>
<td>221</td>
</tr>
<tr>
<td>24</td>
<td>7</td>
<td>246</td>
</tr>
<tr>
<td>26</td>
<td>8</td>
<td>277</td>
</tr>
<tr>
<td>28</td>
<td>11</td>
<td>321</td>
</tr>
<tr>
<td>30</td>
<td>13</td>
<td>346</td>
</tr>
<tr>
<td>32</td>
<td>16</td>
<td>378</td>
</tr>
</tbody>
</table>

*Albumin, 4.4 μM, in aqueous subphase.

*Albumin, 17.6 μM, in aqueous subphase.
18 μM albumin in the aqueous subphase had no effect on the surface area of dioleoylglycerol at 24 to 31 dynes/cm, indicating that this lipid is not removed from the interface by albumin. The effect of albumin on desorption of trioleoylglycerol could not be studied in this manner because albumin (18, 19) has a much higher equilibrium spreading pressure than trioleoylglycerol (Fig. 2), 22 to 23 versus 13 dynes/cm.

Hydrolysis of Trioleoylglycerol by Lipoprotein Lipase and Effect of Albumin on Movement of Lipolytic Products at an Argon-Water Interface—The movement of lipolytic products formed by the action of lipoprotein lipase on trioleoylglycerol at an argon-water interface was studied using the tricomparted trough described above (Fig. 1). The enzyme, 90 or 270 pmol, was added to compartment A where it was stabilized by a trace amount, 2.4 nmol, of albumin. This amount of albumin, producing a concentration of 0.06 μM, could not be expected to affect appreciably desorption of lipid from the interface. Results similar to those shown in Fig. 4 were obtained in complete absence of albumin, but the enzyme was quickly inactivated. C-peptide, used for activation of lipoprotein lipase, was added in all experiments shortly before the enzyme.

The amount of trioleoylglycerol applied, 113 nmol, was 14 times that needed to cover the surface of the aqueous medium (Tris-HCl/CaCl₂/EDTA, pH 7.4) with a monolayer at a pressure of 13 dynes/cm. The excess, probably present as lenses on the surface (20), constituted a substrate reservoir for reactivation at a constant surface area.

The small amount of albumin used for enzyme stabilization induced an increase in surface pressure from 13 to 21 dynes/cm (Fig. 4), suggesting that the protein first penetrated the trioleoylglycerol film and then displaced trioleoylglycerol from the interface, probably into preformed lenses. Concentration of trioleoylglycerol in the aqueous subphase was negligible, <8 × 10⁻¹¹ M, 8 min after adding albumin to compartment A.

Addition of 270 pmol (13.2 μg) of lipoprotein lipase to the aqueous subphase of compartment A increased surface pressure from 21 to 28 dynes/cm in 5 min and to 32 dynes/cm in 22 min and maintained pressure at that level for the rest of the experiment (Fig. 4). Analyses at the end of the experiment showed that oleic acid, monoleoylglycerol and dioleoylglycerol were present in the surface film above compartments A and B (Tables II and III). Although recovery of total lipid from the surface film was incomplete, the amount of oleic acid and monoleoylglycerol recovered was sufficient to cover 44 to 71% of the surface of aqueous phase with a monolayer at a surface pressure of 32 dynes/cm. These findings demonstrate that lipolytic products immediately located and spread throughout the interface. It is likely they also displaced albumin from the interface since their spreading pressures (Fig. 2) were considerably higher than that of albumin (18, 19).

Addition of 360 μmol of albumin to the aqueous subphase of compartment A, 1 min after adding enzyme, decreased at once the effect of lipolytic product on surface pressure, lowering pressure from 23.6 to 22 dynes/cm in 1 min, maintaining it at that level for 9 min, and then lowering it again to 21 dynes/cm at 22 min (Fig. 5). Addition of the same amount of albumin to compartment B, at 1.6 min, had a smaller effect on the rise in surface pressure. It stopped at once further increase in pressure at 25 dynes/cm, maintained pressure at that level for 9 min, and then lowered slowly pressure to 21 dynes/cm at 63 min. These observations suggested that lipolytic products generated by lipoprotein lipase were rapidly desorbed from the interface by albumin and that desorption was delayed when albumin was added to compartment B because time was required for transfer of lipolytic product from compartment A.

