Lipase Activity Measured in Serum by a Continuous-Monitoring pH-Stat Technique—an Update

Norbert W. Tietz, J. Rex Astles,¹ and Denise F. Shuey

Using recent knowledge regarding the roles of colipase, bile acids, Ca²⁺, and emulsifiers, we optimized a previously published pH-Stat method for lipase (EC 3.1.1.3) activity measurements. The recommended assay conditions are: olive oil/triolein, 100 mL/L; sodium glycololate, 35 mmol/L; Ca²⁺, 8.5 mmol/L; and colipase, 6.0 mg/L. The sample volume is 0.10 mL, the reaction pH 9.0, the temperature 30 °C, and the concentration of titrant 15 mmol/L. Hydroxypertol methylcellulose, 20 g/L, replaces acacia as emulsifier to avoid inhibition by excess Ca²⁺. The standard curve is linear to >4566 U/L. The reference interval with olive oil as substrate is 30−235 U/L. Lipase activities with triolein substrate are 9.9% greater than with olive oil. Interference by pancreatic carboxylesterase (EC 3.1.1.1) activity is inhibited by incubating the sample with diisopropylfluorophosphate. Results correlate well with those by the optimized SingleVial² method of Boehringer Mannheim Diagnostics (r = 0.997) and the immunochemical assay of Beckman Instruments, Inc. (r = 0.995). Correlation with the aca method (E.I. DuPont de Nemours & Company) is less satisfactory (r = 0.892), probably owing to loss of colipase in the latter method.

Increased activity of lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) in serum is widely accepted as a good indicator of pancreatitis (1−3). Measurement of lipase is claimed to be more specific than measurement of amylase, which is increased not only in pancreatitis but also in various nonpancreatic abdominal and non-abdominal illnesses (4). There are many published methods for measuring lipase activity in serum, but no one method has been widely accepted. We previously published a continuous-monitoring method in which a pH-Stat is used to add standard NaOH automatically when fatty acids, liberated by the action of lipase, decrease the pH of the reaction mixture (5). Increased understanding of the reaction mechanisms for lipase now makes it possible to improve significantly the specificity and sensitivity of this assay. Such improvements are reported in this communication.

Pancreatic lipase is rapidly and irreversibly inactivated at the interface of water and insoluble substrate because of the unfolding of its three-dimensional structure by the high surface tension at the hydrophobic interface (6). Addition of bile salts or albumin at low concentrations prevents this inactivation, so we added bile salts in a concentration of 2 mmol/L to our previously recommended reaction mixture (5). This method, like other titrimetric assays, detects not only lipase but also post-heparin lipolytic activity from lipoprotein lipase (triacylglycerol-protein acylhydrolase, EC 3.1.1.34) and hepatic lipase. However, the addition of bile acids to the reaction mixture at supramicellar concentrations has been shown to inhibit lipase (7−9) and post-heparin lipolytic activity (10, 11) by physically excluding these enzymes from the substrate−water interface (12−14). Lipase (but not lipoprotein lipase) activity can be fully restored by adding colipase to the reaction mixture. Such restoration is brought about by the formation of a bile acid−lipase−colipase complex that has a high affinity for the substrate−water interface that anchors lipase to the substrate surface (9, 12). Formation of this complex not only increases the specificity of the assay, it also increases the stability of lipase (15).

Because colipase is now recognized as an essential component of a specific lipase assay (16, 17) its concentration in the reaction mixture must be optimized, and its concentration must be adequate to compensate for the different amounts of colipase found in individual serum specimens (18, 19). Unfortunately, most of the experimental work with colipase has been done with purified lipase preparations (7−9) rather than with serum. In 1982, Hockeborn and Rick (10), using a pH-Stat method, showed the beneficial effects of adding colipase to the reaction mixture when serum specimens are analyzed. But in 1984 the same authors were unable to obtain linear reaction rates with their assay and recommended that colipase not be added to the assay until a preparation of consistent purity had become available (20). Junge (21) also added colipase to his assay mixture, but our experiments have shown that his procedure is suboptimal with regard to the bile acid concentration and therefore detects lipoprotein lipase. In addition, the assay is not optimized for Ca²⁺. Thus, there remains an urgent need for a reliable method of measuring lipase activity in serum, a method that takes into consideration the new knowledge regarding the roles of colipase, bile salts, Ca²⁺, and emulsifiers.

