Hormone-sensitive Lipase and Monoglyceride Lipase Activities in Adipose Tissue

MARTHA VAUGHAN, JACOB E. BERGER, AND DANIEL STEINBERG

From the Laboratory of Metabolism, National Heart Institute, National Institutes of Health, Bethesda 14, Maryland

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Several workers (1–3) have reported that lipase activity assayed in homogenates of adipose tissue is increased by exposure of the tissue to epinephrine or to adrenocorticotropic hormone prior to homogenization. In general, the magnitude of the increases observed has been small compared to the large effects of these hormones on glycerol formation by the intact tissue. In studies of whole homogenates or complex fractions thereof, the possibility that several lipase activities are being simultaneously assayed cannot be ruled out. If only one of these activities were responsive to hormonal stimulation, the effects of a hormone on lipase activity as assayed would appear relatively small. The studies reported below were undertaken in an attempt to characterize and to study the hormone-sensitive lipase activity in adipose tissue without interference from other lipases. By devising suitable assay conditions, we have been able to measure a lipase activity that apparently reflects rather well the hormone-sensitive lipolytic activity of the intact tissue. In addition, we have investigated a lipase activity present in adipose tissue of both rats and rabbits which exhibits a marked specificity for hydrolysis of monoglycerides. Evidence indicating that this activity is suitable assay conditions, we have been able to measure a lipase activity that apparently reflects rather well the hormone-sensitive lipolytic activity of the intact tissue. In addition, we have investigated a lipase activity present in adipose tissue of both rats and rabbits which exhibits a marked specificity for hydrolysis of monoglycerides. Evidence indicating that this activity is distinct from the hormone-sensitive lipase activity and probably also from lipoprotein lipase is presented. Preliminary reports of this work have appeared (4–6).

EXPERIMENTAL PROCEDURE

Methods

Epididymal fat pads were obtained from Sprague-Dawley rats (150 to 250 g), killed by decapitation. All animals were fed ad libitum unless otherwise noted. Rabbit adipose tissue for incubation was obtained from the narrow retroperitoneal strips that extend caudad from the perinephric fat body. Incubation of intact fat pads and of 0.5- to 1-cm segments of rabbit fat (200 to 500 mg) was carried out as described previously (7, 8). Adipose tissue was homogenized (after rapid rinsing and blotting, if it had been previously incubated) by hand in a glass tissue grinder with 5 ml of 0.25 M sucrose and 10 mM triethanolamine, pH 7.4, containing 0.1×10−6 M FFA, free fatty acids. The recovery of lactic and pyruvic acids was less than 85% over the range of 0.025 to 0.125 μeq of palmitate added. A sample of the chloroform phase, usually 2.5 ml, was transferred to a round cuvette (12 × 75 mm) containing 0.25 ml of 0.1% sodium diethyldithiocarbamate in 1-butanol. After mixing, the optical density was read at 440 μm. With this method, recovery of palmitic acid added to assay mixtures containing bovine serum albumin, and 20 μmoles of sodium phosphate buffer, pH 7.0. The reaction was stopped at the desired time by addition of 1 ml of a mixture containing 0.9 M triethanolamine, 0.1 N acetic acid, and 5% cupric nitrate solution. Chloroform, 4 ml, was added; the tubes were placed horizontally in a Fisher-Kahn mechanical shaker and agitated vigorously for 30 minutes in the direction of the long axis of the tubes. After brief centrifugation, as much as possible of the aqueous phase and precipitated protein was removed by suction. A sample of the chloroform phase, usually 2.5 ml, was transferred to a round cuvette (12 × 75 mm) containing 0.25 ml of 0.1% sodium diethyldithiocarbamate in 1-butanol. After mixing, the optical density was read at 440 μm. With this method, recovery of palmitic acid added to assay mixtures containing bovine serum albumin, homogenate, and buffer was 85 ± 5% over the range of 0.025 to 0.125 μeq of palmitate added and 0.1% w/v of total fatty acid determined. The color yield per mole of fatty acid was the same for palmitic, stearic, oleic, linoleic, linolenic, and ricinoleic acids in this procedure. The recovery of decanoic acid was less than 90%, and that of octanoic acid only about 20%, of that of C16 to C18 fatty acids. The recovery of lactate and pyruvate acids was less than 90%.