The amount of 3H-labeled lipid desorbed from the interface under various conditions is shown in Fig. 5 and Table II. The data in Table IV indicate that oleic acid and monoleoylglycerol accounted for more than 90% of the lipid desorbed from the interface, as would be expected from the findings with pure lipid films (Fig. 3). Desorption of lipolytic products from the interface and hydrolysis of trioleoylglycerol were both increased by addition of albumin (Table II), and desorption/unit surface area was greater in the compartment containing albumin (Fig. 5). Addition of albumin also decreased the amount of lipolytic product remaining in the surface film (Table II). Tripling the amount of lipoprotein lipase added to compartment A, from 90 to 270 pmol, increased hydrolysis of trioleoylglycerol by only 12% (Table II), indicating that the amount of enzyme used in this study was not limiting.

The effect of albumin on desorption of oleic acid and monoleoylglycerol formed by lipoprotein lipase from trioleoylglycerol is shown in Figs. 6 and 7. As observed with pure lipid films (Table I), desorption of oleic acid in the absence of albumin was many times faster than that of monoleoylglycerol, and desorption of both substances was increased by adding albumin to the aqueous subphase. Also, the enhancing effect of albumin was proportionally greater on monoleoylglycerol than on oleic acid. Addition of albumin to compartment A increased desorption of monoleoylglycerol to compartment A and B by 7-fold, whereas it increased desorption of oleic acid by only 2-fold. Addition of albumin to compartment B, in contrast, increased desorption of monoleoylglycerol 40- to 60-fold to compartment B and 2-fold to compartment A, while it increased desorption of oleic acid 6-fold to compartment C, which constituted 12% of the total surface area and volume of the trough, were not analyzed. They probably were similar in composition to those of compartment B in all experimental groups except IV and V (Tables III and IV).

Fig. 4. Increase in surface pressure induced by the action of lipoprotein lipase (LPL) on trioleoylglycerol spread at the interface between argon and Tris-HCl buffer solution (pH 7.4). The amount of trioleoylglycerol applied (113 nmol in 25 μl of chloroform) was 14 times that needed to cover the aqueous medium in the tricomparted trough (Fig. 1) with a monolayer at 13 dynes/cm. Traces of albumin and C-peptide (apolipoproteins C-I, C-II, and C-III) were added to compartment A in order to stabilize and activate the enzyme, respectively. Surface pressure was measured with a Wilhelmy plate suspended in compartment C and recorded automatically. Temperature ranged from 28 to 30°C. Values are means of three experiments.
TABLE II
Effect of different amounts of lipoprotein lipase and albumin on the amount of $^3$H-labeled lipid recovered in the surface film and aqueous subphase at 63 min

The amount of tri$^3$H]oleoylglycerol spread over the three compartments of the trough was $8,460 \times 10^3$ cpm. See Figs. 4 and 5 for experimental details. The values given are means of two to four experiments/group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Lipoprotein lipase added to A</th>
<th>Albumin in aqueous subphase</th>
<th>Total labeled lipid recovered</th>
<th>Labeled lipolytic product recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount added to compartment</td>
<td>Concentration in compartment</td>
<td>In film above compartment</td>
<td>In aqueous subphase of compartment</td>
</tr>
<tr>
<td></td>
<td>pmol</td>
<td>nmol</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>I</td>
<td>90</td>
<td>0.0</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>270</td>
<td>0.0</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>270</td>
<td>960</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>270</td>
<td>960</td>
<td>0.06</td>
<td>71</td>
</tr>
</tbody>
</table>

Lipolytic product, dioleoylglycerol, monoooleoylglycerol, and oleic acid; based on analyses given in Tables III and IV.

TABLE III
Composition of $^3$H-lipid in surface film recovered from compartments A and B at 63 min

See Table II for experimental details. Values given are means of two to four experiments/group. OA, oleic acid; MOG, monoooleoylglycerol; DOG, dioleoylglycerol; and TOG, trioleoylglycerol.

