Post-heparin serum lecithinase in man and its positional specificity

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ABSTRACT Lecithinase activity in post-heparin serum has been demonstrated. Phosphatidyl choline (PC) can be degraded to lysophosphatidyl choline and fatty acids at a rate of more than 1 μmole/hr per ml of serum in an incubation system containing PC, 0.1 M glycine–NaOH buffer (pH 9.6), and deoxycholate. This activity cannot be found in serum obtained prior to the injection of heparin.

Post-heparin serum lecithinase can be distinguished from the heat-stable pancreatic lecithinase by the markedly different effects of heat, paraoxon, and EDTA, and from serum lecithin:cholesterol acyltransferase by the differential effect of *p*-hydroxymercuribenzoate. In contrast to the acyltransferase and to pancreatic lecithinase, which are active at the β (C-2) position of lecithin, post-heparin serum lecithinase is active at α′ (C-1) position.

KEY WORDS post-heparin serum · man · α′-lecithinase · phosphatidyl choline · egg lecithin · cabbage lecithin · lecithin fatty acids · phospholipase

POST-HEPARIN PLASMA and serum have previously been shown to contain a phospholipase which degrades phosphatidyl ethanolamine (PE) to lysophosphatidyl ethanolamine (LyPE) and fatty acids (FA) (1). In contrast to the site of action of the phospholipase of venom or of heat-treated human pancreatic extract, post-heparin plasma phospholipase activity attacks the FA at the α′ (C-1) position of egg PE (2).

Results in the present study indicate the presence in post-heparin serum of lecithinase activity which degrades phosphatidyl choline (PC) to lysophosphatidyl choline (LyPC) and FA. This enzymatic activity has been measured. Properties which distinguish it from other human enzymes involved in lecithin catabolism are described, and the site of action of the enzyme on its substrate is delineated.

MATERIALS

Pure egg PC was obtained by chromatographic separation of crude egg yolk phospholipid (3) on columns of alumina (Merck) and of 100–200 mesh silicic acid (Mallinckrodt) as described by Rhodes and Lea (4). A major portion of total cabbage PC was obtained by chromatographic separation of a chloroform–methanol (C–M) extract of cabbage on a column of silicic acid (5). "L-α-Dipalmitoyl phosphatidyl choline" (General Biochemicals, Div., Chagrin Falls, Ohio) was purified by silicic acid column chromatography. The separation and purity of these lecithins in column eluates were determined by TLC (6) and by chromatography on silicic acid-impregnated paper (7). The three lecithins had the same R_f by these techniques and were free from other lipids. These fractions were stored in C–M 1:1 at 4°C. The amount of PC used in experimental procedures was calculated from the phosphorus content (7) of these solutions.

Other chemicals used include: sodium deoxycholate (No. S-285, Fisher Scientific Co., Pittsburgh, Pa.); lyophilized *Crotalus adamanteus* venom (Ross Allen's Reptile Institute, Inc., Silver Springs, Fla.); diethyl *p*-nitrophenyl phosphate (paraoxon) (kindly supplied by Dr. Schrader, Farbenfabriken Bayer, Wuppertal-Elberfeld, West Germany); boron trifluoride–methanol (140 g of BF_3 per liter of methanol), and myristic, oleic, and linolenic acids (Applied Science Laboratories Inc., State College, Pa.); *p*-hydroxymercuribenzoate (Sigma Chem-

Abbreviations: C–M, chloroform–methanol; DFP, diisopropyl fluorophosphate; FA, fatty acid(s); GLC, gas–liquid chromatography; LyPC, lysophosphatidyl choline; LyPE, lysophosphatidyl ethanolamine; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; TLC, thin-layer chromatography.

ical Co., St. Louis, Mo.); bovine albumin, fraction V (Armour Pharmaceutical Co., Kankakee, Ill.); and protamine sulfate and diisopropyl fluorophosphate (DFP) (K & K Laboratories, Inc., Jamaica, N.Y.).

