Human milk lipases.
I. Serum-stimulated lipase

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Abstract Lipase activity has previously been demonstrated in human milk. This study shows that there are two separate triglyceride lipases in human milk. One is mainly in the skim milk and is stimulated by bile salts; the other is mainly in the cream and is inhibited by bile salts but stimulated by serum. The serum-stimulated lipase was purified by affinity chromatography on heparin-substituted Sepharose 4B. This gave a 9500-fold purification over whole milk. Although polyacrylamide gel electrophoresis showed that the enzyme was not purified to homogeneity, it had the highest specific activity so far reported for a human serum-stimulated lipase. The purified enzyme was free from bile salt-stimulated lipase activity and had the characteristics of other serum-stimulated or so-called lipoprotein lipases. Thus, it was almost completely inhibited by 1 M NaCl. The purified enzyme was active against tributyrylglycerol also in the absence of exogenous serum factors.

Supplementary key words lipoprotein lipase · purification · bile salt-stimulated lipase

Milk from several species contains a serum-stimulated lipase (lipoprotein lipase, a glyceryl ester hydrolase, EC 3.1.1.3) (1, 2). This may be the only or at least the predominant lipase in the milk of some species (2). In 1901, Marfan demonstrated lipolytic activity in human milk (3). Freudenberg later showed that this activity is stimulated by bile salts (4). Since the serum-stimulated lipases are usually inhibited by bile salts (5, 6), this raised the possibility that human milk contains a serum-stimulated lipase with properties different from those of other serum-stimulated lipases. In a preliminary study, however, we found that human milk contains two lipases, one with the classical properties of a serum-stimulated lipase and another lipase that is stimulated by bile salts (7). The previous few studies on the lipolytic activity in human milk have not taken this important fact into consideration. We have therefore decided to reinvestigate the properties of the human milk lipases, and we now report a study of the serum-stimulated lipase.

Serum-stimulated lipases catalyze the hydrolysis of triglycerides in chylomicra and very low density lipoproteins. The liberated fatty acids are thereby made available for uptake from the blood into the tissues (8, 9). The presence of such enzymes in milk is considered to reflect a leakage of enzyme from the mammary tissue (1, 2), and no functional role for these enzymes in the milk has been proposed. We have previously purified a serum-stimulated lipase from bovine milk (10, 11). The crucial step in this purification was affinity chromatography on Sepharose to which heparin was covalently linked. In view of the key role these enzymes have in the metabolism of plasma lipoproteins, it would be highly desirable to purify and characterize serum-stimulated lipases from human tissues also. This has previously been done with enzyme from postheparin plasma (6). In the present work we have obtained a 9500-fold purification of the serum-stimulated lipase from human milk and report some of its properties.

METHODS

Acetone and diethyl ether were of analytical grade, chloroform and methanol were freshly distilled, and all other organic solvents were of commercial grade. Bovine serum albumin and tributyrylglycerol (more than 99% pure; Sigma Chemical Co., St. Louis, Mo.), heparin, protamine chloride (1%), and Intralipid (10%) (AB Vitrum, Stockholm, Sweden), sodium taurocholate (Koch-Light Laboratories, Colnbrook, England), trioleylglycerol (more than 99% pure; Nu-Chek-Prep Inc., Elysian, Minn.), and tri[9,10-^{3}H]oleylglycerol, 500 mCi/mmole (more than 98% pure; The Radiochemical Centre, Amersham, England), were used in the assay mixtures. Dialyzed human serum was prepared by recalcifying outdated citrated plasma from the local blood bank. After the fibrin clot had been removed, the serum was dialyzed against 0.154 M NaCl and then stored frozen until used.

Abbreviations: SDS, sodium dodecyl sulfate.

TABLE 1. Effect of some substances on the activity of human milk lipases

Substance Added[a] or Omitted[b]	Serum-stimulated Lipase		Bile Salt-stimulated Lipase	
	units/ml	*%*	*units/ml*	*%*
Complete system[c]	1.5	100	68	100
Serum[d]	0.10[b]	6.7	7.6[a]	11
Sodium taurocholate[d]	0.01[a]	<1	0[b]	0
Heparin[d]	1.4[b]	93	68[a]	100
Protamine chloride[d]	0.62[a]	41	12[a]	18
Sodium chloride[d]	0.10[a]	6.7	0	0

[a,b] Substance added to (*a*) or omitted from (*b*) the routine assay system for the respective lipase.

[c] The complete assay system for the respective lipase is described under Methods. Enzyme sources: a dissolved acetone–ether powder (prepared as described in the text) for the serum-stimulated lipase and a dissolved acetone–ether powder prepared from the whey fraction of human skim milk for the bile salt-stimulated lipase.

