monoamines in the central nervous system (14).

Summary. Norepinephrine (NE) levels were determined in the hypothalamus, midbrain, and medulla oblongata of rats following exposure to cold of 1°C for 1, 15, and 30 days. In comparison to room temperature controls, hypothalamic levels were slightly increased following 15 and 30 days at 1°C, while levels in the medulla and midbrain were slightly increased only after 30 days of cold-exposure. These increases were unrelated to differences in body temperature, body weight and the water content of brain between cold-exposed and room temperature rats. It is suggested that the increases in brain NE derive from a basis similar to the increases in peripheral tissue catecholamines previously observed by others to follow prolonged cold exposure.

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Evidence for *in Vivo* Activity of Postheparin Plasma Lecithinase in Man* (32625)

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Plasma obtained after intravenous administration of heparin contains enzymatic activity catalyzing the hydrolysis of triglyceride (TG) *in vitro*. This activity is also apparent *in vivo* and results in a decrease in total plasma TG, an increase in products of TG hydrolysis, and a shift of the plasma lipoprotein spectrum from very low density to higher density fractions (1). From prior studies it appeared that no change occurred in total plasma cholesterol or phospholipid (1).

Recent studies have shown that postheparin plasma also contains enzymatic activity catalyzing the hydrolysis of the phospholipids phosphatidyl ethanolamine or phosphatidyl choline (lecithin) *in vitro* (2,3). Hydrolysis occurs at the α' (C-1) fatty acid with the production of β (C-2) lyso derivatives. Results in the present study indicate that lipoprotein lecithin is hydrolyzed in parallel with TG *in vivo* after intravenous heparin, and thus provide evidence for the *in vivo* action of postheparin plasma phospholipase in man.

Methods. Blood samples were obtained from 12 normal adult male subjects prior to and again after rapid intravenous administration of 5000 units of heparin. In three additional experiments blood was obtained prior to and 20 and 40 min after 10,000 units of IV heparin. Pre- and postheparin bloods were immediately placed in tubes containing sodium oxalate (1 mg/ml) and sufficient diethyl-*p*-nitrophenyl phosphate (Paraoxon) to effect a concentration of 2×10^{-3} *M* (4). Paraoxon was used to inhibit postheparin lipolytic activities and also the fatty acid acyl transferase described by Glomset (3,4). The use of this inhibitor is critical for docu-

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mentation of *in vivo* rather than *in vitro* changes in plasma lipids (4). (Lesser amounts of plasma lysolecithin than are commonly reported were found in blood obtained in this manner.) In addition, 4 subjects with endogenous hyperlipemia maintained on fat-free diets for at least 10 days were given constant infusions of heparin intravenously (25.3 units per M^2 per min) begun 10 min after loading doses (2280 units/M^2). Blood samples (Paraoxon used as described above; anticoagulant EDTA Na_2 1 mg/ml) were obtained prior to and at intervals during the infusion of heparin.

Plasma cholesterol and TG were analyzed after extraction in chloroform-methanol (2:1, v:v) by standard AutoAnalyzer techniques (5). The phosphorus of plasma lecithin, sphingomyelin, and lysolecithin was measured after chromatographic separation of these components in aliquots of the chloroform-methanol extract applied to silicic acid impregnated paper (3). Measurement of the precision of replicate analysis of lecithin from duplicate aliquots of the extract yielded a coefficient of variation of 3.6%.

Plasma very low density lipoprotein (VLDLP) lipids were obtained by centrifugation of 2.0 ml of plasma layered under saline ($d = 1.006$) in a Spinco 50 rotor tube, 15 $\times$ $10^7 g$ min at 5°C. The tubes were sliced and the top 2 ml were aspirated and extracted in chloroform–methanol. Aliquots of the extract were taken for analysis of triglyceride, lecithin, and cholesterol by the previously described methods.

Results. Plasma lecithin decreased 15 min after 5000 units of intravenous heparin in 11 out of 12 subjects (Fig. 1A). For all subjects the average decrease was 3.5 $\pm$ 4.3% (mean $\pm$ SD) ($p < .02$). The expected decrease in plasma TG (27 $\pm$ 14%; $p < .001$) also was observed. In contrast, no significant change in plasma sphingomyelin or cholesterol occurred. Changes in plasma lysolecithin were highly variable and not significant although 8 out of the 12 subjects showed some increase (Fig. 1B).