METHODS

Preparation of Post-Heparin Serum

Post-heparin sera obtained from two normal male subjects, A and B (sera A and B), were used in this study. Blood, 450 ml, was collected in a glass container without added anticoagulant 10 min after rapid intravenous injection of 5000 units of heparin (Invenex Pharmaceuticals, San Francisco, Calif.). The anticoagulant effect of the injected heparin was sufficient to prevent clotting of the plasma of subject B during centrifugation at room temperature, and aliquots were stored at −10°C. However, when each aliquot was thawed, a fibrin clot formed, which was removed before the serum was used. A small amount of fibrin formed during the centrifugation of the blood obtained from subject A, and appreciable hemolysis of red cells occurred. Clotting was complete, and the serum was stored at −10°C, within 4 hr of the time of blood collection. The phospholipase activity (PE substrate) in sera A and B degraded PE in the incubation system previously described (1) at the rate of 30–35 μmoles/hr per ml of serum. Enzyme activity appeared to be stable at −10°C for several months or at 4°C for 1 wk.

Standard Incubation System for Measurement of Post-Heparin Lecithinase

Solvents were removed from appropriate aliquots of lecithin solutions at 55°C under reduced pressure. The PC was then immediately emulsified in a buffered deoxycholate solution with the use of a mechanical tube vibrator for 5–10 min. Each substrate was emulsified for 2 min when the effect of pH or concentration of deoxycholate on enzyme activity was studied. Longer intervals of emulsification were not necessary in the optimal incubation system.

The maximal activity of post-heparin lecithinase was obtained by incubation at 38°C of 2.0 ml of post-heparin serum with 90 μmoles of PC emulsified in a 7.0 ml solution of 0.1 M glycine–NaOH buffer (pH 9.6) containing 80 μmoles of deoxycholate. In studies of factors affecting enzyme activity, the compounds, dissolved in 2.0 ml of glycine buffer pH 9.6, were added to the serum, and the PC and deoxycholate were added in a volume of 5.0 ml.

Assay of Post-Heparin Lecithinase Activity and Its Positional Specificity

The amounts of PC, LyPC, and FA in the incubation system were determined immediately after the addition of serum and at intervals thereafter. Lipid extracts of the incubation medium were prepared by the addition of 1.0 ml to 16.7 ml of C–M 1:1 at room temperature followed by dilution to 25.0 ml with chloroform. PC and LyPC were determined by a chromatographic technique (7), essentially that of Marinetti (8). An aliquot (5–8 ml) of the extract was centrifuged briefly in a capped tube and 300–600 μl of the supernatant solution was applied below the silicic acid of specially prepared silicic acid-impregnated papers (7). 1 ml of this extract could be applied to the available 12 cm^2 area in 2 min. FA were measured by the method of Dole (9), except that the concentration of H_2SO_4 in the extraction medium was doubled to compensate for the alkalinity of the incubation medium.

At intervals, 15-μl aliquots of the incubation medium were applied directly to thin-layer chromatoplates for separation of the LyPC formed and subsequent determination of its FA composition. Results obtained by direct application of the incubation medium were identical with results obtained by preliminary lipid extraction and subsequent chromatography.

Hydrolysis of Lecithin by Venom Phospholipase

For comparative studies, two methods were used for the hydrolysis of lecithin with venom phospholipase. In the method of Saito and Hanahan (10), PC was hydrolyzed in diethyl ether. The dried venom was dissolved in an aqueous solution (containing 220 mM NaCl, 20 mM $CaCl_2$, and 1 mM EDTA, adjusted to pH 7.4 with 0.1 N KOH). Of this solution, 20 μl (30 μg of venom) was added to 1.0–1.4 μmoles of PC in 2.0 ml of ether. After 16 hr of incubation at 22°C, the ether was removed under reduced pressure. The residual lipids were dissolved in 250 μl of C–M 1:1 and the solution was applied to a chromatoplate for separation of LyPC and FA prior to methanolysis.

Venom dissolved in 2.0 ml of 0.1 M glycine buffer pH 9.6 was used for comparison with serum in the aqueous assay system. Since hydrolysis of PC was extremely rapid with 1 mg of venom in the assay, initial (zero time) measures of FA and phospholipid were obtained by addition of enzyme to the Dole or C–M extraction mixture prior to addition of substrate. For determination of the composition of FA released from PC by venom, 15 ml of the 25 ml C–M extract was evaporated under reduced pressure at 38°C, and the concentrate was applied to chromatoplates.