[d] The concentrations of serum and heparin were 0.17 ml and 0.43 IU/ml of assay mixture, respectively. The respective concentrations in the assay mixture of sodium taurocholate, protamine chloride, and sodium chloride were 12 mM, 5×10^{-5} mM, and 1 M. (Values are means of two measurements.)

Assay systems

The serum-stimulated lipase was assayed as described by Egelrud and Olivecrona (11) except that the incubations were performed at pH 8.5 and at 25°C.

Each assay mixture contained 0.5 ml of 1.35 M Tris–HCl buffer, pH 8.5, 0.1 ml of substrate (Intralipid, 10%), 0.1 ml of heparin (20 IU/ml), 1.2 ml of 18.7% bovine serum albumin in 0.154 M NaCl, pH 8.5, and 0.8 ml of dialyzed human serum. Enzyme solution and 0.154 M NaCl were added to give a final volume of 4.7 ml. Incubations were carried out in a water bath with the tubes shaking at 50 strokes/min. After 15 min of preliminary incubation the enzyme was added. Duplicate 1-ml aliquots were withdrawn at zero time and after 60 min. The free fatty acid content was determined by extraction (12) and titration (13). For calibration, a standard amount of palmitic acid was taken through the extraction procedure and titrated.

The activity against tributyrylglycerol was assayed using Radiometer pH-stat equipment (TTA 31 titration assembly, TTT 1c titrator, SBR 2c titrigraph; Radiometer, Copenhagen, Denmark). 1 ml of tributyrylglycerol and 13.5 ml of NaCl solution were first incubated with constant stirring under a stream of nitrogen at 25°C for 10 min. The addition of 0.02 M NaOH to maintain the pH at 8.2 was recorded for a few minutes before the enzyme was added. This value was subtracted when the enzyme activity was calculated.

The bile salt-stimulated lipase was assayed in the following system: 2×10^7 cpm of tri[9,10-^{3}H]oleylglycerol was mixed with 25 mg of unlabeled trioleylglycerol. 1.25 ml of 1 M Tris–HCl, pH 8.0, 1 ml of 10% gum arabic, and 5.5 ml of distilled water were then added, and the mixture was cooled with ice water and sonicated for 4 min at maximal effect in a 60-W disintegrator (MSE Ltd., London, England). To this emulsion 1.25 ml of 1 M NaCl, 1 ml of 10% sodium taurocholate, and 2.5 ml of 18.7% bovine serum albumin, pH 8.0, were added. The assay system contained 150 μl of this mixture and the enzyme source. The total volume was adjusted to 200 μl. The final incubation system was 1.7 mM in trioleylglycerol, 75 mM in NaCl, 12 mM in sodium taurocholate, and 2.8% in bovine serum albumin. Incubations were performed at 37°C in a water bath with the tubes shaking at 50 strokes/min. After a preliminary incubation of 15 min, the enzyme was added. The reaction was stopped after 15 min by adding 3.25 ml of methanol–chloroform–heptane 1.41:1.25:1 (v/v/v) and immediately thereafter 1 ml of a 0.05 M potassium carbonate buffer, pH 10.5 (14). The tubes were vigorously shaken and were then centrifuged at 1000 rpm for 30 min in a Sorvall GLC-1 centrifuge (HL-4 rotor). Under the conditions used, model experiments showed that 80% of the fatty acid soaps but only 2% of the monoglycerides were extracted into the upper phase. These values are higher than those reported for the original method (14). A possible explanation is the rather high concentration of bile salt in our assay. No corrections were made for the small contribution of monoglycerides to the radioactivity in the upper phase. 1 ml of the upper phase was removed to a counting vial containing 5 ml Aquasol, and the radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer, model 3020.

One lipase unit is defined as that amount of enzyme that releases 1 μeq of fatty acid/min. Protein was determined by the method of Lowry et al. (15). No corrections to obtain true protein concentrations were made. The heparin-substituted Sepharose gel (Sepharose 4B; Pharmacia Fine Chemicals, Uppsala, Sweden) was a generous gift from Dr. P.-H. Iverius and was prepared as described previously (16).

SDS gel electrophoresis

Molecular weight was determined by SDS gel electrophoresis according to the standard procedure of Weber, Pringle, and Osborn (17). The calibration proteins used were bovine serum albumin (mol wt 68,000), bovine chymotrypsinogen A (mol wt 25,700), and horse cytochrome *c* (mol wt 11,700). A straight line was obtained when the logarithms of the molecular weights were plotted against the R_F values. Staining and destaining were performed as previously described (11). Gels stained by periodic acid–Schiff reagent were first washed in 40% ethanol and 7% acetic acid, with a small amount of anion exchanger in the bottoms of the tubes, for 24 hr in order to elute the SDS. Staining and destaining were then performed according to Glossmann and Neville (18).