Materials and Methods

Instrumentation

We used a pH-Stat (Radiometer A/S, DK-2400 Copenhagen NV, Denmark; U.S. distributor: The London Company, Cleveland, OH 44145) consisting of a PHM 63 digital pH meter with G2040B and K4040 electrodes, a TTA 60 titration assembly, and a TTT 60 titrator. Parameters were set as follows: temperature setting, 30 °C; titration, upscale; proportional bend, pH-Stat; delay shut-off, infinity; and endpoint, pH 9.00. The temperature in the reaction vessel was maintained at 30 °C by a Leda K-2/RD circulating water bath (Brinkmann Instruments, Inc., Westbury, NY 11590). We added standard sodium hydroxide with an autoburette, Model ABU 12 (Radiometer) equipped with a 250-µL burette and a 25-mL reaction vessel. The delivery speed ranged from 10 to 160. The autoburette was linked through a mechanical connection to the Servograph recorder, type REC 61 (Radiometer), operated with the pen set at 28 mm/full scale and the chart speed set at “free.”
Reagents

Purified olive oil and CO₂-free water. Both reagents were prepared as described in our previous communication (5).

Olive oil (cat no. 0-1500) and triolein (99% pure, cat no. T-7140) were both obtained from Sigma Chemical Co., St. Louis, MO 63178.

Hydroxypropyl methylcellulose, 20 g/L. Dissolve 20 g of hydroxypropyl methylcellulose (Sigma Chemical Co.; cat. no. H-8384, premium grade, USP) in 340 mL of CO₂-free water, heated to 80–90 °C. Use a 1-L beaker, a magnetic stirrer, and a 35-mm stirring bar. When solution is complete, add 640 mL of CO₂-free, chilled water to the beaker and stir. Store at 4–8 °C in a glass-stoppered bottle.

Substrate emulsion. To 28.6 mL of triolein or purified olive oil in a high-speed blender (e.g., Osterizer “Galaxie” 14-speed blender) add 171.4 mL of hydroxypropyl methylcellulose solution, previously cooled to 4–8 °C. Blend at high speed for 5 min, cool in refrigerator for 1 h, and continue the emulsification process at high speed for an additional 5 min. Store the reagent, tightly stoppered, at 4–8 °C. This emulsion is stable for five days.

Because the optimal substrate concentration is not determined by the amount but by the surface area of the olive oil (tri olein), the substrate must be sufficiently emulsified. Confirm the adequacy of the surface area by measuring the lipase activity with either a previously standardized control specimen or with a patient’s specimen that has lipase activity ≥4000 U/L, using substrate concentrations at both 100 and 150 mL/L. No increase in activity should be observed with the higher substrate concentration.

Combined sodium glycocholate, 0.125 mol/L, and CaCl₂, 0.030 mol/L. Dissolve 6.095 g of sodium glycocholate (Behring Diagnostics, La Jolla, CA 92037; cat no. 360512) and 0.4463 g of calcium chloride dihydrate (Fisher Scientific Co., Fair Lawn, NJ 07410; cat no. C-79) in about 80 mL of CO₂-free water, stirring constantly. When solution is complete, transfer the solution to a 100-mL volumetric flask and fill to the mark with CO₂-free water. Stored at 4–8 °C and protected from light, this solution is stable for at least six weeks.

Colipase. Prepare a stock solution of colipase (Boehringer Mannheim Diagnostics, Indianapolis, IN 46250; cat no. 644099) at a concentration of 1200 mg/L in sterile isotonic saline (Travenol Laboratories, Inc., Deerfield, IL 60015; cat no. 1P7124). Lyophilized preparations of colipase are generally only about 60% colipase, so an appropriately greater amount must be weighed out to achieve the indicated concentration. Store this reagent, in appropriate aliquots, at −70 °C. Prepare a working solution of colipase, 600 mg/L, by diluting one part of stock solution with one part of the sterile saline. Colipase solution is stable for longer than a year at −70 °C, and for at least two weeks when refrigerated.