TLC Separation of Lipids

Silicic acid (Mallinckrodt) chromatoplates were prepared as previously described (6). Material in the silicic acid which interfered with measurement of FA methyl esters was effectively moved to the top of the plates with two successive solvent developments: 1 hr with C–M–H_2O

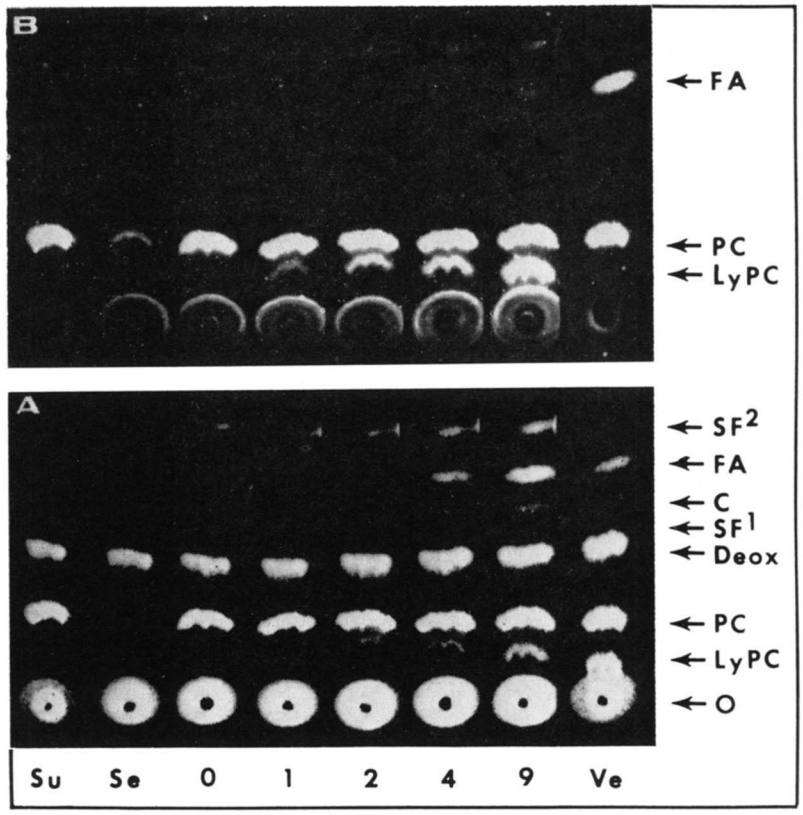

FIG. 1. Thin-layer chromatogram in which the lecithinase activity of post-heparin serum is compared with that of snake venom.

The incubation system of 9.0 ml contained 45 μmoles of PC and 50 μmoles of deoxycholate. Aliquots (10 μl) were applied directly to the plate initially and after intervals of 1, 2, 4, and 9 hr at points marked 0,1, 2, 4, and 9 respectively. 10 μl of the system with buffer instead of serum was applied at Su and 10 μl of the system without added PC at Se. Venom, 3 μg in buffer, was substituted for post-heparin serum and after 2 hr of incubation 10 μl was applied directly at Ve.

The chromatoplate (Mallinckrodt silicic acid) was developed for 5 min with C–M–H_2O 75:25:2 and then with petroleum ether–ethyl ether–acetic acid 80:20:1 for 7 min. The chromatoplate was photographed (part A) under ultraviolet light after it had been sprayed with 0.2% 2′,7′-dichlorofluorescein in ethanol. This plate, rephotographed 17 hr after a 10 min exposure to I_2 vapor, is shown again as part B of the figure.

Abbreviations: *O*, origin; *LyPC*, lysophosphatidyl choline; *PC*, phosphatidyl choline; *Deox*, deoxycholate; SF^1, first solvent front; SF^2, second solvent front including esterified cholesterol; *C*, area for cholesterol and diglycerides; *FA*, fatty acids. Triglycerides, when detectable, appear just above FA.

60:40:3, and 1 hr with petroleum ether–ethyl ether–acetic acid 50:50:1. The plates were not heat-activated.