TABLE 2. Initial purification of serum-stimulated lipase

Step	Volume	Total SSL Activity[a]	Total BSSL Activity[b]	Yield of SSL Activity	Yield of BSSL Activity	Total Protein	Specific Activity of SSL	Purification of SSL
	ml	*units*	*units*	%	%	*mg*	*units/mg protein*	
Milk	2,040	477	83,436	100	100	32,640	0.015	1
Skim milk[c]	1,900	39.6	77,760	8.3	93	23,040	0.002	
Wash water[c]	1,900	37.8	2,707	7.9	3.2	1,094	0.035	
Dissolved acetone–ether powder	133	258	1,982	54	2.4	505	0.51	35-fold

[a] SSL, serum-stimulated lipase.
[b] BSSL, bile salt-stimulated lipase.
[c] Discarded.

RESULTS

Preliminary experiments suggested that the two lipases in human milk differed in several respects (7). These results are confirmed and extended in the present study (**Table 1**). The data presented refer to one representative enzyme preparation for each enzyme. The preliminary observations indicated that the bile salt-stimulated lipase activity was mainly in the skim milk, whereas the serum-stimulated lipase activity was in the cream. When casein was precipitated from the skim milk by exposure to pH 4.6 at 40°C for 30 min, more than 90% of the bile salt-stimulated lipase activity remained in the whey, but no remaining serum-stimulated lipase activity could be demonstrated. Presumably, the serum-stimulated lipase had been inactivated at the low pH. The whey proteins were precipitated with acetone, and the precipitate was extracted with diethyl ether. This gave a convenient, stable preparation that could be used to explore the properties of the bile salt-stimulated lipase (Table 1). When this enzyme preparation was added to the assay system for serum-stimulated lipase, no activity was observed whether serum was present or not. Similarly, no activity was recorded when this enzyme preparation was added to the assay system for bile salt-stimulated lipase if bile salt was omitted (Table 1). Thus, this enzyme seems to have a strong dependency on bile salt for activity against long-chain triglyceride.

The source of serum-stimulated lipase used in the experiments presented in Table 1 was an acetone–ether powder from the cream fraction, prepared as described below. Such a preparation contains both enzyme activities (**Table 2**) but is enriched severalfold in serum-stimulated lipase. Since the bile salt-stimulated lipase showed no activity in the assay for serum-stimulated lipase, the results obtained in Table 1 with this assay system should be representative for the properties of the serum-stimulated lipase. Some additional findings support this conclusion. The bile salt-stimulated lipase showed little or no activity when Intralipid was the lipid substrate in either of the two assay systems used, even when bile salt was added. Furthermore, this enzyme was inhibited by serum (Table 1).

Serum stimulated the serum-stimulated lipase but inhibited the bile salt-stimulated lipase, whereas the reverse was true of bile salts. Protamine and 1 M NaCl were inhibitory to both enzyme activities (Table 1). The effect of heparin (0.43 IU/ml) on the serum-stimulated lipase activity was variable. With the preparation shown in Table 1, it increased activity slightly. In some milks and acetone–ether powders, heparin did not increase the enzyme activity; in others, the effect was more pronounced than with the preparation shown. The effect of heparin was most pronounced when the enzyme was assayed under otherwise suboptimal conditions, e.g., in the presence of high ionic strength (see below). Heparin had no effect on the activity of the bile salt-stimulated lipase either in the routine assay (Table 1) or when the NaCl concentration was raised to 0.3 M, a concentration that reduced the activity to 75% of the optimum.

When a milk sample is tested for lipase activity in the two assay systems, the activity recorded for bile salt-stimulated lipase is much higher than that observed for the serum-stimulated lipase (usually by a factor of 50 or more; see below). In the assay system for bile salt-stimulated lipase, there is no serum, which is required for optimal activity of the serum-stimulated lipase, and, moreover, bile salt is present in a concentration that markedly decreases the activity of the serum-stimulated lipase (Table 1). The much lower activity of serum-stimulated lipase than of bile salt-stimulated lipase in milk, together with the unfavorable assay conditions, makes the contribution of serum-stimulated lipase to the activity recorded in the bile salt-stimulated lipase assay very minor (no more than a fraction of 1%). This conclusion was supported in experiments in which purified serum-stimulated lipase was assayed in this system and found to have no measurable activity. Thus, this assay adequately measures the activity of bile salt-stimulated lipase without significant contribution of the serum-stimulated lipase, and the serum-stimulated lipase assay measures the activity of