During constant infusions of heparin in the hyperlipemic subjects, plasma TG decreased rapidly and reached a constant level after 90 min (Fig. 2). Plasma lecithin decreased in parallel; only a single postheparin plasma lecithin value exceeded the initial control level. After 90 min plasma lecithin had decreased 5–25% while TG decreased 29–60%. Again, plasma lysolecithin levels were quite variable and showed no significant trend.

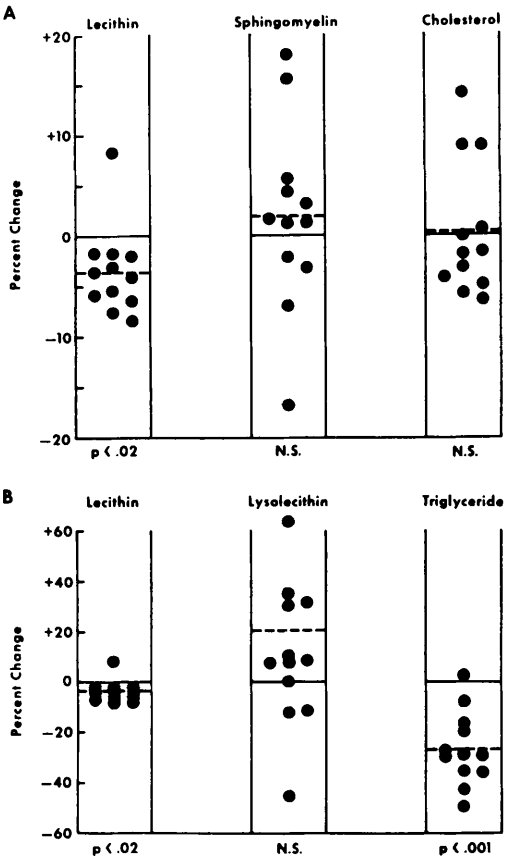

FIG. 1A and B. The effect of intravenous heparin on plasma lipids of 12 normal adults. The percentage change of lipids measured 15 min after 5000 units of heparin is plotted. The changes of lecithin in part A are plotted again on a contracted scale in part B.

A comparison of lipid changes in the plasma and the plasma VLDLP fraction at intervals after 10,000 units of intravenous heparin indicates that most of the changes in whole plasma TG and lecithin are accounted for by decreases in this fraction (Table I). Furthermore, almost identical decreases in VLDLP-TG and -lecithin (71–80% in 20 min) were observed. The small progressive decrease in cholesterol in VLDLP is consistent with established observations that shift of

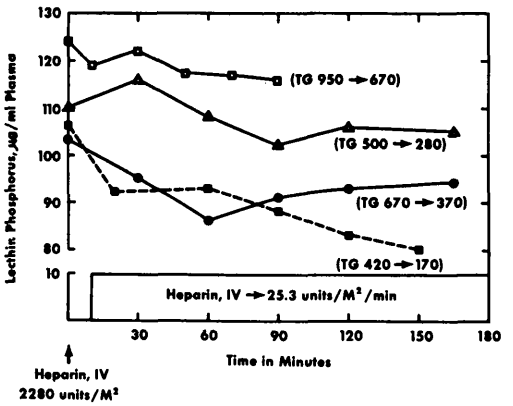

FIG. 2. The effect of intravenous heparin on plasma lecithin and triglyceride in 4 hyperlipemic subjects on fat-free diets. After the initial bood sample, subjects were given a loading dose of 2280 units heparin per M^2, then 10 min later a constant infusion of 25.3 units/M^2 per min was started. The initial and constant levels of plasma triglyceride (mg/100 ml) attained in each subject after 90 minutes are shown in parentheses after the plot of plasma lecithin phosphorus.

lipids of the low density lipoproteins to the more dense fractions occurs in postheparin plasma (1) and provides an index to its magnitude. Thus the measured changes in VLDLP-lecithin and -TG reflect both hydrolysis and the resultant shifts in the lipoprotein density spectrum.

Discussion. These results, obtained with Paraoxon added to blood samples to inhibit *in vitro* enzymatic activity, indicate that postheparin plasma hydrolyzes both lipoprotein-lecithin and -TG *in vivo*. Although the decrease in normal whole plasma lecithin averages only 3.5%, the VLDLP fraction appears to be the major natural substrate in which lecithin may be extensively hydrolyzed. In this fraction, both lecithin and TG appear to be hydrolyzed proportionally. This finding is in accord with results in a study by Lindgren *et al.* (6) of a hyperlipemic subject in whom VLDLP-lecithin and -TG decreased 74 and 85%, respectively, 10 min after 10,000 units of heparin.

