minute prior to treatment with angiotensin amide, contraction was reduced by 50%. Doses of 0.2 and 0.4 mg of 37157 also blocked by 50% the spasm induced by 15 μg of serotonin and doses of one mg completely blocked the serotonin response.

Doses of 5 mg of the compound briefly relaxed the rabbit ileum, followed by a slight decrease in tone. The relaxing action of 0.5 μg of levarterenol was not altered.

The rabbit uterus was contracted with 1, 3, and 5 mg doses of 37157. No alteration of the uterine response to 3 μg doses of levarterenol was noted.

Contractile force, rate, and coronary blood flow of the rabbit heart were decreased by 2 mg of compound 37157. Complete recovery occurred in 8 minutes. A dose of 5 mg stopped the heart for 20 minutes. The voltage of the R wave of the electrocardiogram was decreased.

Summary. It has been shown that haloethynyl derivatives have a hypotensive action in animals with a minimum of ganglionic blockade. Oral administration of compound 37157 resulted in a significant fall in blood pressure in renal hypertensive rats and dogs. In the anesthetized cat, compound 37157 lowered blood pressure and only partially blocked the contraction of the electrically stimulated nictitating membrane. The anesthetized normotensive dog responded with a fall in blood pressure, persisting for 5 hours. Respiration and electrocardiogram were not altered. The pressor response to carotid occlusion was completely abolished. Hypotensive activity could not be elicited in decerebrate cats and dogs, indicating partial central activity. In this preparation the pressor response to levarterenol was augmented. Compound 37157 stimulated the rabbit aortic strip and produced a delayed block of the response to angiotensin amide and levarterenol. The spastic action of angiotensin amide and serotonin on isolated ileum preparations was inhibited by 37157. The isolated rabbit uterus was stimulated. In the perfused isolated rabbit heart, there was a decrease in cardiac contractility, heart rate, and coronary flow.

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New Effects of Sodium Chloride and Protamine on Human Postheparin Plasma 'lipoprotein' Lipase Activity.* (29039)

D. V. DATTA AND H. S. WIGGINS† (Introduced by Sheila Sherlock)
Department of Medicine, Royal Free Hospital, University of London, England

Sodium chloride and protamine have long been thought to be inhibitors of postheparin plasma 'lipoprotein' lipase(1-10). The mechanism of their action is not known. The described inhibition by sodium chloride was not reversed by heparin nor by addition of whole serum or boiled extract of lipoprotein lipase(1). The protamine inhibition was thought to indicate the presence of enzyme bound heparin which is apparently an essential component of the system(1).

The present study reports the effect of sodium chloride and protamine on posthep-

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TABLE I. Effect of Sodium Chloride and Protamine on Postheparin Plasma Lipolytic Activity from 5 Normal Subjects in Presence and Absence of Calcium Chloride.

Assay system	FFA liberated in μEq per 0.1 ml of postheparin plasma in 30 min.*				
Standard assay system	1.32	1.56	2.34	1.86	1.71
Idem with 1 M sodium chloride	1.30	1.53	2.04	1.58	1.80
Idem with 10 mg protamine	1.19	1.60	2.28	1.74	1.60
Idem without calcium chloride	.54	.48	.84	.51	.39
Idem without calcium chloride but with 1 M sodium chloride	1.01	1.17	1.86	1.80	1.62
Idem without calcium chloride but with 10 mg protamine	1.28	1.47	2.31	1.70	1.62

* Coefficient of variation calculated from 40 duplicate determinations 5%.

arin plasma 'lipoprotein' lipase activity in a system recently described(11) which differs from those previously used mainly in the amount of available glyceride.

Material and methods. Blood was collected from an antecubital vein in healthy adults 10 minutes after intravenous injection of 10 units of heparin per kg body weight. It was taken into ice cold test tubes containing 10 to 20 units of heparin and the plasma separated immediately by centrifuging at 0° for 30 minutes at 3000 r.p.m. The separated plasma was used at once or frozen for subsequent use.