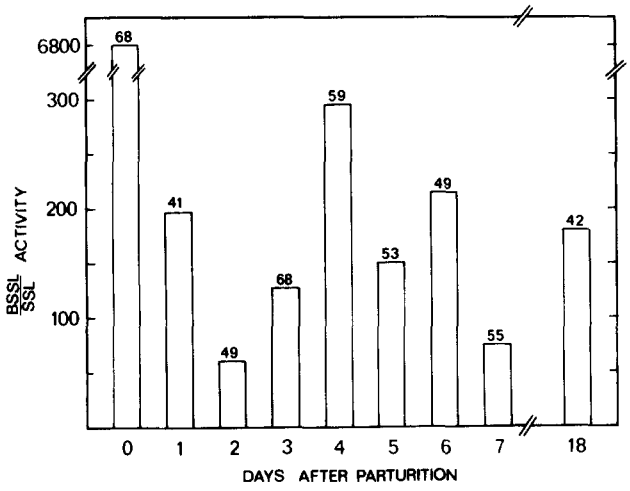

Fig. 1. Ratio of bile salt-stimulated lipase (BSSL) to serum-stimulated lipase (SSL) activity in milk samples from one donor during the first week after parturition. Milk was collected with a breast pump at the end of the meal at approximately 10 a.m. The samples were immediately placed at 4°C and analyzed within 2 hr or were immediately frozen at −20°C and assayed at the end of the period. Milk could be frozen at −20°C without any loss of the two enzyme activities. The values at the tops of the bars are the bile salt-stimulated lipase activities (units/ml of milk).

serum-stimulated lipase without contribution of bile salt-stimulated lipase.

If the two activities belonged to the same enzyme, one would expect to find a constant ratio between them from day to day. However, when determined in milk from one donor, the ratio varied widely (**Fig. 1**). We found a high activity of the bile salt-stimulated lipase even in colostrum (68 units/ml of milk on the day of parturition), confirming the previous results of Freudenberg (4). In contrast, we have always found very low activities of the serum-stimulated lipase in colostrum. Bile salt-stimulated lipase activity has been high in all milk samples that we have studied. In 37 different milk samples collected from 10 different mothers, the lowest value recorded was 33 units/ml of milk. The level of serum-stimulated lipase activity has always been much lower and also seems to be much more variable, both from day to day in milk from the same donor (Fig. 1) and between donors. These facts, as well as the observation (Table 2) that the bile salt-stimulated lipase is mainly found in the skim milk whereas the serum-stimulated lipase is mainly in the cream, strongly suggest that these two activities belong to two different enzymes.

Initial purification of serum-stimulated lipase

Milk from one mother was collected every fourth hour and immediately placed at 4°C. At the end of a 24-hr period the samples were pooled and frozen at −20°C. They were kept frozen until a few days later, when milk samples from two consecutive 24-hr periods were pooled and used.

2040 ml of milk was centrifuged at 20,000 *g* for 30 min at 4°C. The skim milk (1900 ml) was decanted, the pellet was removed, and the cream was dispersed in distilled water (1900 ml) and centrifuged again at 20,000 *g* for 15 min. To the washed cream, 3.5 l of acetone (25 vol) was added slowly while stirring at room temperature. 1 hr after the last addition of acetone, the whole mixture was filtered through a Büchner funnel, and the collected precipitate was washed with 1.4 l (10 vol) of diethyl ether. The precipitate (hereafter called the acetone–ether powder) was dried overnight in vacuo at 4°C and stored at −20°C until used a few days later. Such a preparation can be stored for months without loss of activity.

The milk contained 175 times more bile salt-stimulated lipase activity than serum-stimulated lipase activity as assayed under our routine conditions (Table 2). The skim milk (discarded) contained only 8.3% of the serum-stimulated lipase activity but 93% of the bile salt-stimulated lipase activity (Table 2). The cream was washed with distilled water to lower the ionic strength because this increased the recovery of enzyme activity in the following step. If the cream was washed in 0.154 M NaCl in 5 mM Veronal–HCl buffer, pH 7.4, and then dissolved in a small volume of the same buffer, the enzyme was completely destroyed during the precipitation with acetone. The delipidation of the washed cream with acetone and with ether was done at room temperature to increase the solubility of the lipids. The acetone–ether powder contained 54% of the initial serum-stimulated lipase activity with a 35-fold purification. Although it contained only 2.4% of the bile salt-stimulated lipase activity, this activity was still higher than that of the serum-stimulated lipase (Table 2). The enzyme was brought into solution from the acetone–ether powder with the aid of sodium deoxycholate (see below). In the absence of this detergent there was a lower recovery of enzyme activity. Moreover, the presence of the detergent was necessary for the enzyme to bind to the heparin–Sepharose, and it reduced the nonspecific binding of protein to the gel. If the acetone–ether powder was first dissolved with the aid of sodium deoxycholate and was then again precipitated with acetone and washed with diethyl ether, the preparation obtained was readily dissolved without detergent in 0.154 M NaCl in 5 mM Veronal–HCl, pH 7.4. Such a preparation binds to the heparin–Sepharose also in the absence of sodium deoxycholate. A second acetone precipitation, however, gave poorer recovery of enzyme activity with no higher specific activity.