The abbreviations used are: ACTH, adrenocorticotropic hormone; TSH, thyroid-stimulating hormone; FFA, free fatty acids; DFP, diisopropyl fluorophosphate.
than 2% of that of the long chain fatty acids. Although complete recovery of FFA from pure solutions of the sodium or potassium salts was obtained with only brief manual mixing of phases, the vigorous mixing mentioned above was necessary for complete recovery from mixtures containing albumin. Recovery was incomplete if larger amounts of buffer (phosphate or Tris) were used in the assay. Lipolytic activity measured in Assay II is recorded as microequivalents of FFA produced per g of tissue per 10 minutes.

In these assays, no exogenous substrate was added. As discussed later, the homogenate itself provided essentially saturating amounts of triglyceride substrate. Activity measured under these conditions will be referred to as hormone-sensitive lipase activity, since these conditions were found to be optimal for demonstrating the increments in lipase activity due to hormone treatment. It has not been established, however, that there is only one lipase contributing to the formation of glycerol and FFA under these assay conditions.

Whereas addition of exogenous triglycerides caused little, if any, increase in assayable lipase activity, addition of exogenous monoglycerides gave large increments. What will, for convenience, be referred to as monoglyceride lipase activity was assayed usually in 1 ml of a mixture containing 8 mg of monostearin, 30 mg of bovine serum albumin, and 20 μmoles of Tris buffer, pH 7.9 to 8.0. Activity is expressed as microequivalents of FFA produced per g of tissue (wet weight) per 10 minutes. No correction was made for production of FFA from hydrolysis of endogenous substrate in these assays.

**Materials**

Bovine serum albumin (Fraction V) was purchased from the Armour Pharmaceutical Company. This material was extracted by the method of Goodman (12) to remove FFA. The preparations used in these studies contained less than 1.0 μeq of FFA per g of albumin. The margaric acid which was used as a standard was pure as determined by gas-liquid chromatography. The other acids used in checking the FFA method were commercial preparations. Concentrations of solutions of these reference fatty acids were determined by titration according to the method of Dole (13).

Monostearin, technical grade (Eastman Organic Chemicals), was dissolved in hot ethanol and crystallized at ice bath temperature. The material so collected was then recrystallized from ethanol and air-dried at room temperature. Only monoglyceride was detected on thin layer chromatography of this material. Suspensions were prepared by placing 32 mg of recrystallized monostearin per ml of distilled water in an all-glass homogenizer and heating for 1 minute in a 60° water bath. The pestle was then inserted and the glyceride dispersed by homogenizing for about 15 seconds with the homogenizer removed from the bath. For each 5 ml of suspension, 0.3 ml of albumin, 150 mg per ml in water, was then added, and homogenization was continued for an additional 5 seconds. The resulting suspension was then diluted 1:1 with 0.08 M Tris containing albumin, 120 mg per ml (pH 7.9), to provide the final substrate mixture (0.04 M Tris; albumin, 60 mg per ml; and monostearin, 16 mg per ml). Each assay tube contained 0.5 ml of this mixture, plus enzyme in a total final volume of 1.0 ml. The substrate suspension separated on standing but dispersed readily when mixed before pipetting.

2 This method yielded falsely high values for FFA in serum, probably owing to the presence of phospholipids.

Diolein, described as 99% pure, was purchased from the Hormel Institute. On thin layer chromatography, it was found to be predominantly the 1,3-isomer. Suspensions of diolein were prepared as described above except that all procedures were carried out at room temperature.

The 1- and 2-isomers of monolein and monostearin were kindly supplied by Dr. F. H. Mattson, Research Division, Proctor and Gamble Company, Cincinnati, Ohio. The 1-mono- stearin contained about 96%, and the 2-mono- stearin, 2% of the 1-isomer according to Dr. Mattson. Suspensions of these were prepared immediately before use. Isomerization was minimized by suspending 2-monolein as described above except that all manipulations were done in an ice bath. The other monoglycerides were homogenized at room temperature. Efdol (Schenlabs Pharmaceuticals, Inc.) contained 50% coconut oil, 12.5% suerose, 1.5% glyceryl monostearate, and 2.0% polysorbate 80.