Sodium hydroxide, 1.000 mol/L. Prepare this solution with sodium hydroxide pellets (Fisher Scientific Co., cat no. S-318) and standardize.

Sodium hydroxide, 30 and 15 mmol/L. Prepare from sodium hydroxide stock solution, 1.000 mol/L, by appropriate dilution with CO₂-free water. Confirm the titer of the 15 mmol/L solution by titration against a standard solution of potassium hydrogen phthalate.

Hydrochloric acid, 100 and 50 mmol/L. Prepare both solutions by appropriate dilution of standard HCl with CO₂-free water.

Heparin, sodium (1000 USP units/mL; Elkins-Sinn, Inc., Cherry Hill, NJ 08034).

Diisooxypropylfluorophosphate, 3 mmol/L. In aqueous solution, this has been recommended as a carboxylesterase inhibitor (21A, 21B). Prepare an aqueous solution of diisooxypropylfluorophosphate (Sigma Chemical Co., cat no. D-0879), approximately 3 mmol/L, and store in a brown bottle at 4–8 °C. Refrigerated in the dark, this solution is stable for five days.

Diethyl-4-nitrophenyl phosphate (paraaxon, cat no. D-4503), eserine (cat no. E-8375), phenylmethyisulfonyl fluoride (cat no. P-7626), p-arsanilic acid (atolyl, cat no. A-9258), and p-hydroxymercurobenzoic acid (cat no. H-0642) were all from Sigma Chemical Co. Lanthanum chloride (cat no. L-9) was from Fisher Scientific Co.

Release of Lipoprotein Lipase

Six volunteers had fasted for 12 h and also remained seated for at least 20 min before the sampling procedure, to decrease hemocencentration. Blood was then sampled before and 10 min after an intravenous injection of sodium heparin, 10 units per kilogram body weight, to release lipoprotein lipase (17).

Preparation of instruments

Adjust the titrator settings, set the recorder to specifications listed above, and fill the burette with 15 mmol/L sodium hydroxide. Standardize the pH meter at 30 °C with a standard buffer. After each measurement, rinse the reaction vessel, the electrode, and all parts under the cover of the reaction vessel with de-ionized water. Keep the electrodes wet at all times to prevent the drying of olive oil or triolein on the electrode surface. Between runs, place the electrodes into pH 4 buffer (potassium biphthalate; Fisher Scientific, cat no. SO-B-98). If results are not reproducible within the desired precision goal or if reaction-rate curves are not linear, soak the glass electrode overnight in HCl, 0.10 mol/L. At the end of each working day, dry the electrodes with acetone and clean them with petroleum ether to remove all lipid particles. Place the glass electrode in pH 4 buffer or in 0.10 mol/L HCl solution, as needed. Place the reference electrode into saturated KCl solution. Meticulous cleaning and conditioning of the electrodes are essential for satisfactory performance of the procedure.

Assay

Final concentration in reaction mixture: substrate (tri olein or olive oil), 100 mL/L; Na glycocholate, 35 mmol/L; CaCl₂, 8.5 mmol/L; and colipase, 6 mg/L.

1. With a 10-mL syringe, dispense 7.0 mL of substrate solution into the reaction vessel.

2. Add 2.5 mL of sodium glycocholate–CaCl₂ solution.

3. Add 0.10 mL of colipase, 600 mg/L.

4. Secure the reaction vessel in its proper place. Guide a gentle but constant stream of nitrogen gas over the surface of the mixture by inserting an appropriate tube through the port in the cover of the reaction vessel. This purge removes CO₂ gas from the reaction mixture and prevents CO₂ absorption from the atmosphere.