Heparin–Sepharose chromatography

2.80 g of acetone–ether powder was gently stirred in 140 ml of 0.05 M NH_4OH–NH_4Cl, containing 0.5% sodium deoxycholate and 0.5 mM potassium oleate, pH 8.5, for 7 hr at 4°C. The dispersion was centrifuged at 35,000 *g* for 30 min. This gave 133 ml of a clear supernate con-

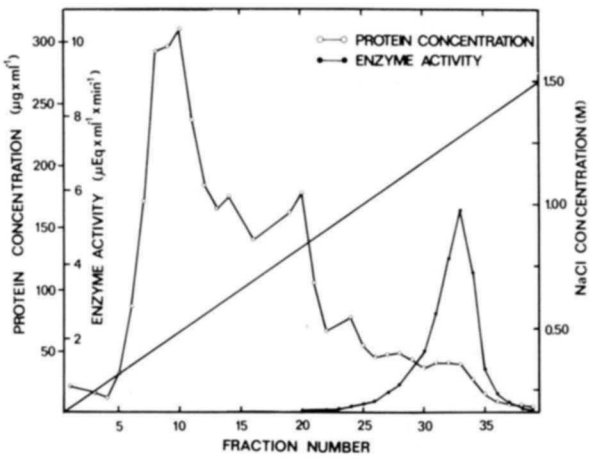

Fig. 2. Chromatography profile on heparin–Sepharose of dissolved acetone–ether powder from washed cream. The flow rate through the column was 20.6 ml/hr. The volume of the fractions was 5.1 ml.

taining 258 serum-stimulated lipase units and 505 mg of protein. 125 ml was applied to a column (25 × 1 cm) of heparin–Sepharose equilibrated with the same buffer. The column was then washed with 60 ml of 0.154 M NaCl in 5 mM Veronal–HCl, pH 7.4. After this wash, 28% of the applied serum-stimulated lipase activity and 85% of the applied bile salt-stimulated lipase activity had been eluted from the column. When this first eluate was again applied to the column, 30% of the serum-stimulated lipase activity did not bind to the column, while 40% of the activity was eluted with the Veronal–HCl buffer containing 1.50 M NaCl. The column was eluted with 205 ml of a linear salt gradient (0.154–1.50 M NaCl) in 5 mM Veronal–HCl, pH 7.4 (**Fig. 2**). Fractions 26–36 contained 46% of the applied enzyme activity. The total recovery from the chromatography was 80%. The specific activities of fractions 31–35 were 66, 103, 140, 139, and 77, respectively. No bile salt-stimulated lipase activity was found in these fractions. In fractions 33 and 34 the enzyme was thus purified about 9500-fold over whole milk. In a previous similar experiment the same degree of purification was obtained.

Purity of the enzyme

SDS–polyacrylamide gel electrophoresis of fractions 31–36 showed that the enzyme was not purified to homogeneity. These fractions all contained two major and some minor components (**Fig. 3**). The major components had apparent molecular weights of about 88,000 and 63,000, respectively. If the specific activity of the serum-stimulated lipase in human milk is not much higher than that in bovine milk (highest specific activity observed: 467 units/mg of protein at 37°C [11]), one of these major bands should correspond to the enzyme protein. The intensity of stain for the component with the lower molecular weight seemed to correspond in its distribution in the fractions to the distribution of the enzyme activity. The bovine milk

