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Measurement of Serum Lipase Activity with the Oxygen Electrode

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We describe the measurement of lipase (triacylglycerol lipase; EC 3.1.1.3) in serum by continuous monitoring of relative rates of oxygen consumption with a polarographic oxygen electrode. The reactions described by Proelss and Wright (*Clin. Chem.* **23:** 522-531, 1977) are used: trilinolein, with lipase catalysis, yields linoleic acid, which reacts with oxygen in the presence of lipoxygenase. Lipase activity is measured by comparing the zero order reaction rate of the specimen with that obtained with linoleic acid standards. Results for lipase (y) correlated well with those by the copper-soap method of Myrtle and Zell (x) (*Clin. Chem.* **21:** 1469-1473, 1975): $y = 0.33x - 11$; $r = 0.98$; $n = 34$. Results are unaffected by specimen dilution, and the standard curve is linear to 520 U/L. Day-to-day precision (CV) is 10.4% for normal activities, 6.1% for above-normal activities. We believe the method offers a precise, practical approach to lipase analysis.

Additional Keyphrases: *pancreatic disease · lipids · polarographic electrode · lipoxygenase · kinetic enzyme assay*

For longer than 40 years, serum lipase has been measured as an aid in diagnosis and monitoring of pancreatic disorders. Because the common view is that lipase is less sensitive than amylase as an indicator of acute pancreatitis and because most laboratories cannot provide emergency lipase analysis, this test has had relatively little use. In clinical studies, the issue of diagnostic sensitivity is clouded by methodological difficulties. Lipase assay may be more useful clinically than is commonly realized. Its use as an emergency test has been largely precluded by technical difficulties and the long incubations involved in most existing methods.

Proelss and Wright (*1*) recently published a rapid method for serum lipase in which a trilinolein emulsion is the substrate in the reactions:

$$\text{Trilinolein} \xrightarrow[\text{pH 8.9}]{\text{lipase}} \text{1,2-dilinolein} + \text{linoleic acid}$$

$$\text{Linoleic acid} + O_2 \xrightarrow{\text{lipoxygenase}} \text{linoleic acid hydroperoxide}$$

After a fixed incubation interval, linoleic acid hydroperoxide was used to oxidize Fe^{2+} to Fe^{3+}, which was reacted with thiocyanate to produce a red complex that was quantitatively monitored.

We demonstrate here the measurement of serum lipase activity by continuous monitoring of relative rates of oxygen consumption with a polarographic oxygen electrode during the lipoxygenase reaction. Lipase activity is measured kinetically during the zero-order phase of the reaction.

Materials and Methods

Apparatus

Reactions were carried out in the reaction chamber of a Glucose Analyzer (Beckman Instruments, Inc., Fullerton, CA 92634) with use of the oxygen electrode supplied with the instrument. Reaction-chamber temperature was 33 °C. We changed the electrode membrane daily. Glucose Analyzer electronics were not suitable for our purposes, so we connected the electrode to a Model 174 Polarographic Analyzer (Princeton Applied Research, Princeton, NJ 08540). The polarograph was operated in the d.c. mode at a fixed applied potential of +0.560 V; full-scale recorder current ranges were 2.0 to 4.0 nA.

We modified the sample-injection head on the Glucose Analyzer to exclude atmospheric oxygen from the reaction chamber during the reaction, because we desired to follow oxygen consumption for several minutes. We added samples through a glass tube (i.d. 3 mm, o.d. 6 mm, length 13 mm), which was inserted through the sample injection head to protrude about 2 mm into the reaction chamber. The V-shaped expansion on the injection head was sealed with a silicone-based cement. We did not plug the small hole in the tube during analysis because results were unaffected by leaving it open.

Reagents

Lipoxygenase stock solution. Dissolve 85 mg of lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.99.2.1, Type I, cat. no. L-7127; Sigma Chemical Co., St. Louis, MO 63178) in 10 mL of working buffer. Discard any residue that may appear on overnight refrigeration, by centrifugation. The reagent is stable for two weeks when stored at 4 °C.