<table>
<thead>
<tr>
<th>Group</th>
<th>Compartment A</th>
<th>Compartment B</th>
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<tr>
<td></td>
<td>OA</td>
<td>MOG</td>
</tr>
<tr>
<td>I</td>
<td>9.9</td>
<td>7.2</td>
</tr>
<tr>
<td>II</td>
<td>15.7</td>
<td>9.5</td>
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<td>III</td>
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<td>5.7</td>
</tr>
<tr>
<td>V</td>
<td>11.9</td>
<td>10.2</td>
</tr>
</tbody>
</table>

TABLE IV
Composition of $^3$H-lipid in aqueous subphase of compartments A and B at 63 min

See Table II for experimental details. Values given are means of two to four experiments/group. OA, oleic acid; MOG, monoooleoylglycerol; DOG, dioleoylglycerol; and TOG, trioleoylglycerol.

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<th>Compartment B</th>
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<tr>
<td></td>
<td>OA</td>
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</tr>
<tr>
<td>I</td>
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<td>4.7</td>
</tr>
<tr>
<td>II</td>
<td>94.9</td>
<td>2.9</td>
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<tr>
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<tr>
<td>IV</td>
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<td>8.6</td>
</tr>
<tr>
<td>V</td>
<td>90.1</td>
<td>7.8</td>
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Fig. 5. Effect of albumin on surface pressure and desorption of $^3$H-lipid from surface to aqueous subphase during hydrolysis of tri$^3$H]oleoylglycerol by lipoprotein lipase (LPL). Trioleoylglycerol was spread 13 min, and traces of albumin and C-peptide were added to compartment A 10 and 6 min before addition of 90-70 pmol of lipoprotein lipase, respectively (see Fig. 4). Albumin (960 nmol) for trapping lipolytic product was added as indicated by arrows at either 1.0 or 1.5 min after addition of enzyme. Temperature was 28-30°C. Other data from these experiments are given in Tables II to IV and Figs. 5 and 6.
Lipolysis and Lipid Movement in a Membrane Model

No Albumin

3.6

3.3

3.0

Compartment LPL -

*A

1

270

pmol

2.7

0

0 I pm0

2.4

1; 90

pmol

2.1

1.8

1.0

0.6

0.3

0.2

0.1

0

TIME AFTER ADDITION OF LIPOPROTEIN LIPASE

TO COMPARTMENT A (min)

FIG. 6. Transfer and desorption of oleic acid formed at the surface by action of lipoprotein lipase (LPL) on trioleoylglycerol. Albumin (960 nmol) for trapping oleic acid was added to the aqueous subphase of compartment A at 1.0 min and to compartment B at 1.6 min after addition of enzyme. Concentration of oleic acid in the aqueous subphase can be calculated from the amount desorbed/unit surface area, in nanomoles/cm², and the average depth of the aqueous subphase, 0.95 cm. See Fig. 5 for other details of experiment.

FIG. 7. Transfer and desorption of monooleoylglycerol formed at the surface by action of lipoprotein lipase (LPL) on trioleoylglycerol. Albumin (960 nmol) for trapping monooleoylglycerol was added to the aqueous subphase of compartment A at 1.0 min and to compartment B at 1.6 min after addition of enzyme. Concentration of monooleoylglycerol in the aqueous subphase can be calculated from the amount desorbed/unit surface area, in nanomoles/cm², and the average depth of the aqueous subphase, 0.95 cm. See Fig. 5 for other details of experiment.