One-dimensional separation of PC, LyPC, and FA was accomplished with the successive use of two solvent systems: system A was C–M–H_2O 75:25:2 and system B was petroleum ether–ethyl ether–acetic acid 85:15:1. These were used as follows: (*a*) for FA released by venom (aqueous medium), 3 min of A and 6 min of B; (*b*) for LyPC and FA released by venom (ether medium), 6 min of A and 8 min of B; (*c*) for residual PC and the LyPC released by post-heparin serum, 10 min of A. The lipid areas were located by spraying the chromatoplate with Rhodamine 6G, 0.05% in 95% ethanol. The areas that contained lipids were transferred to special tubes (11) for methanolysis. The Rhodamine 6G was recovered in the aqueous phase after methanolysis and hence did not contaminate the methylated FA.

Preparation of Fatty Acid Methyl Esters and GLC

Lipid FA were methanolyzed by the method of Morrison and Smith (12), except that the methyl esters were recovered in petroleum ether. This solution was treated with anhydrous Na_2SO_4 and $NaHCO_3$, centrifuged, transferred to another tube, and evaporated with nitrogen at 30–35°C. The esters were redissolved in 20–100 μl of petroleum ether and analyzed by GLC.

GLC was carried out on a 6 ft ethylene glycol succinate polyester column at 175°C with an argon ionization detector containing a ^{90}Sr source (Barber-Colman series 5000). Relative mass distributions were calculated by tri-

angulation. Analysis of National Institutes of Health standards (A–D) for GLC showed reliable linearity of detector response.

Butylated hydroxytoluene was tested as an antioxidant to lipid peroxidation. It was added (0.005%) to solvents used prior to TLC, to solvent systems used for TLC, and to the methanolysis reagent. GLC results were similar to those obtained without the use of the antioxidant.

Total FA composition of each type of lecithin determined by methanolysis after recovery from chromatoplates was identical with that determined by direct methanolysis. Similarly, FA composition of LyPC formed by the action of venom or serum on PC was not altered by TLC. This was tested by fractionation on silicic acid columns (4) of the LyPC formed in each reaction followed by methanolysis, both directly and after recovery of the TLC scrapings that contained LyPC. FA composition of LyPC was identical by either method except when mixtures containing a relatively high proportion of PC to LyPC (10:1) were analyzed. Then the area of the plate containing LyPC appeared to be contaminated with PC.

RESULTS

Incubation of post-heparin serum with a PC substrate results in the release of FA and the formation of LyPC (Fig. 1A). This lecithinase activity could not be detected in the plasma of five additional subjects obtained prior to the administration of heparin, but was apparent in all subjects 6–10 min after heparin injection.

The optimal pH range for this post-heparin serum activity was found to be 9.3–9.6 (Fig. 2), similar to that observed for the hydrolysis of PC in glycine–NaOH buffer by snake venom (3).

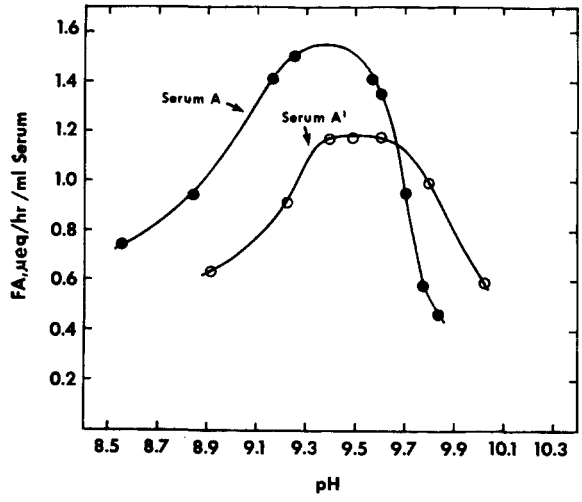

FIG. 2. Effect of pH on post-heparin serum lecithinase activity. Two studies of the effect of pH used post-heparin serum collections A′ and A. The incubations with 2.0 ml of serum A′ were in the standard 9.0 ml system described under Methods. Incubations with 2.0 ml of serum A were in a 7.0 ml system with PC (45 μmoles) and deoxycholate (50 μmoles) in 5.0 ml of glycine buffer. The FA released were measured after 90 min of incubation.