Triolein, described as 99% pure (Hormel Institute), was homogenized with 0.04 M sodium phosphate buffer, pH 7.0, containing albumin, 60 mg per ml. The final substrate mixture contained about 30 mg per ml of triolein, and 0.5 ml of the mixture was used in a total volume of 1 ml for each assay. Olive oil (Fisher Scientific Company) extracted twice with alkaline ethanol (14) replaced the triolein in this substrate mixture for some studies.

**RESULTS**

Assay of Hormone-sensitive Lipase Activity—In studies of the hormone-sensitive lipase activity, no exogenous substrate was added, i.e., the adipose tissue homogenate provided both enzyme and substrate.

The rate of FFA production was constant for at least 40 minutes in Assay II with the amounts of homogenate employed. In most assays, the incubation period was 10 minutes, occasionally longer when very low activity was expected. With a 10-minute incubation period, FFA production was a linear function of the amount of homogenate added over a range representing 12 to 60 mg of tissue.

When homogenates with activities differing by a factor of 2 (prepared from one control fat pad and from one previously exposed to ACTH) were mixed in varying proportions, the observed lipolytic activities were equivalent to those calculated from the activities of the homogenates assayed separately (Table I).

The pH optimum for lipase activity in Assay II was about 7.5 (Fig. 1). In early studies, it had appeared to be nearer pH 7.0, and for this reason, the pH 7.0 buffer was used in most of assays, giving activities approximately 20% below maximum. The activity was somewhat greater in sodium phosphate buffer than it was in Tris buffer, as shown in Fig. 1 and in Table II.

Some lipolysis can be observed in homogenates of adipose tissue in the absence of any added fatty acid acceptor. We have observed widely varying effects with different samples of bovine serum albumin. Several years ago, we found that a preparation of Fraction V from bovine serum caused essentially complete inhibition of lipolysis in homogenates of adipose tissue (15). Treatment of the albumin to remove FFA did not prevent the inhibition. The albumin used in the studies reported here (Lots W18602 and X30210, Fraction V from bovine serum, Armour Pharmaceutical Company) considerably enhanced fatty acid production in Assay II as shown in Fig. 2. The same experiment
TABLE I
Lipase activity of mixtures of two homogenates with different activities

Paired fat pads were incubated for 2 hours in 3 ml of Krebs’ bicarbonate medium. ACTH, 0.6 unit, was added to one flask 5 minutes before the tissues were removed and homogenized in 0.154 M KCl (1 ml of homogenate contained 100 mg of tissue). Homogenate A was prepared from incubated control fat pad; Homogenate B was prepared from ACTH-treated fat pad.

<table>
<thead>
<tr>
<th>Homogenate added</th>
<th>FFA produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>ml</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0.09</td>
</tr>
<tr>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0.22</td>
</tr>
<tr>
<td>0.2</td>
<td>0.22</td>
</tr>
<tr>
<td>0.3</td>
<td>0.33</td>
</tr>
<tr>
<td>0.3</td>
<td>0.23</td>
</tr>
<tr>
<td>0.2</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* Assay II was used as described under “Methods.”
† Calculated = sum of activities of indicated volume of each homogenate assayed separately.

The pH optimum for FFA production with monostearin was about 8 (Fig. 4). These data were obtained with Tris buffer. Addition of sodium phosphate (final concentration, 10^{-2} M) to the system at pH 7, 7.5, or 8 did not enhance FFA production.

TABLE II
Effect of buffer on hormone-sensitive lipase activity

Assay II (20 mg of tissue incubated for 20 minutes) was performed with buffers as indicated. FFA production was determined by titration (13), since recovery of FFA in the colorimetric procedure may be low at higher buffer concentrations.

<table>
<thead>
<tr>
<th>Final molarity of pH 7.0 buffer</th>
<th>Sodium phosphate</th>
<th>Tris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative lipase activity</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Arbitrarily set equal to 100 to permit combining of data from two different experiments.