5. Adjust the pH of the mixture to about 9.00 with NaOH, 30 mmol/L. Mix for 4 or 5 min to assure that all CO₂ is removed from the reaction vessel and that temperature equilibrium is reached. The temperature can be monitored with a thermistor (Fisher Scientific Co., cat no. 1517624) connected to a Model 45TA Tele-Thermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH 45387) and inserted through a port in the top of the reaction vessel.
6. Add 0.10 mL of NaCl, 155 mmol/L, to the blank, and 0.10 mL of specimen to the unknown.
7. Readjust the pH with either HCl, 50 mmol/L, or NaOH, 30 mmol/L, until the pH meter reads between 8.99 and 9.00.
8. Reset the burette volume counter to 0.00, and simultaneously push the start button of the titrator and start a stop watch. Note the burette reading every minute until a constant rate is maintained during at least 5 min. Check the reaction-rate curve on the recorder-chart paper by placing a ruler next to the line to confirm linearity, and determine the consumption of NaOH per minute for the time during which the reaction rate curve was linear. For example, if the reaction is linear during the interval 3 min to 8 min, subtract the burette reading at 3 min from the burette reading at 8 min and divide by 5 min.
9. Determine the blanks in duplicate at the beginning of the first run of the day and recheck at several intervals during the day. If duplicates do not check within the desired precision, additional blanks should be titrated; if not satisfactory, electrode maintenance may be required.

Calculations

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\text{Lipase activity, U/L} = \frac{(T/t_f - B/t_b) \times 0.015 \times 10^4}{(T/t_f - B/t_b) \times 150}
\]

where U is defined as micromoles of free fatty acids formed per minute; T and B represent the volume of a 15 mmol/L solution of NaOH, in microliters, used to titrate the test and blank, respectively; \(t_f\) and \(t_b\) are the reaction times, in minutes, for the test and blank, respectively; and \(10^4\) converts sample volume to liters, for a 100-\(\mu\)L sample.

**Results with the Proposed Method**

**Optimization of Reaction Parameters**

**Hydroxypropyl methylcellulose.** This emulsifier is a cellulose ether with no ionic charge, and thus it will not complex with metallic salts and ionic organic compounds. It stabilizes the emulsion by decreasing the surface and interfacial tensions and by increasing the viscosity of the water phase (22). We confirmed the earlier reports of Sémeriva et al. (23), who found a 20 g/L solution most effective. Concentrations of 30 and 40 g/L render the reaction mixture too viscous and cause uneven mixing of the titrant and thus irregular reaction rate curves.

**Triolein or olive oil emulsions.** We determined optimal concentrations of substrate by measuring the lipase activity with emulsions containing 50, 80, 100, 120, and 150 mL of triolein or purified olive oil per liter. We obtained maximum activities with substrate emulsions containing 80 mL/L of triolein or olive oil, and with the higher substrate concentrations listed above. A concentration of 100 mL/L was selected to provide for excess substrate. We optimized the olive oil substrate concentration by using specimens with slightly and with greatly increased lipase activities, in all cases using hydroxypropyl methylcellulose concentrations of 20 g/L.

**Olive oil vs triolein.** Lipase activities in 26 sera from hospital patients, varying in activity from 55 to 3544 U/L, were compared by using purified olive oil and triolein, 99%, both at a final concentration of 100 mL/L. The mean activity measured by using triolein was 9.9% greater than that obtained with olive oil as substrate. The regression equation was \(y\) (triolein) = 1.1x (olive oil) + 5.4, the correlation coefficient \((r)\) was 0.998, and \(S_{xy} = 45.8.\)

These findings are in contrast to those of Hockeborn and Rick (10), who found the same activities with olive oil and triolein. The disparity is perhaps due to differences in the qualities of olive oil (especially) and triolein. These authors used triolein, 95% (Serva no. 37085), and triolein, 99% (Calbiochem no. 6450), as opposed to the 99% triolein (Sigma) we used in our experiments. Triolein is the preferred substrate when highest accuracy is desired, but olive oil, because of its lower cost, is preferred for routine assays and has been used in these experiments except when otherwise specified.

**pH optimum.** The pH-optimization curves showed a broad plateau between pH 8.8 and 9.2. A reaction pH of 9.0 (±0.1) is optimal, because it is near the maximum activity for all specimens tested. There is, however, little change in activity from the optimal between pH 8.8 and 9.2.