Linoleic acid stock solution, 36 mmol/L. Dissolve the contents of a 1-g ampule (Sigma no. L-1376) in 100 mL of absolute ethanol. Store at 4 °C.

Trilinolein stock solution, 50 mg/L. Dissolve the contents of a 500-mg ampule (Sigma no. T-3252) in 10.0 mL of ethanol/acetone (3/2 by vol). Store the solution at 4 °C.

Working buffer. Dissolve 1.5 g (3.6 mmol) of sodium deoxycholate in 1 L of tris(hydroxymethyl)aminomethane, 50 mmol/L. Adjust the pH to 8.9 at 25 °C with concentrated HCl. Store the solution at 4 °C.

Substrate emulsion. Each day, prepare fresh batches of substrate by adding 0.15 mL of trilinolein stock solution to 12.5 mL of working buffer. Homogenize (10-15 strokes) with a tissue homogenizer (Corning Glass Works, Corning, NY 14830). Prepare and combine several substrate batches if needed, and gently bubble nitrogen through combined batches for 10 min, to remove oxygen and thus minimize substrate deterioration. Seal tightly and equilibrate to temperature in the storage compartment of the Glucose Analyzer until use.

Chamber reaction mixture. Before use, add 50 μL of stock

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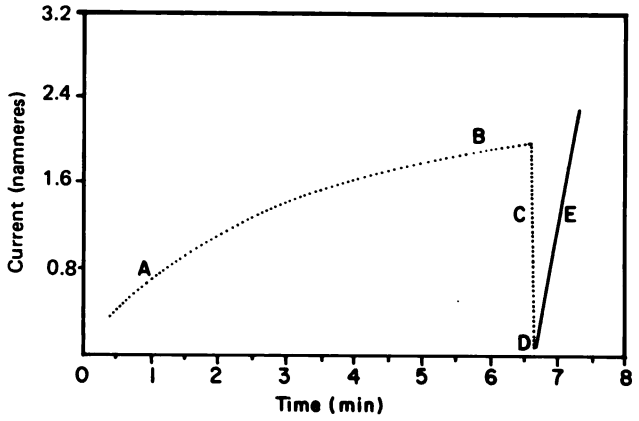

Fig. 1. Electrode response vs time for a typical analysis
A-B: reagent-electrode equilibration period; *C*: recorder reset to baseline; *D*: sample injection; *E*: reaction rate due to sample lipase.

lipoxygenase to each 100 mL of substrate emulsion. Mix gently.

Procedure

Add reaction mixture, equilibrated to temperature, to the reagent bottle used with the Analyzer. Draw portions into the reaction chamber by using the reagent plunger; about 4 mL *completely* fills the reaction chamber.

After filling the reaction chamber, allow the stirred reaction mixture and electrode to equilibrate for about 4 min, until a stable slope (rate) is observed on the recorder (Figure 1, region *B*). This background rate is subtracted from the zero-order rate obtained after serum or standard is injected. Reset the pen to baseline and inject 0.1 mL of standard or sample. A constant slope is soon obtained (Figure 1, region *E*), which represents the zero-order reaction rate. Drain and refill the chamber with a fresh reaction mixture for each specimen analyzed. Calculate lipase activity by comparing the rates of oxygen consumption by linoleic acid standards and unknowns. We prepare a calibration curve daily, using dilutions of stock linoleic acid.

Calculations

1. Compute the corrected relative rate of oxygen consumption, IR, for each standard and unknown by using the slopes from regions *B* and *E* (Figure 1):

$$IR\ (\text{scale units/min}) = (B)(V_B)(C_B) - (E)(V_E)(C_E)$$

where B, E = slopes in regions *B* and *E* (in scale units/min); V_B, V_E = respective full-scale recorder voltages (in V); and C_B, C_E = chart speed divided by 120 (the speed usually used, in cm/h).