Fig. 1. Lipolysis as a function of pH in Assay II. •—•, 20 µmoles of sodium phosphate buffer; ○—○, 20 µmoles of Tris buffer. pH was determined on mixtures of homogenate, albumin, and buffer, duplicating the assay tubes but not incubated.

Fig. 2. Effect of bovine serum albumin (Fraction V) on lipolysis in Assay II. Each assay tube contained, in addition to the indicated amount of albumin (<0.3 µeq of FFA per g), 20 µmoles of sodium phosphate buffer, pH 7.0, 30 µmoles of KCl, and 18 mg of homogenized adipose tissue (4.5 µeq of FFA per g). Incubation was at 37° for 20 minutes.

Fig. 3. FFA produced as a function of amount of commercial monostearin added. Each assay tube contained 20 µmoles of Tris buffer, pH 7.9, 30 mg of albumin, 9 mg of homogenized adipose tissue, 30 µmoles of KCl, and monostearin as indicated. Final volume, 1 ml.
in contrast to the effects of phosphate on the hormone-sensitive lipase activity. The presence of 30 mg per ml of bovine serum albumin, used in these assays, increased FFA production more than 5-fold over that obtained in the absence of albumin. Further increase of the amount of albumin in the assay to 60 mg per ml did not further enhance FFA production. Under the conditions adopted for assay of monoglyceride lipase activity, the rate of FFA production was a linear function of homogenate concentration and was constant for at least 20 minutes.

A large fraction of the monoglyceride lipase activity of fresh homogenates could be recovered in extracts of acetone powders prepared from rat or from rabbit adipose tissue. With these extracts, the rate of hydrolysis of monostearin was much greater than that of di- or triolein, as shown in Table III. In contrast, pancreatin hydrolyzed the triolein and diolein substrates more rapidly than it did monostearin.

The commercial monostearin used as substrate in these assays was presumed to be an equilibrium mixture, i.e. approximately 12% 2-monostearin and 88% 1-monostearin (16). Since, as shown in Fig. 3, the amount of monostearin required to approach substrate saturation was very large compared to the amount hydrolyzed, it was considered that the fatty acids released might be derived in the main from 2-monostearin present in the mixed substrate.

The hydrolysis of 1- and 2-monoleoin by homogenates of rat epididymal fat was investigated at substrate concentrations ranging from 1.4 to 22.4 mm. Saturation of the enzyme with 2-monoleoin was attained at the lowest substrate concentration tested. At this level, the rate of fatty acid production from 2-monoleoin was more than twice that observed with 1-monoleoin. The rate of hydrolysis of the latter increased with substrate concentration over the entire range explored. At the pH and temperature used in the assay, there is rapid isomerization of 1- and 2-monoleoin (16). The extent to which fatty acid production in assays with “1-monoleoin” may be attributable to hydrolysis of 2-monoleoin cannot be determined with certainty.

The rate of isomerization of 1- and 2-monoleoin is very slow at the temperature and pH used for the assay. With these substrates and with enzyme derived from homogenates of fresh fat or from aqueous extracts of acetone powder, the rate of hydrolysis of the 2-isomer was twice that of the 1-isomer. The amount of substrate cleaved during these assays was a very small fraction of the substrate added and in no case exceeded the amount of 2-monoleoin present in the original substrate mixture. The possibility that further isomerization had occurred during storage and handling cannot be ruled out. In one experiment in which fresh enzyme was added to the assay system three times during a 2-hour period, the total quantity of fatty acid produced during hydrolysis of 1-monoleoin corresponded to approximately twice the calculated amount of 2-monoleoin present in the substrate as added. Fatty acid production represented hydrolysis of about 5% of the total monoglyceride per hour. If isomerization of 1-monoleoin to 2-monoleoin were to take place at this rate, the observed results might represent hydrolysis of newly isomerized 2-monoglyceride. Alternatively, the enzyme may in fact attack 1-monoleoin but at a rate significantly slower than that at which it attacks the symmetrical isomer.

Hydrolysis of Ediol and Other Exogenous Substrates—As shown in Fig. 5, glycerol production was not proportional to homogenate concentration when Ediol was used as substrate (Assay I). Since homogenates of adipose tissue rapidly hydrolyze monoglycerides, it seemed probable that the relatively greater glycerol production at low concentrations of homogenate was due to rapid hydrolysis of the monostearin contained in Ediol. Based on the stated concentration of monostearin in Ediol it was calculated that about 0.05 mole of monostearin was present in each assay.