For the demonstration of maximal activity, deoxycholate was required (Fig. 3). Although the deoxycholate requirement was not in constant proportion to the amount of PC substrate, the absolute requirement increased with the amount of PC until a molar ratio of approximately 1:1 was reached. Maximum enzymatic activity occurred with 90 or 120 μmoles of PC at optimal deoxycholate levels. At each of three amounts of serum tested in the standard assay system (Fig. 4), enzymatic activity was constant for 2 hr and this activity was approximately proportional to the amount of serum added. The reaction rate slowly declined after 2 hr.

The activity of post-heparin serum is compared with the action of venom phospholipase in the aqueous assay medium in Table 1, part A. The incubation of different amounts of venom (0.3, 3, 30, 300 μg) produced 7, 34, 71, and 92% hydrolysis of PC substrate. In each incubation, the amount of FA released was accompanied by an equivalent increase of LyPC and decrease of PC, as measured by quantitative chromatography. In contrast, with post-heparin serum, a larger quantity of FA was released and a smaller quantity of LyPC formed than was expected from the decrease in PC substrate, which sug-

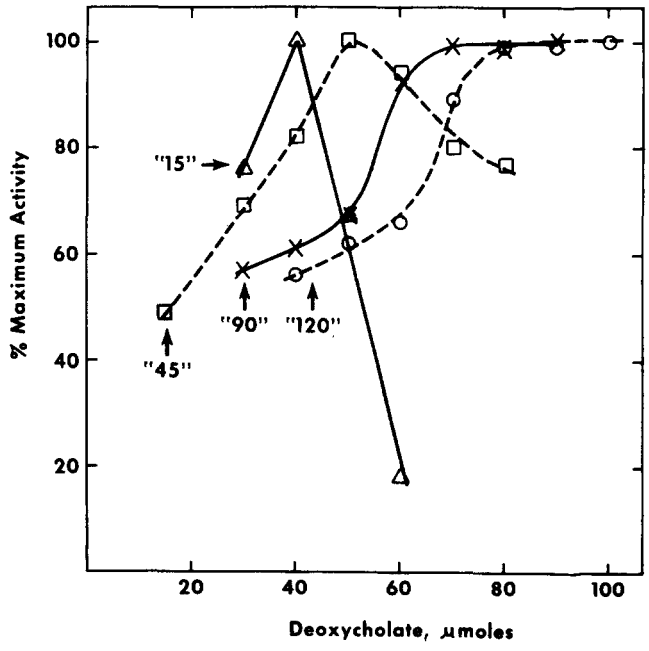

FIG. 3. Effect of varying the amounts of deoxycholate on the post-heparin serum lecithinase activity.

As shown in the figure in quotation marks, the incubation system contained 15, 45, 90, or 120 μmoles of PC substrate. The optimal effect of added deoxycholate, from 20 to 100 μmoles, was determined for each amount of PC substrate by measuring FA released in each incubation during a 2 hr incubation. The maximum rates of activity for 15, 45, 90, and 120 μmoles of PC substrate were 0.79, 0.96, 1.24, and 1.25 μeq of FA per hr per ml of serum respectively.

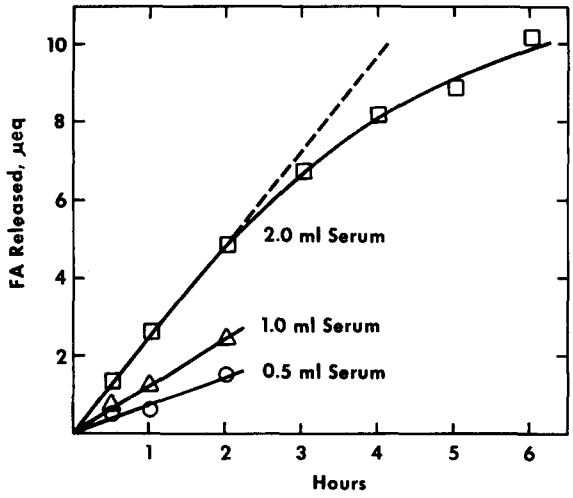

FIG. 4. Time course of the lecithinase reaction with each of three concentrations of serum.