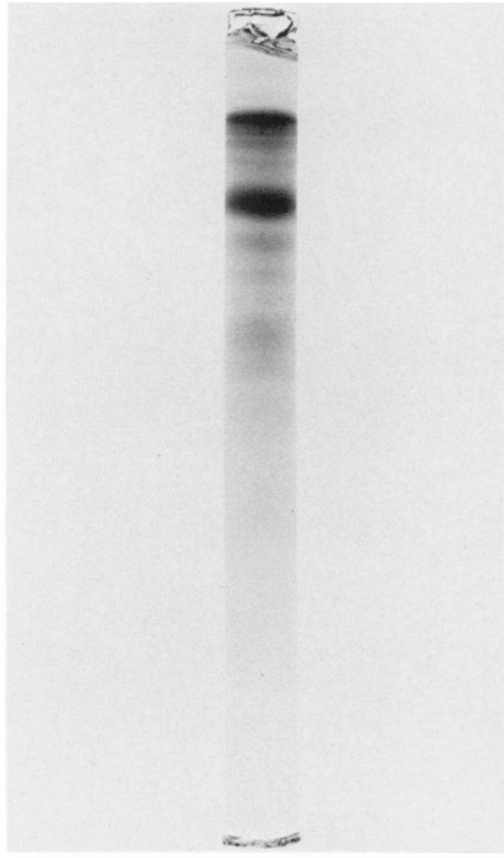

Fig. 3. SDS–polyacrylamide gel electrophoresis of purified human milk serum-stimulated lipase after treatment with 2-mercaptoethanol. 1.5 ml of fraction 34 (40 μg of protein) from the heparin–Sepharose chromatography was dialyzed against 0.05 M NH_4OH–NH_4Cl, 0.1% SDS, pH 8.5, and then against the same buffer diluted 10-fold and finally lyophilized. The treatment with 2-mercaptoethanol and the electrophoresis were performed as described under Methods. The gels were 75 × 7 mm and the current 8 mA/tube. Migration was towards the anodic end (the lower part of the photograph).

serum-stimulated lipase could be stained by the periodic acid–Schiff method and thus contained carbohydrate (11). It was recently shown that a serum-stimulated lipase purified from pig adipose tissue binds to a concanavalin A–Sepharose gel, suggesting that this enzyme also contains carbohydrate (19). The human milk serum-stimulated lipase also binds to concanavalin A–Sepharose and thus probably is a carbohydrate-containing protein. Of the components shown on the gel (Fig. 3), only the one with an apparent molecular weight of 63,000 (lower major band) was stained with the periodic acid–Schiff method. The minimum molecular weight of the bovine milk lipase was estimated to be 62,000–66,000.

Effect of serum on the enzyme activity

Serum-stimulated lipases have a low activity against emulsions of long-chain triglycerides, but the activity is stimulated severalfold by addition of serum or certain serum factors (6, 9, 20). The serum-stimulated lipase from

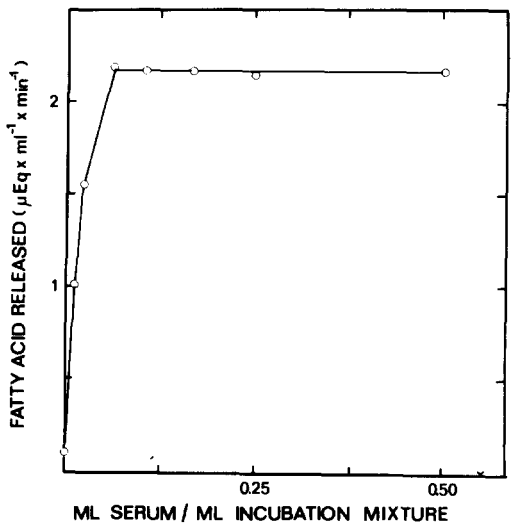

Fig. 4. Effect of serum on the activity of serum-stimulated lipase. 50 μl of enzyme (fraction 31) was incubated with different amounts of serum or 5% bovine serum albumin in 0.154 M NaCl. The volume of serum and/or 5% albumin was 2.4 ml in a total assay volume of 4.7 ml.

human milk had a significant activity against Intralipid even in the absence of serum, but the activity was stimulated by serum at all stages of the purification procedure. The activities in the absence of serum were for milk, 15%, and for the dissolved acetone–ether powder, 6.5% of the respective optimal serum-stimulated activities. For the purified serum-stimulated lipase, the maximal activity was obtained with 0.06 ml of serum/ml of assay mixture (**Fig. 4**). This corresponded to a 20-fold stimulation by serum.

The purified bovine milk lipase is active against a variety of lipid substrates even in the absence of added serum factors (21). **Fig. 5** shows that the purified human milk enzyme is capable of splitting tributyrylglycerol in the absence of added serum. Under the conditions of this particular experiment (see legend to the figure), the activity was 53% of the optimal serum-stimulated activity against Intralipid.