When the amount of glycerol derivable from the monostearin was...
glycerol production was related to homogenate concentration by a straight line passing through the origin (Fig. 5). The “corrected” rate of glycerol production was no greater than that observed in the absence of Ediol. It is possible, of course, that the findings in the presence of Ediol are not due to hydrolysis of monoglycerides. The effects of the polyoxyethylene sorbitan monostearate contained in the Ediol are, for example, unknown.

Addition of emulsions of tripalmitin in Assay I did not increase glycerol production. Addition of emulsions of triolein led to extra fatty acid production by whole homogenates of adipose tissue, but this extra production of FFA was not a function of the amount of homogenate used, as shown in Fig. 6. Regardless of the amount of tissue present (from 20 to 100 mg), about 0.13 μeq of extra FFA was produced in the presence of triolein. This amount of FFA corresponds to only a few tenths of 1% of the total added fatty acid ester. When olive oil, repeatedly extracted with alkaline ethanol to remove lower glycerides, was substituted for triolein, there was even less extra FFA production. It seems most probable that the extra FFA released in the presence of the triolein preparation resulted from hydrolysis of some minor component of the added substrate, not triolein itself. We have never unequivocally demonstrated extra FFA production attributable to hydrolysis of added triglycerides.

Effect of Hormones on Activity of Lipase—In Table IV, two experiments with paired fat pads are summarized to indicate the manner in which the studies of hormone-induced changes in lipase activity in tissues were made. The experiment with Rat 1 indicates the decrease in lipase activity that occurs during incubation of the fat pad in the absence of hormones. The experiment with Rat 2 is representative of those described below. The hormone, in this case epinephrine, was added to one of the paired fat pads for the final 10 minutes of a 90-minute incubation period. This brief exposure increased the lipase activity at pH 7 (Assay I) from 1.4 to 3.4 μmoles of glycerol released per g of tissue in 20 minutes.

The extent of the decrease in lipase activity during incubation in the absence of hormones was variable from one group of tissues to another, probably related at least in part to the handling of the rats immediately before decapitation. On several occasions, with tissues from groups of rats that had been disturbed or excited, no decline in lipase activity was observed during 90 minutes of incubation. In one group of three rats, the lipase activity declined to 55 ± 3% of its initial value in 90 minutes. In some studies, little or no detectable lipase activity (i.e., less than 10% of the initial) was observed after 3 hours of incubation. Usually, however, the activity remaining after 3 hours was approximately 20 to 30% of the original activity. The mean of the differences between paired control and hormone-treated tissues ± standard error of the mean.

Example of studies with paired fat pads to show changes in activity of hormone-sensitive lipase

In the first experiment, one fat pad was homogenized and assayed immediately after excision. The contralateral fat pad was incubated in 3 ml of Krebs’ bicarbonate medium containing bovine serum albumin, 30 mg per ml, for 90 minutes before it was homogenized and assayed (Assay I). In the second experiment, both fat pads were incubated for 90 minutes and epinephrine, 0.5 μg per ml, was added to one tissue for the last 10 minutes of incubation.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Fat pad</th>
<th>Treatment before homogenization</th>
<th>Lipase activity μmoles glycerol/g/10 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Right</td>
<td>None</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Incubation for 90 min</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>Right</td>
<td>Incubation for 90 min; epinephrine added after 80 min</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Incubation for 90 min</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Table IV

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Concentration</th>
<th>No. of pairs</th>
<th>Lipolytic activity Control ± Δ due to hormone μmoles glycerol/g/10 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>17 μg/ml</td>
<td>12</td>
<td>1.0 ± 0.4 +1.4 ± 0.2</td>
</tr>
<tr>
<td>ACTH</td>
<td>0.2 unit/ml</td>
<td>7</td>
<td>1.6 ± 0.2 +2.2 ± 0.2</td>
</tr>
<tr>
<td>TSH</td>
<td>10 μg/ml</td>
<td>3</td>
<td>1.6 ± 0.2 +1.3 ± 0.5</td>
</tr>
<tr>
<td>Glucagon</td>
<td>5 μg/ml</td>
<td>3</td>
<td>1.8 ± 0.2 +3.0 ± 0.7</td>
</tr>
</tbody>
</table>

* Mean.
† Mean of differences between paired control and hormone-treated tissues ± standard error of the mean.
were immediately transferred to tubes containing the components were added 3 minutes before the end of incubation. The tissue in the absence of added monostearin.