TABLE V

<table>
<thead>
<tr>
<th>Group</th>
<th>Lipoprotein lipase added to aqueous subphase of compartment A</th>
<th>Albumin added to aqueous subphase of compartment B</th>
<th>Oleic acid in aqueous subphase of compartment A</th>
<th>Oleic acid in aqueous subphase of compartment B</th>
<th>Oleic acid in aqueous subphase of compartments A &amp; B</th>
<th>Monoleoylglycerol in aqueous subphase of compartment A</th>
<th>Monoleoylglycerol in aqueous subphase of compartment B</th>
<th>Monoleoylglycerol in aqueous subphase of compartments A &amp; B</th>
<th>Ratio of monoleoylglycerol/oleic acid in aqueous subphase of compartments A</th>
<th>Ratio of monoleoylglycerol/oleic acid in aqueous subphase of compartments A &amp; B</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>90 pmol</td>
<td>2.4 nmol</td>
<td>38.1 nmol</td>
<td>6.8 nmol</td>
<td>44.9 nmol</td>
<td>2.0 nmol</td>
<td>0.2 nmol</td>
<td>2.2 nmol</td>
<td>0.05</td>
<td>0.03</td>
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<td>43.0 nmol</td>
<td>7.4 nmol</td>
<td>50.4 nmol</td>
<td>2.0 nmol</td>
<td>0.2 nmol</td>
<td>2.2 nmol</td>
<td>0.04</td>
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</tr>
<tr>
<td>III</td>
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<td>962.4 nmol</td>
<td>93.8 nmol</td>
<td>21.7 nmol</td>
<td>115.5 nmol</td>
<td>17.4 nmol</td>
<td>2.3 nmol</td>
<td>18.7 nmol</td>
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<td>0.11</td>
</tr>
<tr>
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<td>2.4 nmol</td>
<td>66.7 nmol</td>
<td>35.0 nmol</td>
<td>101.7 nmol</td>
<td>6.5 nmol</td>
<td>11.6 nmol</td>
<td>18.2 nmol</td>
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<td>0.33</td>
</tr>
<tr>
<td>V</td>
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<td>960 nmol</td>
<td>69.4 nmol</td>
<td>42.8 nmol</td>
<td>112.2 nmol</td>
<td>6.0 nmol</td>
<td>14.2 nmol</td>
<td>20.2 nmol</td>
<td>0.09</td>
<td>0.33</td>
</tr>
</tbody>
</table>

The amounts of oleic acid and monooleoylglycerol recovered in the aqueous subphase of compartments A and B are given in Table V. In the absence of albumin (Groups I and II), 85 to 90% of both oleic acid and monooleoylglycerol were recovered in compartment A and 10 to 15% in compartment B, and the molar ratio of monooleoylglycerol to oleic acid in the aqueous subphase was about 0.04 in all compartments. When albumin was added to compartment A (Group III), the proportion of each lipid recovered in compartment A was the same, 85%, but the molar ratio of monooleoylglycerol to oleic acid was increased to 0.19 in compartment A, to 0.11 in compartment B, and to 0.17 in compartments A and B combined. When albumin was added to compartment B (Groups IV and V), most of the oleic acid was still recovered in compartment A, whereas 67% of the monooleoylglycerol was recovered in compartment B. Accordingly, the molar ratio of monooleoylglycerol to oleic acid was higher in compartment B than in compartment A, 0.33 versus 0.10, even though the ratio for compartments A and B combined was not changed. These findings indicate that hydrolysis of trioleoylglycerol occurred primarily in compartment A and that proportionally more monooleoylglycerol than oleic acid was transferred to compartment B in Groups IV and V.

DISCUSSION

The purpose of the present work was to test with a simple membrane model the hypothesis that lipolytic products are transported by lateral movement in cell membranes. Lipolytic
products were generated by the action of lipoprotein lipase on trioleoylglycerol spread on the surface of aqueous medium, pH 7.4, in a special tricomparted trough (Fig. 1). The amount of trioleoylglycerol applied (in chloroform) was 14 times that needed to cover the surface at collapse pressure, 13 dynes/cm (Fig. 2). Consequently, most of the trioleoylglycerol applied probably formed small lenses (20) on the surface of the aqueous medium in all three compartments. The trace of albumin (2.4 nmol) used to stabilize the enzyme was eight times that which would be needed to cover the aqueous subphase with a monolayer of albumin at 21 dynes/cm (18). The increase in surface pressure when albumin was added (Fig. 4) indicates that albumin displaced trioleoylglycerol from the interface, probably into existing lenses. The small amount of C-peptide added for activation of the enzyme caused a small immediate increase in surface pressure (Fig. 4) indicating that C-peptide had entered the interface (21). It is likely that the albumin monolayer, containing C-peptide, extended under the trioleoylglycerol lenses to form an interfacial plane between the lipid and the aqueous subphase.