Failure to detect a significant increase of plasma lysolecithin concomitant with hydrolysis of lecithin may reflect the limits of analytical technique with minor amounts of lipid phosphorus, rapid turnover of lysolecithin in plasma (7), or the presence of a lysolecithinase.

TABLE I. The Effect of 10,000 Units of Intravenous Heparin on Total Plasma and Plasma Very Low Density Lipoprotein Fraction Lipids in Three Normal Subjects.

Subj.	Post-heparin (min)	Plasma						VLDLP					
		Lecithin		Triglyceride		Cholesterol		Lecithin		Triglyceride		Cholesterol	
		μg P/ml	% Δ	mg/100 ml	% Δ	mg/100 ml	% Δ	μg P/ml	% Δ	mg/100 ml	% Δ	mg/100 ml	% Δ
A	0	51		47		150		2.4		25		9	
	20	46	−10	28	−40	160	+7	0.7	−71	7	−72	8	−11
	40	50	− 2	28	−40	158	+5	0	−100	4	−84	6	−33
B	0	54		49		129		2.6		28		9	
	20	48	−11	32	−35	140	+9	0.7	−73	6	−79	4	−56
	40	51	− 6	28	−43	139	+8	0.6	−77	2	−93	3	−67
C	0	56		45		175		1.7		20		5	
	20	58	+ 4	34	−24	180	+3	0.4	−76	4	−80	3	−40
	40	57	+ 2	32	−29	180	+3	0.3	−82	3	−85	2	−60

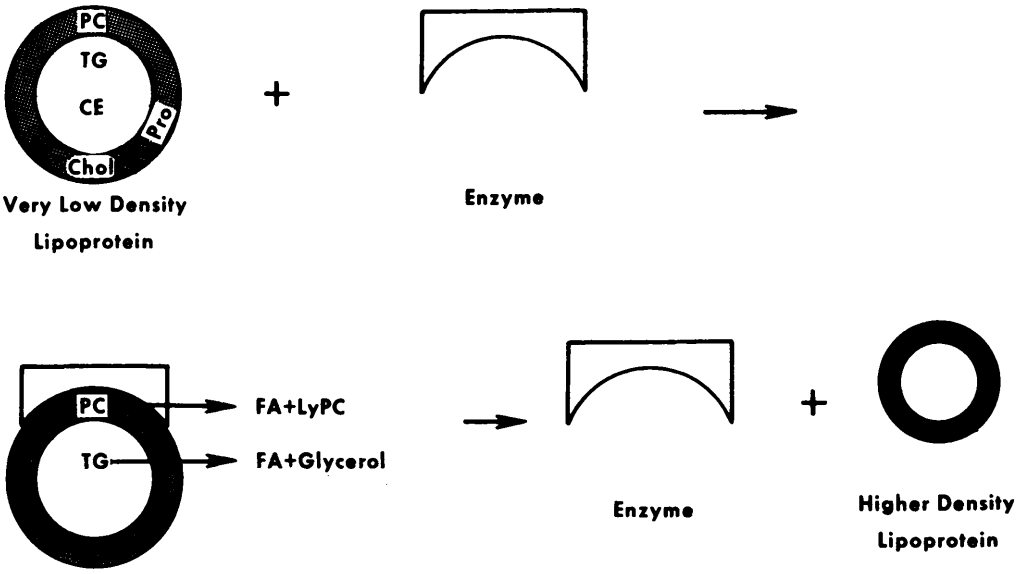

FIG. 3. A hypothetical model of the action of the postheparin enzyme(s) on plasma very low density lipoprotein.

The presence of an α' fatty acid specific phospholipase *in vivo,* releasable into the circulation after heparin, is in accord with related observations that fatty acids of phospholipids are metabolized independently (8), and that both α' and β lysophospholipids are present in plasma and tissues (9).

The measurement of postheparin plasma lecithinase activity in VLDLP may provide a clue to the mechanism for the dismantling of large TG-rich lipoproteins. If one assumes a structure for chylomicrons proposed by Zilversmit (10), the enzyme could hydrolyze both the lecithin on the surface and TG in the core and yield a higher density lipoprotein (Fig. 3).

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