**Ca\(^{2+}\) optimization.** The role of Ca\(^{2+}\) in lipase activity measurements has been debated for many years. It is now reasonably well established that Ca\(^{2+}\) has one or more of the following roles: it protects the active center against poisoning by reaction products through precipitation of free fatty acids as calcium soaps at the substrate interface; it interacts with emulsified substrate particles to reduce their negative electrostatic charge; it facilitates adsorption of the enzyme onto the substrate in the presence of bile salts; and it shortens the lag phase (24–27). Calcium concentrations between 8 and 9 mmol/L were found optimal for all specimens regardless of their lipase activity or the disease state (Figure 1); therefore we chose a concentration of 8.5 mmol/L for use in our proposed method.

The inhibition of lipase activity by Ca\(^{2+}\) concentrations of ≥9.0 mmol/L is of interest because gum acacia, used hitherto as an emulsifier in lipase assays, may contain substantial amounts of calcium and magnesium. The solution of gum acacia used in our experiments (cat. no. G-85, Fisher Scientific Co.) contained, per liter, 13.5 mmol of Ca\(^{2+}\), 7.4 mmol of Mg\(^{2+}\), and 15 mmol of K\(^+\). By contrast, hydroxypropyl methylcellulose contains none of these ions. We believe that these contaminants are responsible for the lower activity observed with gum acacia emulsions as compared with emulsions prepared with hydroxypropyl methylocellulose.

**Fig. 1.** Ca\(^{2+}\) optimization curves prepared by using four sera with different lipase activities.
Glycocholate concentration. The bile salt concentration in the reaction mixture should exceed the critical micellar concentration in order to "clear" the substrate surface of proteins, to inhibit the activity of interfering enzymes, and to form the lipase–colipase–bile salt complex. Figure 2 shows results of experiments with sera from an individual before (curves A and B) and after administration of heparin (curves C and D). Curve A demonstrates the complete inhibition of lipase by sodium glycocholate concentrations of ≥30 mmol/L. The addition of colipase, 6 mg/L (curve B), restores lipase activity to a level exceeding that seen in curve A. This increase in activity is probably ascribable to saturation of the enzyme with cofactor, protection of the enzyme from inactivation, and improved anchoring of the enzyme to the substrate. Post-heparin specimens (curves C and D) show considerably higher lipolytic activity at low concentrations of bile salts, presumably owing to the release of lipoprotein (and possibly hepatic) lipase. Adding bile salts to the reaction mixture to give a concentration ≥35 mmol/L causes complete inhibition of all lipolytic activity (curve C). However, addition of colipase, 6 mg/L (curve D), restores lipase activity to near that seen in pre-heparin specimens. We conclude from these experiments that bile salt concentrations of 35 mmol/L in the presence of colipase, 6 mg/L, successfully inhibit all post-heparin lipoprotein lipase activity. Comparison with pre-heparin values indicates that lipase activity is fully restored by the presence of colipase. The results of these experiments were confirmed on pre- and post-heparin specimens from five other individuals. (See also Colipase concentration, below.)

Although deoxycholate has been recommended by other authors (16, 28) for use in lipase activity measurements, it is unsuited for our reaction mixture. Optimal calcium concentrations of 8.5 mmol/L in the presence of deoxycholate produce a heavy precipitate that interferes with titration of the reaction mixture. Neumann et al. (29) also found that deoxycholate precipitates with calcium concentrations >2 mmol/L. We observed precipitates in the reaction mixture proposed by Neumann et al. even at Ca** concentrations of 1 mmol/L.

Hockeborn and Rick (10) reported difficulties with specific lots of glycocholate. We checked various lots of this reagent, including the lot (Behring lot no. 810044, cat. no. 360512) that was used unsatisfactorily by these authors, but we saw no differences in results with our assay system.

Colipase concentration. Although glycocholate optimization experiments (Figure 2) established the need for a colipase concentration of 6 mg/L, we conducted additional experiments with reaction mixtures containing 3, 6, and 12 mg of colipase per liter. Lipase activity was determined by using specimens with moderately and greatly increased lipase activity. These experiments confirmed that a colipase concentration of 6 mg/L is adequate to maintain optimal lipase activity, even in specimens with a very high (2400 U/L) lipase activity.