2 ml of undiluted serum, and serum diluted 1:2 and 1:4 with glycine buffer, were each incubated in the standard 9.0 ml system. The total FA released were measured after intervals of 0.5–6 hr of incubation.

gests that additional FA were released from LyPC by other enzymes present in the serum.

Post-heparin serum lecithinase appears to have a specific site of action at the α' (C-1) position of the lecithin molecule. This is suggested by iodine vapor staining of a thin-layer chromatogram (Fig. 1B) which quenches the fluorescence of lipids containing saturated FA (Vogel, W. C., and E. L. Bierman, unpublished data). Snake venom action, which releases mainly unsaturated FA from the β (C-2) position of egg PC (13, 14), produced a strongly fluorescent FA spot. In contrast, the action of the serum enzyme resulted in a strongly staining LyPC area, which suggests that the unsaturated FA on the β-position of PC remain with the LyPC molecule.

The α'-specificity of this enzyme was confirmed by analyses (GLC) of the fatty acid composition of lecithin and of the products formed during hydrolysis, and compared by similar studies with the known β-specificity of venom (13, 14) (Table 1, part B). The activity of the post-heparin enzyme resulted in the formation of LyPC that contained FA similar to those released by the venom enzyme and therefore is specific for the α'-position. This specificity was also found with cabbage lecithin, a substrate that contains a large proportion of unsaturated FA at the α'-position.

TABLE 1 FATTY ACID COMPOSITION OF PRODUCTS OF ACTION OF SNAKE VENOM PHOSPHOLIPASE A AND OF POST-HEPARIN SERUM ON EGG AND CABBAGE PC

Part A (aqueous system)

	Concn	Substrate	Time	ΔFA	ΔLyPC	ΔPC	ΔPC*
			hr		*μmoles*		%
Venom	0.3 μg	Egg PC	2	+3.3	+3.4	−3.1	7
"	3 μg	"	2	+15.5	+13.3	−15.9	34
"	30 μg	"	2	+32.0	+32.4	−29.3	71
"	300 μg	"	2	+41.4	+37.8	−40.2	92
Serum A	2 ml	Egg PC	2	+4.5			
"	"	"	5	+9.2	+6.6	−7.1	16
"	"	"	10	+16.6	+12.9	−14.1	31
"	"	"	15	+22.1	+15.3	−18.2	40
Serum B	2 ml	Cabbage PC	10	+11.6	+6.5	−6.8	13

Part B

					Fatty Acid Distribution†				
	Medium	Substrate	Product	Hydrolysis	16:0	18:0	18:1	18:2	18:3
				%					
Venom	Ether	Egg PC	LyPC (C-1)	100	67	27	6		
			FA (C-2)	100	3	<1	62	35	
"	Aqueous	"	FA	92‡	2	<1	63	35	
Serum A	"	"	LyPC	40‡	6	2	56	36	
Venom	Ether	Cabbage PC	LyPC (C-1)	100	35	4	9	21	31
			FA (C-2)	100	2	<1	3	32	63
Serum B	Aqueous	"	LyPC	13‡	7	2	5	32	54

The incubation system as described under Methods contained 45 μmoles of PC and 50 μmoles of deoxycholate. Palmitoleic and arachidonic acid, 1 and 4% respectively of the egg PC FA, were not included in the calculations. No correction was made for the minor quantity of FA in endogenous serum LyPC.

* Calculation based on actual chromatographic measurement of PC in the complete system.

† Fatty acids are designated by number of carbon atoms:number of double bonds.

‡ Maximal hydrolysis from data in Part A.

A possible contribution of post-heparin triglyceride lipolysis to FA released was negligible, since there was scant hydrolysis of olive oil substituted for PC in this assay and the serum triglyceride concentration (20 mg/100 ml) was too small to contribute significant quantities of FA, even if the triglycerides were totally hydrolyzed. There was no evidence of reacylation of LyPC with FA in the medium: myristic or linolenic acid (10 μmoles), added to the system containing egg PC, were not detected in the FA of LyPC or residual PC substrate after 15 hr of incubation.

Control incubations of pre-heparin plasma or serum yielded only negative or small positive values. Qualitative TLC analysis of prolonged incubations (16–23 hr) of pre-heparin plasma or serum in the standard system did not show hydrolysis of PC substrate.