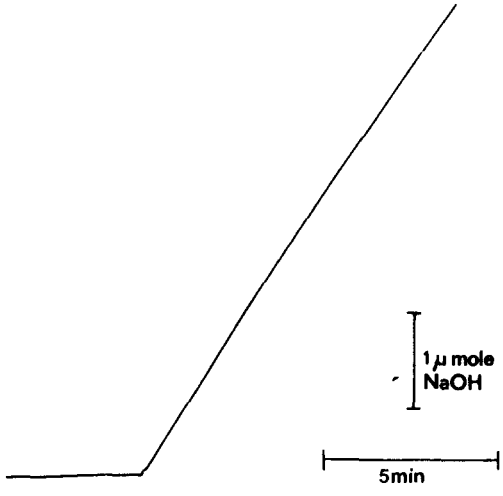

Fig. 5. pH-stat recording of the hydrolysis of tributyrylglycerol in the absence of serum factors. 0.5 ml of fraction 31 was incubated in a system that contained 1 ml of tributyrylglycerol in a total volume of 15 ml. The final concentration of NaCl was 0.1 M and the pH was kept at 8.2 by titration with 0.02 M NaOH.

Stability of the serum-stimulated lipase

Serum-stimulated lipases are known to be rather labile. This was also true for the purified human milk enzyme. If the enzyme as it eluted from the heparin–Sepharose was immediately frozen at −20°C, more than 80% the initial activity remained after a few days. When stored at 4°C, 50% of the activity was lost in 1 hr.The inactivation was even more rapid at 25°C and 37°C. The enzyme reaction was, however, linear for at least 1 hr both at 25°C and at 37°C (**Fig. 6**). This was probably due to stabilizing effects of components in the assay system. A linear relationship was also obtained between the amount of enzyme added and the liberation of fatty acids in the interval used (**Fig. 7**).

The serum-stimulated lipase from human milk was very sensitive to high ionic strength in the assay system. The activity of the purified enzyme was reduced to less than 3% when incubated in the presence of 1 M NaCl. This is a property shared with other serum-stimulated lipases (11, 22, 23). When the ionic strength of the assay system was increased, there was a corresponding decrease in the enzyme activity (**Fig. 8**). At moderate ionic strengths (0.1–0.4 M NaCl), the decrease in the enzyme activity was less when heparin was added to the incubation system (Fig. 8). This effect of heparin was statistically significant ($0.01 < P < 0.05$) when tested with a sign test. At high ionic strengths, heparin had no effect on the activity. At low ionic strengths, heparin itself (in the concentration used) decreased the enzyme activity (Fig. 8). These effects of heparin are in agreement with those reported for the bovine milk serum-stimulated lipase (24).

DISCUSSION

Serum-stimulated lipases (lipoprotein lipases) are supposed to have a key role in the utilization of lipoprotein triglyceride fatty acids by several tissues (8, 9). In the guinea pig (25, 26), the rat (27), and the cow (28), the serum-stimulated lipase activity in the mammary gland is greatly increased during lactation. This correlates well with the high demand for fatty acids for synthesis of milk triglycerides. In some species, the enzyme is also found in the milk (1, 2). However, milk fat is not a good substrate for these lipases, and it appears unlikely that they have a physiological function in milk (1, 7, 25). It has been suggested that the appearance of the enzyme in milk is a re-

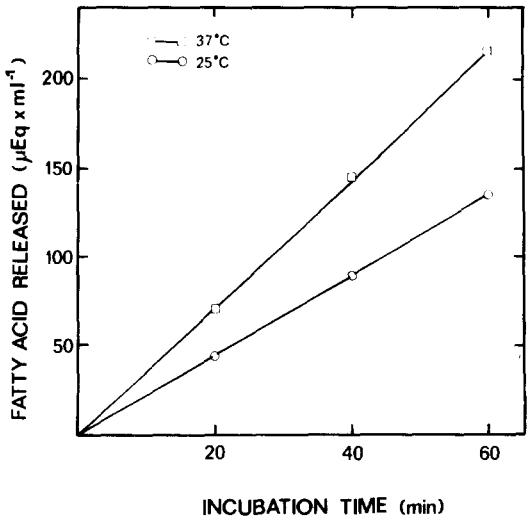

Fig. 6. Time course of the liberation of fatty acids at 25°C and at 37°C by purified enzyme in the routine assay system (100 μl of fraction 31 in a total assay volume of 9.4 ml).