2. Plot the calibration curve (Figure 2). For the *x* axis, U/L = (linoleic acid, μmol/L) × (total volume/sample volume).

3. Compute lipase activity from the calibration curve. (Alternatively, only one standard may be used, with a ratiometric calculation.)

Results

Reaction Conditions

The trilinolein substrate emulsion contains some sites that are susceptible to attack by lipoxygenase in the absence of any added lipase. With only substrate in the reaction chamber, lipoxygenase, added in the specified amount, results in an instantaneous upward pen deflection of about 20 scale units. After this sharp rise, a small constant rate of oxygen consumption is observed (Figure 1, region *B*). This background rise ascribable to lipoxygenase activity was also apparent when

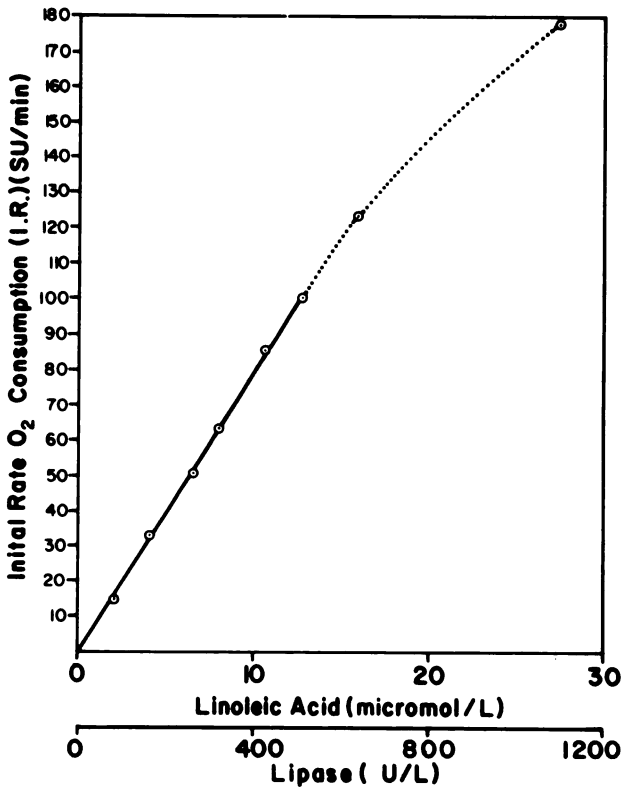

Fig. 2. Typical lipase calibration curve
SU: scale (arbitrary) units

we examined the method of Proelss and Wright (*1*). In their endpoint approach, lipoxygenase was added to the blank; we found this blank contributed about 85% of the total absorbance at 100 U/L. We avoided much of the lipoxygenase blank activity in our kinetic approach by combining lipoxygenase with substrate before we added serum; the blank then contributed about 25% of the rate at 100 U/L. To compensate for possible loss of substrate due to lipoxygenase activity, we used double the substrate and half the lipoxygenase concentrations that Proelss and Wright used.

Calibration

To standardize this system, we add known concentrations of linoleic acid to the reaction mixture and plot observed rates vs concentration (Figure 2). The standard curve is linear to 13 μmol of linoleic acid per liter of reaction mixture, equivalent to 520 U of lipase per liter.

We attempted calibration using only lipoxygenase to react

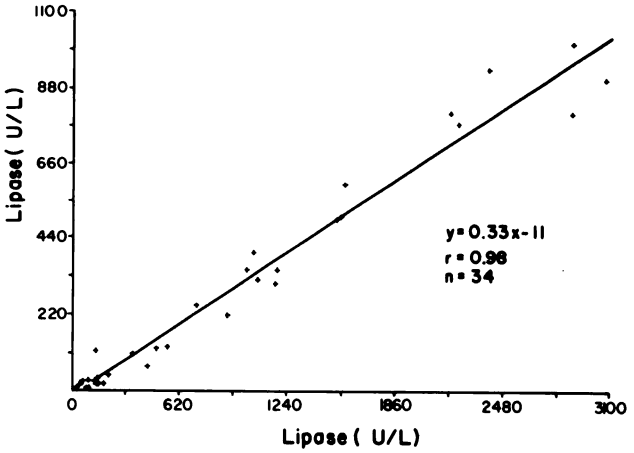

Fig. 3. Comparison of results by the present method (*y*) with those by the copper-soap method (*x*) (*2*)