Factors that might influence lecithinase activity of post-heparin serum were tested (Table 2). The observed heat lability and the inhibiting effect of paraoxon, DFP, or protamine have been reported by various investigators for post-heparin lipase (15); these factors also affect phospholipase activity with PE substrate (1). The slight effect of 1 M NaCl upon the lecithinase activity is compatible with the observation of Datta and Wiggins (16) that either activation or inhibition of post-heparin lipase activity may be obtained with protamine or 1 M NaCl. These agents appear to act on the substrate and not on the enzyme.

TABLE 2 EFFECT OF ENZYME INHIBITORS AND OTHER FACTORS INFLUENCING LECITHINASE ACTIVITY OF POST-HEPARIN SERUM*

Reagent or Treatment		Activity
		% of control†
Heating of serum plus buffer (pH 9.2)	45 °C	77
	55 °C	13
	61 °C	6
Paraoxon	0.044 mM	10
	0.44 mM	5
DFP	0.44 mM	20
Protamine sulfate	1.6 mg/ml serum	40
NaCl	1.0 M	113
Diethyl ether	10%	−7
Methanol	ca. 8%	13
Ethanol	ca. 8%	18
Oleic acid	1.11 mM	69
	3.33 mM	52
Al^{+++}	1.0 mM	74
p-Hydroxymercuribenzoate	1.0 mM	83

Heat treatments were of equal volumes of serum and glycine buffer of pH 9.6 for 15–20 min. Reagent concentrations are expressed for complete system. Reagent, in glycine buffer pH 9.6, was added to serum before the addition of substrate. Serum and paraoxon, DFP, protamine, or *p*-hydroxymercuribenzoate were incubated for 15 min at 38 °C before the substrate was added. Sodium chloride, 4.5 M in glycine buffer pH 9.6, was added to the substrate before addition of serum. Incubation in the presence of ether was at 32 °C. With methanol and ethanol, 1.0 ml of the alcohol was slowly added to a mixture of 2.4 ml buffer and 2.4 ml serum, and 4.0 ml of this mixture was incubated with the substrate. Activity measurements were made from 60-min incubations.

* Serum A, in standard incubation system (2 ml of serum in total of 9 ml).

† Control: 1.2 μeq of FA per hr per ml of serum.

Enzyme activity was completely inhibited by the addition of ether. Methanol and ethanol inhibited the enzyme, and with these alcohols or glycerol there was no appreciable formation of esters with the fatty acids, as was observed with PE substrate (2). Ultrasonic treatment of the serum or substrate did not increase activity. Dipalmitoyl lecithin, cabbage lecithin, and human lipoprotein lecithin were suitable substrates.

Agents that affect the heat-stable lecithinase of human pancreas (17) or duodenal contents (18) were also tested. Only studies made after heat treatment (55 °C) of enzyme preparations were compared since unheated pancreatic material has been found to contain α'- and β-specific phospholipase activities (19). The addition of fatty acids inhibits both post-heparin and duodenal lecithinase activity (18). Al^{+++} (1.0 mM) moderately inhibits the post-heparin lecithinase. Activity was unchanged (within 15% of the control) by added EDTA (0.01–10.0 mM), Ca^{++} (2.5–10.0 mM), Mg^{++} (2.5–5.0 mM), Hg^{++} (0.5–1.0 mM), Zn^{++} (0.1 mM), CN^- (1.0 mM), and albumin (0.5–1.0%). These results are in contrast to the effects of these agents on the activity of the pancreatic enzyme: activation by Al^{+++} and 1% albumin (17); marked inhibition by Ca^{++} and Zn^{++} (17, 18); complete inhibition by 1.0 mM EDTA (17, 18); and no effect of paraoxon (17).

p-Hydroxymercuribenzoate, a potent inhibitor of lecithin:cholesterol acyltransferase (20), had little effect on the post-heparin lecithinase. However, paraoxon inhibited both activities. These results are summarized in Table 3.