In our membrane model, trioleoylglycerol was converted by lipoprotein lipase to amphiphilic dioleoylglycerol, monoo leoylglycerol, and oleic acid. These products immediately accumulated in the interface (Table VI), increased surface pressure to 25 dynes/cm in 2 min and to 32 dynes/cm in 22 min, and displaced albumin from the interface (18, 19). When surface pressure exceeded 25 dynes/cm, some of the dioleoylglycerol formed was probably forced into lenses, making it less susceptible to enzyme action (Groups I and II, Table III). The interface above compartments A and B was large enough to accommodate 26 nmol of oleoyl moieties at 25 dynes/cm (Fig. 3). Thus, this level of surface pressure at 2 min after adding enzyme indicates that 8% of the trioleoylglycerol applied was hydrolyzed during the first 2 min of the experiment (Fig. 4). Subsequent hydrolysis was slower and dependent on transfer of lipolytic product from the interface (Table VI). Despite slow desorption of product in the absence of albumin (Fig. 5), surface pressure remained high because the rate of product formation was faster than the rate of desorption (Groups I and II, Table VI). Addition of albumin to the aqueous subphase accelerated desorption of oleic acid and monoo leoylglycerol (Figs. 6 and 7) and, thereby, increased hydrolysis of trioleoylglycerol (Groups III to V, Table VI). Our findings demonstrate that lipoprotein lipase acts as an acyl pump by converting triacylglycerol to amphiphilic lipids which locate in the interface between triacylglycerol and water and that this action is enhanced by removal of the products from the interface.

When albumin was added to compartment A 1 min after addition of enzyme, oleic acid and monoo leoylglycerol in the interface desorbed immediately into the aqueous subphase, lowering surface pressure from 23.6 to 22 dynes/cm in 1 min (Fig. 5). Thereafter, lipolytic products desorbed from the interface as soon as they formed (Group III, Table VI), maintaining surface pressure constant at 22 dynes/cm (Fig. 5). The rate of desorption and surface pressure both decreased at 22 min, reflecting a marked reduction in rate of hydrolysis of trioleoylglycerol (Table VI). It is not apparent why lipolysis slowed down at 22 min. Substrate was not limiting because the amount hydrolyzed at 22 min was only 29% of that applied, or 44% of that in compartment A, assuming uniform distribution of trioleoylglycerol at the surface (29% × 58.2 cm²/38.5 cm²). Lipoprotein lipase probably was in excess since tripling the amount added increased hydrolysis only 12% (Tables II and VI). Also, albumin was in excess as indicated by the low ratio of fatty acid to albumin in compartment A, <0.1 (Fig. 6). It is unlikely that cofactor apolipoprotein C-II was limiting because nearly 300 pmol, contained in 10 μg of C-peptide, was used to activate up to 270 pmol of enzyme (14, 21, 22). Reentry of albumin into the interface when surface pressure fell to 21 dynes/cm may have disrupted or interfered with formation of enzyme-cofactor-substrate complex at the trioleoylglycerol-water interface. Unlike the case with chylomicrons and artificial emulsions, only a small percentage of the interfacial plane at the surface of the aqueous phase was in direct contact with triacylglycerol lenses; the rest was in contact with argon. The same phenomena probably occurred when albumin was added to compartment B (Groups IV and V, Table VI).

The immediate rise in surface pressure when lipoprotein lipase was added to compartment A (Fig. 4) and the appearance of lipolytic product in the surface film of compartment B (Table II) indicate that lipolytic product spread rapidly throughout the interface. Transfer of product to the aqueous subphase of compartment B was increased severalfold by adding albumin to compartment B (Table V). Also, rates of desorption of oleic acid and monoo leoylglycerol from interface to aqueous subphase of compartment B were increased 6- and <40-fold, respectively (Figs. 6 and 7). It is possible that a small amount, 1 to 2%, of the albumin added to either com-