Lipase activities are not corrected for FFA production observed described in "Methods." Note that the so-called monoglyceride lipase activities were not corrected for FFA production observed in the absence of added monostearin.

Table VI

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Concentration</th>
<th>Lipolytic activity</th>
<th>μg FFA/g/10 min</th>
<th>Δ due to hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>17 μg/ml</td>
<td>2.8</td>
<td>+5.6 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td>0.2 unit/ml</td>
<td>1.0</td>
<td>+1.8 ± 0.59</td>
<td></td>
</tr>
<tr>
<td>TSH</td>
<td>10 μg/ml</td>
<td>0.6</td>
<td>+3.0 ± 0.72</td>
<td></td>
</tr>
<tr>
<td>Glucagon</td>
<td>5 μg/ml</td>
<td>0.3</td>
<td>+3.6 ± 0.73</td>
<td></td>
</tr>
</tbody>
</table>

Table VII

Effect of norepinephrine on lipase activity

Paired fat pads were incubated for 2 hours in 3 ml of Krebs' bicarbonate medium containing bovine serum albumin, 30 mg per ml. To one of each pair of flask, 5 μg of norepinephrine were added 3 minutes before the end of incubation. The tissue was rinsed, blotted, and homogenized. Samples of homogenate were immediately transferred to tubes containing the components of Assay II or of the monoglyceride lipase assay system as described in "Methods." Note that the so-called monoglyceride lipase activities are not corrected for FFA production observed in the absence of added monostearin.

Table VIII

Effect of isopropanol and of dithiophosphate fluoride on activity of adipose tissue lipases

DFP in isopropanol, or isopropanol alone, was added to the assay mixture just before the homogenate was added (no preincubation) or was added to the homogenate in a total volume of 0.5 ml and preincubated at 37°C for the times indicated. The other components of the assay system were then added in a volume of 0.5 ml, and production of FFA during a subsequent 10-minute incubation period was determined. Assay II was used.
probably because fat layers so prepared disperse more readily and completely in the assay system. Usually, 60 to 70%, occasionally up to 95%, of the original activity was recovered and most of this was present in the fat layer, as shown in Table IX.

**TABLE IX**

**Distribution of lipase activity in homogenates of rat and rabbit adipose tissue**

Fat pads were homogenized in 0.154 M KCl (1:10, w/v). Samples of homogenate (0.2 ml) in tubes improvised from polyethylene tubing were centrifuged for 10 to 30 minutes at 15,000 X g (20°). Tubes were sliced with a Stadie-Riggs blade to separate fat and fluid layers. These and the small amount of sediment in the bottom of the tube were transferred as completely as possible to the assay tubes.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Tissue</th>
<th>Lipolytic activity (µg FFA/g/10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Assay I</strong></td>
</tr>
<tr>
<td>1 (rat)</td>
<td>Whole homogenate</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Fluid</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td>Sediment</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td>Fluid + sediment</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td>Fat + fluid</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Fat + sediment</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Fat + fluid + sediment</td>
<td>1.7</td>
</tr>
<tr>
<td>2 (rat)</td>
<td>Whole homogenate</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Fluid</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td></td>
<td>Sediment</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td>Fat + fluid</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Fat + sediment</td>
<td>2.7</td>
</tr>
<tr>
<td>3 (rabbit)</td>
<td>Whole homogenate</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Fluid</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td>Sediment</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

**FIG. 7.** Effect of temperature on lipolysis. *A,* Assay II with 20 mg of tissue per assay, incubated for 10 minutes; *B,* assay for monoglyceride lipase activity with 10 mg of tissue per assay, incubated for 10 minutes. Data from several different experiments were combined by adjusting the activity of each homogenate at 37° to an arbitrary value and correspondingly correcting the assays performed at other temperatures.