DISCUSSION

The incubation conditions necessary for the demonstration of post-heparin phospholipase activity on a lecithin substrate differ from those previously demonstrated for hydrolysis of PE [pH 9.1; albumin–$(NH_4)_2SO_4$ buffer] in that deoxycholate was required and glycine buffer at a slightly higher pH gave optimal activity. Hydrolysis of PE by the post-heparin enzyme will also occur in the deoxycholate-containing medium, but PC was not degraded in the albumin–$(NH_4)_2SO_4$ medium used for assay of PE breakdown. Presumably, deoxycholate influences the molecular arrangement or charge of lecithin in aqueous solutions (3, 21) and the optimal amount required increases with the quantity of lecithin in the medium.

All post-heparin lipolytic enzyme activities demonstrated thus far (triglyceride, PE, PC substrates) are in-

TABLE 3 COMPARISON OF PROPERTIES OF ENZYMES IN MAN THAT CATALYZE THE CONVERSION OF LECITHIN TO LYSOLECITHIN

	Pancreatic Lecithinase (17, 18)	Post-Heparin Lecithinase	Lecithin:cholesterol Acyltransferase (20)
Optimal pH	8.8–9.0	9.4–9.6	7.3–8.0
Deoxycholate	Required	Required	
Heat stability at 55 °C	Stable	Labile	Labile
Positional specificity	β or C-2*	α' or C-1	β or C-2
p-Hydroxymercuribenzoate 1.0 mM		Slight inhibition	Complete inhibition
Paraoxon 0.1 mM	No effect	Complete inhibition	" "
EDTA 0.01 mM	Activation	No effect	No effect
EDTA 1.0 mM	Complete inhibition	" "	" "
Ca^{++} 1.0 mM	Inhibition	" "	
Zn^{++} 0.1 mM	"	" "	
Al^{+++} 1.0 mM	Activation	Slight inhibition	
Albumin 1%	"	No effect	
CN^- 1.0 mM	"	" "	

"No effect" = enzymatic activity with the treatment was 85–115% of control activity.
* Specific for the β or C-2 position if prepared by appropriate heat treatment (19).

activated by heat, DFP, or paraoxon. As previously observed with PE phospholipase (2), post-heparin lecithinase is specific for the α' (C-1) position of the phospholipid substrate, with either predominantly saturated or unsaturated FA at this position. However, the presence of methanol, ethanol, or glycerol in the medium employed with PC substrate did not result in the formation of methyl or ethyl esters of FA or of monoglyceride as was observed previously with PE substrate (2).

Factors (Ca^{++}, Mg^{++}, Hg^{++}, albumin) which affected the rate of activity of β-specific pancreatic lecithinase did not influence α'-specific post-heparin activity. The post-heparin serum lecithinase can be distinguished from the pancreatic enzyme by the markedly different effects of heat, paraoxon, and EDTA, and from serum lecithin:cholesterol acyltransferase by the different effect of *p*-hydroxymercuribenzoate. Furthermore, only the post-heparin enzyme is specific for fatty acids at the α' (C-1) position of the phospholipid substrate.

α-Specific lecithinase activity has now been demonstrated in several tissues of other species (22, 19); the source of the enzyme released into the circulation after heparin administration remains unknown.

Despite differences in incubation conditions, it appears likely that post-heparin phospholipase activity observed with either PE or PC substrate results from the action of the same enzyme, since differential effects of heat and inhibitors could not be demonstrated.

The interrelation of post-heparin lipase and phospholipase activities is also of interest. Preliminary observations (23) have indicated that phospholipase (PE substrate) and lipase (triglyceride substrate) activities cannot be separated from each other by several physical fractionation methods tried (ultracentrifugation, zone electrophoresis, gel filtration). Furthermore, differential variation of enzymatic activities with changes in nutritional status did not occur. Recently De Haas, Sarda, and Roger (24) observed that a highly purified lipase preparation from pig pancreas also readily hydrolyzed phospholipids at the α'-position. At present, however, the question of whether a single macromolecular aggregate, released into the circulation after heparin administration, is responsible for different enzymatic activities is not yet resolved. It is of interest that the phospholipid substrates degraded are surface ("membrane") components (25) of triglyceride-rich lipoproteins. Phospholipid degradation in parallel with triglyceride lipolysis provides a potentially convenient mechanism for the dismantling of large lipoproteins.

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