The standard assay was 0.3 ml sonicated coconut oil emulsion (15 mg of glycerides), 0.1 ml 0.5 M calcium chloride, 0.5 ml 0.1 M tris buffer pH 8.9 and 0.1 ml postheparin plasma (total volume 1 ml). This was incubated in a metabolic shaking water bath at 37°C for 30 minutes. The free fatty acids liberated were measured by Dole's method (12). Solutions of 2 M sodium chloride and 3% protamine were prepared in 0.1 M tris buffer. When either of these substances was required in the medium an appropriate amount of one of the solutions replaced the 0.1 M tris buffer in the standard assay system. The emulsions were prepared by emulsifying mechanically at 70°C, 30 g of coconut oil B.P., 12.5 g sucrose, 1.5 g glycerol monostearate and 1 g Tween 60 in water to make 100 ml. This emulsion was then subjected to ultra sound for 30 minutes and diluted with 5 volumes of physiological saline. A similar procedure was used when emulsions were made with the different constituents referred to in the text. Low density lipoproteins and chylomicrons were prepared from fresh blood as described by Korn(13).

In the partition experiments the phases employed were heptane (5 ml) and aqueous solutions of 10 mg/ml protamine or albumin in 0.1 Molar tris buffer pH 8.9 (5 ml) at room temperature. 1μEq palmitic acid was added in the heptane. The phases were equilibrated in a shaker for 8 hours and the free fatty acids in the heptane and the water phase were measured by titration. There was no visible evidence of denaturation of the albumin. All reagents used were of analytical quality.

Results. When 1 M sodium chloride or 10 mg/ml protamine (final concentrations) were present in the standard assay system no significant effect was seen on the release of free fatty acids (Table I). Moreover, each of these reagents, at the concentration stated, could replace the calcium chloride in the assay system (Table I). This suggested that sodium chloride and protamine remove the free fatty acids formed from the reacting system, preventing inhibition of the enzyme activity by product accumulation.

In the case of sodium chloride this removal was probably due to the common ion effect, resulting in precipitation of the sodium soaps. This view is supported by the observation that a 0.1 μEq/ml solution of palmitic acid in 0.1 M tris buffer pH 8.9 forms a precipitate in presence of 1 M sodium chloride.

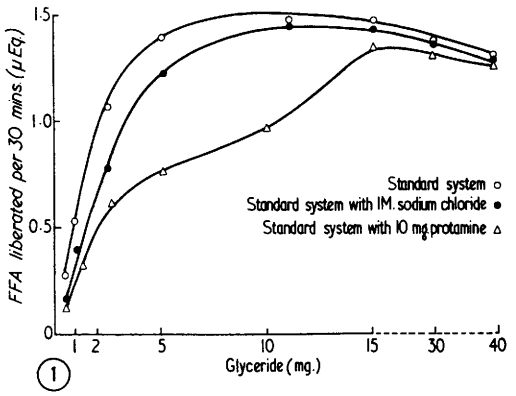
Protamine may bind the fatty acids in a similar way to albumin. This hypothesis is supported by the results shown in Table II from which it is clear that the presence of protamine in the aqueous phase greatly in-

TABLE II. Partition of Palmitic Acid Between Heptane and Aqueous Tris Buffer Containing Protamine or Albumin.

Aqueous phase	Free fatty acid heptane phase μEq	Free fatty acid water phase μEq
Tris buffer 0.1 M	.79	.17
Protamine 10 mg/ml in .1 M tris buffer	.24	.79
Albumin 10 mg/ml in .1 M tris buffer	.09	.89

creases the partition of the palmitic acid into this phase in the same way, but not to the same extent, as albumin.