<table>
<thead>
<tr>
<th>Group</th>
<th>Lipoprotein lipase added to aqueous subphase of compartment A</th>
<th>Amount of lipolytic product in interface and aqueous subphase of compartments A and B combineda</th>
<th>2 min</th>
<th>11 min</th>
<th>22 min</th>
<th>42 min</th>
<th>63 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol</td>
<td>nmol</td>
<td>pmol</td>
<td>nmol</td>
<td>pmol</td>
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<td>pmol</td>
</tr>
<tr>
<td>I</td>
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<td>0</td>
<td>26</td>
<td>1</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>270</td>
<td>962.4</td>
<td>0</td>
<td>17</td>
<td>9</td>
<td>67</td>
<td>44</td>
</tr>
<tr>
<td>III</td>
<td>270</td>
<td>270</td>
<td>270</td>
<td>270</td>
<td>270</td>
<td>270</td>
<td>270</td>
</tr>
</tbody>
</table>

* Lipolytic product, dioleoylglycerol, monoo leoylglycerol, and oleic aid.
* Sub, aqueous subphase. Estimated from surface pressure (Fig. 5) and compression isotherms of pure lipids (Fig. 9)
* Sub, aqueous subphase. Calculated from data in Fig. 5 and Table IV.
* Albumin, 960 nmol, was added to compartment A at 1 min after addition of enzyme to compartment A.
* Difference between estimated amount in interface in absence of albumin (26 nmol) and estimated amount desorbed during the first minute after addition of albumin (9 nmol).
* Based on amount of lipolytic product recovered in surface film at 63 min (Table II).
* Albumin, 960 nmol, was added to compartment B at 1.6 min after addition of enzyme to compartment A.

**Table VI**

Effect of different amounts of lipoprotein lipase and albumin on hydrolysis of trioleoylglycerol

See Table II for experimental details.
partment A or B might have diffused via the aqueous phase to the other compartment (15, 16) and, thereby, enhanced desorption of oleic acid and monoooleoylglycerol in the second compartment (Figs. 6 and 7). Similarly, a small amount of lipase might have diffused from compartment A to compartment B. However, when albumin was added to compartment B, about two-thirds of the oleic acid formed was recovered in compartment A, whereas two-thirds of the monoooleoylglycerol formed was recovered in compartment B (Table V), indicating that hydrolysis occurred primarily in compartment A and that proportionally more monoooleoylglycerol had been transferred to compartment B. Transfer of lipolytic product to compartment B by diffusion in the aqueous subphase seems unlikely because the concentrations of both monoooleoylglycerol and oleic acid in the aqueous subphase were higher in compartment B (13.5 ml) than in compartment A (39 ml) (Figs. 6 and 7). These findings are consistent with the view that oleic acid and monoooleoylglycerol formed in compartment A are transferred to compartment B by lateral movement in the interface. Such movement would be enhanced by gradients of concentration of lipolytic product in the interface resulting from the generative action of lipoprotein lipase in compartment A and the trapping action of albumin in compartment B.

We conclude that amphiphilic acyl lipids formed by the action of lipoprotein lipase on triacylglycerol immediately locate and spread at the interface between triacylglycerol/gas and water. As lipolytic products crowd the interface, surface pressure increases, hydrolysis slows down, and substances with lower spreading pressures are displaced from the interface. Although lipolytic products composed of long chain acyl groups have a strong tendency to remain in the interface, a small fraction of fatty acid and a much smaller fraction of monoacylglycerol desorb to the aqueous subphase. Albumin in the aqueous subphase accelerates desorption of both fatty acid and monoacylglycerol from the interface and, thereby, enhances lipolysis. When albumin is not contiguous with the site of hydrolysis, fatty acid and monoacylglycerol move laterally in the interface to the area of contact with albumin and there desorb from the interface. This system of transport mimics in part that proposed for transfer of lipolytic products from chylomicrons to tissue cells in vivo (2, 9). In the latter, lipolytic products move in a continuous interface of chylomicron surface film and cell membranes to endoplasmic reticulum of cells where they are re-esterified to triacylglycerol and separate from the interface, forming lipid droplets (lenses) between bilayers of reticular membrane. Although lateral movement of lipolytic products in the continuum could be affected quantitatively by cell membrane constituents such as phospholipid and cholesterol, the physicochemical principles demonstrated in the membrane model would still apply.

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