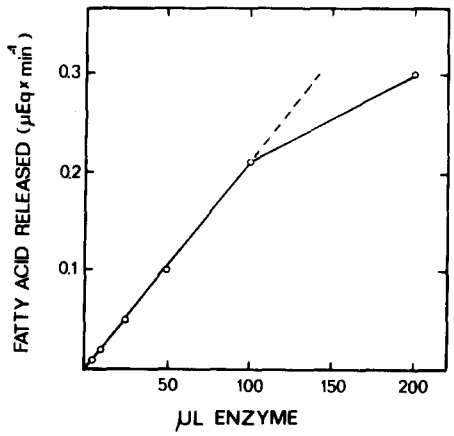

Fig. 7. Determination of the linear range of the routine assay procedure. 5–200 μl of fraction 31 was incubated as described under Methods, with the exception that corrections were made so that after addition of enzyme there was the same concentration of NaCl in all incubation mixtures.

sult of cell damage during the secretion of milk from the mammary gland (1, 2). Most, if not all, of the lipolytic activity in guinea pig milk is probably due to serum-stimulated lipase (2). This may also be true for bovine milk (21). The present study demonstrates that, in contrast to milk from these species, human milk contains at least two triglyceride lipases. A serum-stimulated lipase is present in amounts that vary considerably between individual milk samples, but the activity is sometimes almost comparable to that found in bovine milk. In addition, human milk contains a lipase that is stimulated by bile salts and, under optimal conditions, has a much higher activity than that of the serum-stimulated lipase.

In human milk, almost all of the serum-stimulated lipase activity is in the cream fraction (Table 2), whereas in bovine milk only about one-third of the lipase activity is in the cream and the rest is bound to the casein micelles in the skim milk (1). Therefore, the purification of the human enzyme required quite different initial steps compared with the bovine milk enzyme, which was purified from skim milk (11). However, in both cases the major purification was obtained by affinity chromatography on heparin–Sepharose. A triglyceride lipase was recently purified from human postheparin plasma by affinity chromatography on heparin–Sepharose (29). This enzyme was eluted from the column at a lower ionic strength than the bovine and the human milk lipases, was not inhibited by 1 M NaCl, and was not stimulated by serum (29). Thus, it did not have the classical properties of a serum-stimulated lipase. However, a serum-stimulated lipase with such properties has been purified from both rat (30) and human (6) postheparin plasma. These purifications were obtained by a quite different procedure. The serum-stimulated lipases purified from human postheparin plasma and from human milk have several characteristics in common, but the data are insufficient to permit a detailed comparison. Although the human milk lipase was not purified to homogeneity, it had a specific activity six times higher than the postheparin plasma enzyme when calculated at 37°C. It is not known to what extent the purified enzyme preparations may have contained inactive enzyme protein together with active.

With the technique described in the present paper, the serum-stimulated lipase can be purified free from activity of the bile salt-stimulated lipase. Moreover, the serum-stimulated lipase in human milk may well be a homoge-

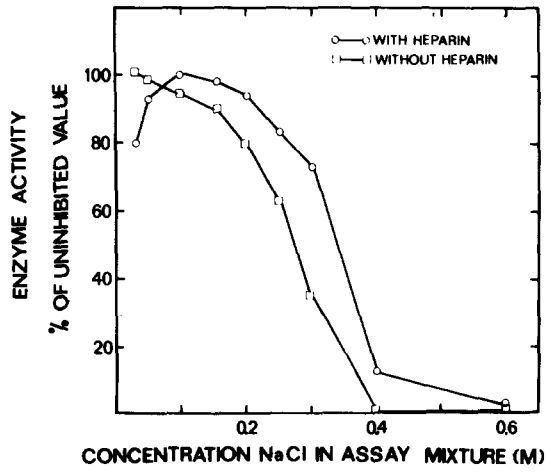

Fig. 8. Effect of heparin on the activity of serum-stimulated lipase at different concentrations of NaCl in the assay mixture. Heparin was added in a concentration of 0.43 IU/ml of assay mixture. The value obtained when the enzyme (25 μl of fraction 33) was incubated in 0.1 M NaCl with the addition of heparin was considered as uninhibited value or 100% activity. The concentration of NaCl in the enzyme source was assumed to be 1.25 M. The data are means of two values. Similar curves were obtained in experiments with two other purified enzyme preparations.

neous enzyme originating from one tissue, i.e., the mammary gland. It has the properties of most other serum-stimulated lipases, i.e., it is inhibited by 1 M NaCl and by protamine, it is active against chylomicra,[1] and its activity against Intralipid as substrate is stimulated by serum or certain polypeptides[1] from the serum lipoproteins. The activity of the enzyme in milk is variable, but in many cases milk is a richer source of the enzyme than human postheparin plasma. In a few milks, we have found activities up to half of those usually found in pooled bovine milk, one of the richest sources of the enzyme known so far. We believe that milk is a good starting material for the preparation of a purified human serum-stimulated lipase for further studies.

[1] Hernell, O., and T. Olivecrona. Unpublished observations.

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