Table 1. Precision Data

	Lipase, U/L	
	Normal	Above-normal
Within-run		
Mean	44	320
SD	1.9	14.3
Range	41–45	304–335
CV, %	4.4	4.5
n	4	4
Day-to-day		
Mean	39	321
SD	4.1	19.5
Range	33–44	302–348
CV, %	10.4	6.1
n	4	4

with linoleic acid standards, but rates were higher than when the complete reaction mixture was used.

Method Comparison

We analyzed 34 patients' specimens with normal and above-normal serum lipase activities by the present method (y) and by the copper-soap method of Myrtle and Zell (x)(2), run at 33 °C (Figure 3). The regression line is described by the equation $y = 0.33x - 11$; $r = 0.98$ (range: 0–1000 U/L, or 0–3100 copper-soap U/L).

We also analyzed 14 specimens by the present method (y) and by the lipoxygenic method of Proelss and Wright (x)(*1*). The resulting regression equation is $y = 0.41x - 16$; $r = 0.95$ (range: 0–620 U/L).

Precision

Within-run precision (CV) for the present method is approximately 4.5% (Table 1); between-run precision varies from 10.4% in the normal range to 6.1% for above-normal samples. Such precision is routinely attainable if a standard curve is run daily to compensate for variable electrode response.

Effect of Dilution

We assayed 16 above-normal specimens directly by the copper-soap method (x)(2), and by the present method (y) after fivefold dilution with a 20 g/L solution of bovine serum albumin. Linear regression of the results produced the equation $y = 0.33x + 0.5$ ($r = 0.96$), in close agreement with the correlation found for undiluted samples ($y = 0.31x - 11$). These results suggest that the lipoxygenic assay is independent of dilution effects.

Discussion

Problems inherent in serum lipase analysis have been well documented. This method avoids many of the inherent problems of spectrophotometric or titration methods, and potentially is rapid and practical.

We made some modifications to the colorimetric endpoint lipoxygenic method of Proelss and Wright (*1*). The good correlation we obtained with the copper-soap method (2) over a wide range of lipase activity indicates that these changes are acceptable. A major modification was to include the lipoxygenase coupling enzyme in the reaction mixture before adding the serum, resulting in a substantial reduction in the blank signal.

Both the colorimetric and electrochemical procedures confirm that lipoxygenase has some activity against trilinolein in the absence of lipase. Unsaturated fatty acid impurities in the trilinolein could account for this activity. However, part of this oxygen consumption in the electrochemical method may be caused by electrochemical reaction at the electrode, or altered oxygen solubility. Turner et al. (*3*) have demonstrated that solutes can decrease oxygen solubility and that the oxygen electrode may consume oxygen at significant rates when measured rates are low. This may explain our qualitative observation that adding trilinolein stock solution to working buffer produces an immediate loss of oxygen from the solution.

Additionally, although oxygen consumption by the electrode is usually considered negligible, it becomes significant when relatively low amounts of dissolved oxygen are present, and the constant background rate may be ascribable to it.

With our method, several patients' specimens with above-normal lipase activity demonstrated a continuous increase in the rate of O_2 consumption, no steady-state slope being reached. We do not know the reason for this; it may occur with other lipase methods but only be observable in a kinetic method.

Our method could be readily applied in routine clinical chemistry laboratories if appropriate apparatus were available from manufacturers; such equipment is not currently available in a convenient configuration, but could be readily constructed. The lipoxygenic oxygen rate method offers an accurate, precise, practical approach to measurement of serum lipase.

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