**DISCUSSION**

Hollenberg, Raben, and Astwood (1) first showed that the lipolytic activity of whole homogenates of rat epididymal adipose tissue could be increased by prior incubation of the intact tissue with ACTH or epinephrine. The present results confirm and extend their findings. Glucagon, norepinephrine, and TSH have also been shown to be capable of stimulating lipase activity. The percentage increments in hormone-sensitive lipase activity due to the several hormones studied, with the incubation conditions and assay conditions described here, are comparable to the percentage increments in rate of glycerol release from intact adipose tissue induced under similar conditions by these same hormones (7). It seems reasonable to conclude that activation of the enzyme system is necessary but not sufficient for the observed increase in the rate of lipolysis.
of the hormone-sensitive lipase system accounts wholly or in large part for the fat-mobilizing action of epinephrine, norepi-
nephrine, ACTH, glucagon, and TSH.

The present results cannot readily be compared with those of Riazz (2) and of Björntorp and Furman (3), who have also re-
ported epinephrine stimulation of lipase activity in rat adipose tis-
tue. These authors studied changes in the lipase activity of the fluid layer below the fat cake in an assay system containing
Etidol. In the experiments reported above, this fluid layer con-
tained only a small fraction of the total lipase activity of the
homogenate. The pH optimum for the lipase activity in the
fluid layer is similar to that of the hormone-sensitive lipase ac-

tivity. There is an increment in activity after exposure of the
tissue to epinephrine and it may be that the same enzyme system
is partitioned between the fat layer and the infranatant fluid.

As we have previously reported, the increased rate of glycerol
release induced by hormones in vitro may be transient (7). For
example, fat pads incubated in the presence of ACTH, 0.04 unit
per ml, produced 2 μmoles of glycerol per g of tissue during the
first 30 minutes of incubation but released none at all during the
second 30 minutes. It was shown that addition of more ACTH
at 30 minutes restored lipolysis during the second half of the in-
cubation to levels comparable to those seen in the first 30 min-
utes, indicating that the tissue was still able to respond. The
arrest of lipolysis presumably reflects the presence of a system
for rapid inactivation of the hormone-activated lipase system.

The nature of this inactivating system is under study.

It will be seen that there is a general similarity between the
behavior of the hormone-activated lipase system and the hor-
monated phospholipase system in adipose tissue (17). In both
cases, the activity of the tissue assayed immediately after
exivision is at a high level and then falls progressively, although
at quite variable rates, during subsequent incubation. Large
hormone effects can be observed only after the activity of the
control tissue has been allowed to decline during an incubation
in the absence of hormone (1, 2).

It seems most likely that in vivo the active enzyme-inactive
enzyme equilibrium is a dynamic one. Administration of hor-
monc at any time causes the activation process to predominate.
It is somewhat paradoxical that it is difficult to effect a large
increment in lipase activity with freshly removed tissue even
though hormone administration effects a decided increase in ac-
tivity in vitro as evidenced by increased glycerol and FFA release.
It is possible that in the process of killing the animal, removing
the tissues, and preparing homogenates for assay, the enzymes
may undergo some degree of factitious activation.

The studies reported above establish the presence in rat adipose
tissue of a lipase with a high degree of specificity for monoglyc-
zerides. Direct comparison with crude pancreatic lipase,
with the use of the same substrates, showed that this specificity
was not based on different physical properties of the glycerides
used. The activity toward monoglycerides changed little, if at
all, as a result of exposure of the tissue to hormones which, under
the same conditions, caused marked activation of the hormone-
sensitive lipase system. It is further differentiated from the lat-
ter by its higher pH optimum, by its very different temperature-
activity curve and by its stability in the presence of isopropanol.
Like lipoprotein lipase, it is sensitive to DFP and its pH optimum
is close to that for lipoprotein lipase. Unlike lipoprotein lipase,
however, it is readily extracted from acetone powders by water
or simple buffers, it has very little activity toward triglyceride substrates, and it is not activated by addition of serum. It is
concluded that there is a distinct monoglyceride lipase in rat and
rabbit adipose tissue. The role of this enzyme in fat mobiliza-
tion remains to be established. It may be responsible for the

further degradation of monoglycerides generated by the action
of the hormone-sensitive lipase. If so, it is unlikely ever to be
rate-limiting, since its activity is so much greater than that of
the hormone-sensitive enzyme system.