Linearity. We checked the linearity of the method by analyzing a serum specimen with 4566 U/L of lipase activity and making serial dilutions with either heat-inactivated serum or NaCl, 155 mmol/L, or by taking a smaller sample. We observed linearity throughout the entire range, regardless of the diluent or sample size used.

The ability of the system to accept sera diluted with either heat-inactivated serum or NaCl solution is a significant advantage over previous assay systems adapted to the pH-Stat technique (5, 10). This improvement, brought about by the change in glycocholate concentration and the addition of colipase, also decreases the relative dependence of the system on the use of a consistent sample size. However, a substantial increase in sample size—e.g., to 200 μL—results in a slight decrease in activity, possibly owing to the competition between serum proteins and lipase for the substrate surface.

Precision. In Table 2 we compare day-to-day and within-day precision for serum specimens with different lipase activities.

Reaction temperature. In accordance with the recommendations of the International Federation for Clinical Chemistry (IFCC), the method was optimized at 30 °C. However, lipase activity measurements performed at 25, 30, 35, 37, and 40 °C indicated a linear increase in activity between 25

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**Table 1. Lipase Activities (U/L) Compared with Acacl and Hydroxypropyl Methylcellulose as Emulsifiers and Olive Oil as Substrate**

<table>
<thead>
<tr>
<th>Acacl</th>
<th>Hydroxypropyl Methylcellulose</th>
<th>Difference, %</th>
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<tbody>
<tr>
<td>160</td>
<td>183</td>
<td>14.4</td>
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<tr>
<td>543</td>
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<td>8.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
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**Table 2. Precision Studies with Two Serum Specimens**

<table>
<thead>
<tr>
<th>Within-day precision</th>
<th>Day-to-day precision</th>
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<tr>
<td>n</td>
<td>16</td>
</tr>
<tr>
<td>X, U/L</td>
<td>15</td>
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<tr>
<td>SD, U/L</td>
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<tr>
<td>CV, %</td>
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and 37 °C. The rate of increase with increase in temperature becomes smaller at a temperature of 40 °C, as an Arrhenius plot showed. These findings are in contrast to our earlier experiments (5), in which we observed temperature stability only up to 30 °C. This increase in stability can be attributed to the presence of colipase, which stabilizes lipase at the substrate–water interface (16). The Q_{10} for lipase between 25 and 35 °C, as measured with our reaction mixture, is 1.7.

**Effect of NaCl on Lipase Activity**

Arzogouli et al. (30) reported that adding sodium chloride to the reaction mixture in both the Boehringer Mannheim Diagnostics (Indianapolis, IN 46250) turbidimetric lipase procedure and a slightly modified titrimetric procedure (31) results in an increase in activity that is affected by both the sodium chloride concentration and the type of lipase isoenzyme present. According to these authors, lipase activities of sera from healthy subjects and from patients without pancreatitis peak in the presence of NaCl, 80 mmol/L. However, sera from patients with pancreatitis exhibited the greatest increase in activity in the presence of NaCl, 140 mmol/L. Arzogouli et al. therefore recommended that sodium chloride be added to the reaction mixture as a means of differentiating hyperlipasemia resulting from acute pancreatitis from that attributable to other causes. When we added NaCl to our recommended reaction mixture, regardless of the NaCl concentration or the type of specimen used, there was a decrease in lipase activity. Thus this approach, when applied to our method, does not aid in the differentiation of the causes of hyperlipasemia.

Schandi and Pittner (32) demonstrated with immobilization techniques that both alkali-earth and alkali metal ions—especially Ca\(^{2+}\), Mg\(^{2+}\), and Na\(^{-}\)—stabilize the active center of the enzyme and promote adsorption of the enzyme onto the substrate surface. These authors found calcium ions to be particularly effective in this regard. Because our proposed method is optimized with regard to calcium ions, and because calcium ions are more effective stabilizers than sodium ions, our method does not benefit from the presence of additional sodium ions.