Since both protamine and sodium chloride have been reported as inhibitors of lipoprotein lipase(1-10), experiments were done to find the cause of this discrepancy. When the glyceride concentration was decreased (Fig. 1) progressive inhibition by protamine was observed suggesting that the substrate concentration is an important factor in determining the action of protamine. Sodium chloride also showed some inhibition at lower concentrations of substrate but at higher concentrations no inhibition was observed. The slight inhibition by sodium chloride observed at low concentrations of the triglyceride emulsion might be due to an interaction between components in the plasma and the added emulsion. Results are shown in Fig. 2 where the proportion of plasma to added emulsion is increased by adding preheparin plasma to the reaction system. In the presence of a high concentration of plasma, sodium chloride acts as an inhibitor. The excess plasma has no effect on the activity found in the absence of sodium chloride. These results indicated that the effects of sodium chloride and protamine on the activity of 'lipoprotein' lipase were more likely to be due to reactions with the substrate than the enzyme.



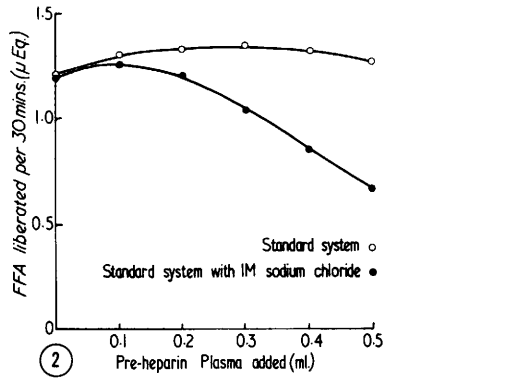


FIG. 1. Effect of variation of substrate concentration on the action of 1 M sodium chloride and 10 mg protamine per ml on postheparin 'lipoprotein' lipase activity.

FIG. 2. Effect of addition of 'preheparin' plasma from the same subject on the action of 1 M sodium chloride on postheparin 'lipoprotein' lipase activity. To accommodate extra plasma, the final volume in all cases was made 1.2 ml.

In Table III the effect of various emulsifying agents on the activity of 'lipoprotein' lipase and the response of the system to 1 M sodium chloride is shown. Although the same enzyme source and the same amount of triglyceride are present in each experiment the amount of free fatty acid released varies widely, being greatest with the Tween 60 stabilized emulsion and least with the chylomicron, low density lipoprotein, and lecithin stabilized emulsion. Systems containing these 3 substrates also show profound inhibition by sodium chloride, whereas the non-ionic detergent stabilized substrates are unaffected and indeed in the absence of calcium chloride the sodium chloride restores full activity.

To determine the contribution that the emulsifying agents make to the free fatty acids released in the standard systems, experiments were done with emulsions in which liquid paraffin replaced the coconut oil. The results (Table IV) indicated that although the monoglyceride may contribute to the free fatty acids released, the Tween 60, which is

TABLE III. Effect of 1 M Sodium Chloride on Postheparin Plasma 'Lipoprotein' Lipase Activity in Presence of Different Emulsifying Agents.

Substrate (final glyceride concentration 15 mg/ml)	Emulsifying agents	(1) Standard system	(2) Standard system plus 1 M sodium chloride	(3) Standard system without calcium chloride	(4) Standard system without calcium chloride plus 1 M sodium chloride
		Free Fatty Acids Liberated			
Chylomicrons	—	.34	.03	.25	.03
Low density lipoproteins	—	.37	.02	.32	.03
Coconut oil	Lecithin (ionic)	.47	.04	.35	.04
" "	Tween 60 (non ionic)	1.53	1.47	.50	1.51
" "	Collone (non ionic)	.79	.77	.26	.78
" "	*C.P.G.E. 10 (non ionic)	.76	.74	.22	.79

The emulsions were stabilized with Lecithin 1.5%, Tween 60 1%, Collone 2%, C.P.G.E. 10 3%
* Cetostearyl ether of polyethylene glycol with chain length of 10 ethylene oxide units.

also a fatty acid ester, makes only a small contribution. This confirms that in the standard system the majority of the fatty acids titrated after the incubation originate from glyceride ester bonds.