The monoglyceride lipase moreover cleaves 2-monoglycerides
much more rapidly than it does 1-monoglycerides. It is difficult
to be certain of the true extent of its activity toward 1-monogly-
cerides because of the ready isomerization of monoglyceride
substrates. This positional specificity together with the fact
that monoglyceride lipase activity can also be extracted from
acetone powders with ammonium hydroxide suggests an ex-
planation for the discrepancy between the apparent positional
specificity of post-heparin lipase in serum and that of lipoprotein
lipase extracted from acetone powders of adipose tissue. Borg-
ström and Carlson (18) have noted that the serum enzyme
catalyzes predominantly an exchange of labeled fatty acids into
the 1-position of triglycerides; Korn (19) found that there was
little or no accumulation of lower glycerides during the action
of acetone powder extracts and concluded that tissue lipoprotein
lipase cleaved fatty acids from the 2-position almost as readily
as it did from the 1,3-positions. If the monoglyceride lipase re-
ported here were present in “lipoprotein lipase” preparations
made from adipose tissue, the apparent lack of specificity might
reflect the combined activities of the two enzymes. If heparin
accelerates predominantly or exclusively the release into the
serum of lipoprotein lipase and not of the monoglyceride lipase,
the activity in post-heparin serum may better reflect the specific-
ity of lipoprotein lipase per se.

Stimulation of release of FFA from rabbit adipose tissue by
ACTH has been previously reported (20). In addition we have
demonstrated a similar effect of glucagon and of epinephrine.
Effects of epinephrine were observed only when ascorbic acid was
present in the medium, presumably interfering in some way with
inactivation of the hormone.

We have previously observed that small amounts of epinephrine are rapidly inactivated during incubation with rat adipose tissue (7).
The apparent unresponsiveness of rabbit adipose tissue to epinephrine in the studies of Rudman, Brown, and Malkin (20) perhaps reflects a greater
capacity of adipose tissue from this species to inactivate epi-
nephrine. It seems probable, on the other hand, that the basic
mechanisms for lipase activation are quite analogous in the two
species.

**SUMMARY**

Homogenates of the epididymal fat pad of the rat were shown
to contain, in addition to lipoprotein lipase, a hormone-sensitive
lipase activity with a pH optimum near 7.5 and a monoglyceride
lipase activity with a pH optimum near 8.0.

The activity of the hormone-sensitive lipase system decreased
progressively during incubation of intact fat pads but could then
be restored by exposure of the tissue to epinephrine, norepineph-
rine, adrenocorticotropin hormone, thyroid-stimulating hormone,

*Unpublished results.*
or glucagon for as little as 3 minutes prior to homogenization. These results are consonant with earlier observations on the time course of glycerol release in the absence and in the presence of hormones. The rapid changes in rates of glycerol release from intact adipose tissue incubated in the presence of hormones apparently reflect activation of the lipase system described here. The tissue must also contain a system for rapid inactivation of the hormone sensitive lipase.

Whereas incubation of the intact tissue with norepinephrine increased the hormone-sensitive lipase activity 2- to 3-fold, the monoglyceride lipase activity was not appreciably altered. The latter activity was retained in aqueous extracts of acetone powders in which the hormone-sensitive lipase activity was not demonstrable. The monoglyceride lipase activity against 2-monoglycerides was shown to be much greater than its activity against 1-monoglycerides.

In homogenates of rat or of rabbit adipose tissue most of the hormone-sensitive lipase activity and the monoglyceride lipase activity was found in the floating fat fraction after centrifugation at 15,000 $\times g$ for 30 minutes.

The stimulating action of adrenocorticotropic hormone on the rate of release of free fatty acid from retroperitoneal adipose tissue of the rabbit was confirmed and it was shown that, at high concentrations, epinephrine and glucagon were also effective, presumably by way of a similar lipase-activating mechanism.

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