**Reference Interval**

We established the normal reference interval by analyzing sera from 116 rested, fasting laboratory employees (Figure 3). The central 95th percentile of results was 30 to 235 U/L with olive oil as substrate. Values obtained with triolein as substrate were ~9.9% higher.

![Fig. 3. Histogram of lipase activity values obtained for 116 healthy adults](image)

**Inhibition of Carboxylesterase Activity**

If the procedure is used as a reference method, pancreatic carboxylesterase activity can be inhibited by pre-incubating 200 μL of serum with 40 μL of diisopropylfluorophosphate stock solution for 30 min at room temperature. Use 100 μL of the inhibited sample for the assay and make a mathematical correction for the dilution. If the method is used for clinical purposes, diisopropylfluorophosphate need not be added because pancreatic carboxylesterase is increased in the same clinical conditions as lipase; in addition, there is little (<12%) interference by this enzyme under the proposed reaction conditions. Alternatively, carboxylesterase interference can be eliminated by replacing sodium glycocholate with taurodeoxycholate, 35 mmol/L (33). In this case, however, lipase activity is decreased by about 45%.

Junge (21) reports that “nonspecific pancreatic carboxylesterase” (no EC no.) is also detected by methods involving the use of triglyceride substrates. However, use of carboxylesterase inhibitors (diisopropylfluorophosphate and eserine) does not inhibit this enzyme, suggesting that it may not be a carboxylesterase. With use of other inhibitors, the enzyme behaves similarly or identically to lipase. Diisopropylfluorophosphate, eserine, phenylmethylsulfonyl fluoride, atoxyl, and LaCl\(_3\) do not inhibit either enzyme, whereas p-hydroxymercuribenzoic acid partly inhibits both enzymes. Paraoxon inhibits the nonspecific carboxylesterase totally but causes only partial inhibition of lipase and nonlinear reaction kinetics. Thus, the identity of this enzyme is not clear and it cannot be ruled out that it is a lipase isoenzyme.

**Method Comparisons**

The proposed method was compared with a “SingleVial” method (Boehringer Mannheim Diagnostics), modified as described previously (34). We measured the activity in sera from 151 hospitalized patients with normal, moderately, and greatly increased lipase activities. As reported in our previous communication (34), results by these methods agreed well except in the low portion of the reference interval. The correlation coefficient (r) is 0.997, and the regression equation is y = 0.57x + 52.2 (S_{xy} = 63.9).

We also compared our proposed procedure with the "aca" method (DuPont Co., Wilmington, DE 19898) and an immunochemical assay developed by Beckman Instruments, Inc. (35). Experiments were carried out with the same serum specimens used in the comparison with the modified Boehringer Mannheim Diagnostics method. The correlation coefficient for the pH-Stat vs Beckman method is 0.995 (S_{xy} = 42.9). A graphic representation of the data is given in Figure 4. The correlation coefficient for the pH-Stat vs the "aca" method is r = 0.892 (S_{xy} = 17.4) (see Figure 4). The relatively poor correlation of the "aca" method with other methods is attributed to the lack of collapse in the reaction mixture of the "aca" method. This interpretation agrees with results reported by Junge et al. (19), who found a significantly better performance of the "aca" method when collapse was added to the "aca" reaction mixture.

Colipase used in our experiments was kindly provided by Dr. K. Wulff, Boehringer Mannheim GmbH, Tutzing, F.R.G., and by Dr. Stephan Hesse, Vice-President, Boehringer Mannheim GmbH, Mannheim, F.R.G. Human pancreatic carboxylesterase (EC 3.1.1.1; M, 100 000) was kindly provided by Dr. Dominique Lombard, Marseilles, France, and nonspecific pancreatic carboxylesterase (no EC number; M, 54 000) by Dr. W. Junge, Kiel, F.R.G.
Fig. 4. Comparison of lipase activities from 151 hospitalized patients measured with the pH-Stat method and (top) the immunochromatographic assay developed by Beckman Instruments, Inc. (35) or (bottom) the DuPont aca method.

References