Discussion. The inhibitory effect of protamine has been variously explained. It has been suggested that it is due to a direct effect on heparin, the latter being essential for the stability of the enzyme(10), that heparin provides a link between the enzyme and the lipoprotein moiety of its substrate (9) or that it forms an integral part of the enzyme(5). Such inhibitors might competitively replace the heparin which is normally bound to the enzyme(1). The inhibition of postheparin plasma 'lipoprotein' lipase activity by protamine, a known heparin antagonist, is inconclusive evidence for participation of heparin in the enzyme action since protamine also exerts a direct effect on the chylomicron substrate(15,16). However, the fact that under appropriate conditions no inhibition of 'lipoprotein' lipase by protamine could be demonstrated may be taken to mean that, under these conditions at least, heparin is not an obligatory component of the reacting system.

TABLE IV. Contribution of Emulsifying Agent to Free Fatty Acids Released by Lipoprotein Lipase.

Substrate	Free fatty acid released per 0.1 ml plasma μeq 30 min.
Standard	1.47
Paraffin oil and Tween 60 + monostearine	.59
Paraffin oil + monostearine	.49
Paraffin oil + Tween 60	.21

The present study indicates that the effect of sodium chloride on release of free fatty acids by postheparin 'lipoprotein' lipase depends on the conditions of assay. Using the system previously described(11) sodium chloride had no significant effect on the so-called 'lipoprotein' lipase. When a system similar to those used by other workers who have described an inhibitory effect of sodium chloride(1-10) was used, inhibition was found. Since in the present work the difference between the lecithin stabilized coconut oil emulsion and the Tween 60 stabilized coconut oil emulsion (Table III) is only in the physical form of the substrate, one must conclude that inhibition by sodium chloride of 'lipoprotein' lipase activity is determined by the physical nature of the substrate. Although electrolytes are known to aggregate emulsions, particularly those stabilized by ionic detergents(14), the inhibitions observed are too large to be accounted for by this. One must postulate that some alteration is induced at the oil water interface by the relative large concentration of sodium chloride.

No attempt has been made to study the effect of the chemical nature of the triglyceride used as substrate and it is not unlikely that different results might be obtained if corn oil, consisting of longer chain unsaturated triglycerides, were used in place of co-

conut oil, which is composed largely of shorter chain saturated triglycerides.

The observation that sodium chloride and protamine can effect the removal from the system of fatty acid released by 'lipoprotein' lipase in the absence of other fatty acid acceptors indicates the care that must be taken in interpreting studies of this kind. If the appropriate conditions are chosen sodium chloride or protamine can be shown to stimulate, inhibit or have no effect on 'lipoprotein' lipase activity.

Summary. The effect of sodium chloride and protamine on the activity of postheparin 'lipoprotein' lipase was studied. Chylomicrons, low density lipoproteins and coconut oil emulsions stabilized with various emulsifying agents were used as substrate. Inhibition, stimulation, or no effect could be observed depending on the system used. The results are interpreted as indicating that where sodium chloride or protamine inhibit 'lipoprotein' lipase the site of the action is more likely to be at the oil-water interface of the substrate emulsion than on the enzyme molecule.

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Leukokinetic Studies. VIII. A Search for an Extramedullary tissue Pool of Neutrophilic Granulocytes.* (29040)

D. R. BOGGS, J. W. ATHENS, O. P. HAAB, S. O. RAAB, G. E. CARTWRIGHT AND M. M. WINTROBE
Department of Medicine, University of Utah College of Medicine, Salt Lake City

Considerable controversy has revolved about the anatomic location of storage reservoirs of mature granulocytes. Craddock and co-workers demonstrated a reserve supply of mature granulocytes in the bone marrow from which cells are released into the blood as needed(1). Their work has been amply confirmed in this(2) and other(3) laboratories. Debated, however, is the question of whether there is or is not a second, and perhaps even larger(4), reserve of mature granulocytes located in extramedullary tissue. Such an extramedullary granulocyte reserve might serve merely as a source of supply from which cells would return to the blood(5) or it might represent a source of supply of cells needed in the tissues(6). The latter hypothesis, that mature neutrophils leave the blood and enter an extramedullary reserve from which

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