Human Endogenous Plasma Lipemia Clearing and Lipolytic Activity After Tricalcium Phosphate Adsorption. (23233)

H. ENGELBERG*
Division of Laboratories, Cedars of Lebanon Hospital, Los Angeles, Calif.

Nilsson and Wenckert(1) demonstrated that heparin was adsorbed to known prothrombin adsorbents. Subsequently Nikkila and Pesola(2) reported that post-heparin clearing factor was adsorbed on tricalcium phosphate gel. Human endogenous plasma lipemia clearing factor has been found in some subjects(3). Further work(4) demonstrated its lipolytic nature and inhibition characteristics which were similar to the post-heparin enzyme (lipoprotein lipase) and different from those of pancreatic lipase. In the present study the effect of tricalcium phosphate adsorption on clearing and lipolytic activity of human post-heparin plasma, pancreatic lipase and endogenous plasma lipolytic activity has been investigated. The results add further evidence of the identity between the endogenous plasma lipolytic enzyme and the post-heparin factor, and support the conclusion that heparin is normally a component part of lipoprotein lipase essential to or stabilizing its activity.

Methods. Tricalcium phosphate gel was prepared as described by Quick(5). (This must be completely salt-free or clearing factor adsorption does not occur.) Blood was obtained using chilled syringes and oxalated tubes, and the plasma separated in refrigerated centrifuge. (Citrate interferes with adsorption of heparin on the gel, and heparin was not used as anticoagulant because of the remote possibility of slight *in vitro* clearing factor formation.) One ml of plasma was added to 0.1 ml of gel and the mixture allowed to stand for 30 minutes at 4°C with frequent shaking. In studies of clearing activity, one ml of the original plasma or one ml of the supernatant liquid after adsorption were mixed with .4 ml of a .25% suspension of Ediol. Optical density changes were followed for one hour at 25°C using a Coleman Jr. spectrophotometer at 540 mμ. In the investigations of lipolysis a series of tubes containing one ml aliquots of plasma before and after adsorption were incubated at 37°C for 15, 30 and 60 minutes with .2 ml of human low density serum lipoproteins† added to each tube. It had been previously determined that at this level of added lipoproteins the amount of fat substrate does not affect the rate of lipolytic reaction. Unesterified fatty acids were determined using the method of Borgstrom(6) which we found to have a maximum deviation from the mean of .05 Meq/l. Calculation of rate of release of unesterified fatty acids per hour was based upon the maximum increment of fatty acids found in any time period. This assumption is justified since in this *in vitro* system the end products of the reaction (glycerol and free fatty acids) are not removed and these inhibit further lipolysis(7). Esterified cholesterol was also measured before and after one hour incubation since we have found that occasionally an increase in cholesterol ester occurs which, if undetermined, might mask a moderate degree of fatty acid release. Human pancreatic lipase was obtained from fresh autopsy material.‡

Results. Table I shows the effect of tricalcium phosphate gel adsorption upon lipemia clearing and lipolytic activity of post-heparin plasma and pancreatic lipase. There is a marked reduction of activity in the former but no decrease or perhaps an increase in the fat splitting action of the pancreatic lipase. Occasionally activity of the post-heparin factor is completely removed by the gel.

Table II presents the results of optical den-

* Supported by U.S.P.H.S. grant.

† Ultracentrifugally separated and supplied by the Inst. of Medical Physics through the courtesy of Mr. D. Spector. The lipoproteins represent a 5-fold concentration of predominantly Standard Sf 12-400 lipoproteins in their original sera.

‡ Supplied through cooperation of Dr. N. Friedman, and extracted by the Southern California Gland Co.

TABLE I. Effect of Tricalcium Phosphate Adsorption on Lipemia Clearing and Lipolytic Activity of Plasma Plus Human Pancreatic Lipase, and of Human Post-Heparin Plasma.

Preparation	$CA_3(PO_4)_2$ Adsorption	Incubation time at 25°C Optical density readings* 0	10′	20′	30′	60′
Pre-heparin plasma	B	.37	.36	.35	.35	.34
+ human pan. lipase	A	.40	.38	.37	.37	.34
Post-heparin plasma (100 mg I.V.)	B	.46	.43	.39	.36	.28
	A	.49	.49	.49	.49	.48

		Incubation time at 37°C Unesterified fatty acids† 0	15′	30′	60′	Rate of release
Pre-heparin plasma	B	1.2	1.3	1.3	1.4	.4/hr
+ human pan. lipase	A	1.0	1.5	1.5	1.3	2.0
Post-heparin plasma (100 mg I.V.)	B	2.0	3.1	3.9	4.2	4.4
	A	1.5	1.7	1.8	1.8	.8

* Read in Coleman Jr. Spectrophotometer at 540 mμ.
† Values in milliequivalents/liter.
B = before.
A = after.

sity studies before and after adsorption on the gel in patients who had endogenous lipemia clearing factor in the plasma. With the exception of subject E.M. the post-adsorptive plasma had less or no clearing activity. The plasma of subject E.M. was repeatedly tested and in no instance did gel adsorption affect activity, although it was inhibited by sodium taurocholate but not by protamine sulfate. No release of free fatty acid could be demonstrated when his plasma was incubated with human lipoproteins so that this probably represents an instance of non-lipolytic clearing. In several instances in other subjects (M.A., J.S., C.S., A.K.) the clearing activity which was adsorbed in the phosphate gel was inhibited by 5% sodium taurocholate, TEPP, molar NaCl and protamine, which inhibit post-heparin factor but do not affect pancreatic lipase activity(4).

Studies in 14 individuals of the release of unesterified fatty acids before and after plasma adsorption on tricalcium phosphate are shown in Table III. In 3 of the 14 subjects (M.E., E.H. and B.L.) endogenous lipemia clearing activity had been found on previous occasions, but the others had not been tested before. Four of these showed an increase in unesterified fatty acids, and in 2 (S.B. and W.N.) the increase in esterified cholesterol masked a release of the fatty acids. In 8 of the 9 subjects in whom lipolysis occurred, the activity was either completely or almost completely removed by adsorption with the gel. In one, M.E., although the rate of release of fatty acids was apparently unchanged, there is evidence of a more rapid inactivation of the lipolytic activity upon incubation. The measurements of cholesterol ester reveal that in every instance a slight decrease was found after adsorption, and that less esterification occurred in the adsorbed plasma.

TABLE II. Effect of Tri-Calcium Phosphate Adsorption on Human Endogenous Plasma Lipemia Clearing Activity. Optical Density Readings at 540 mμ. Tubes Contained 1 ml Plasma Plus 0.4 ml .25% Ediol.

Patient Sex age	$Ca_3(PO_4)_2$ Adsorption	Incubation time at 25°C 0	10′	30′	60′
L.D.	B	.54	.46	.30	.40
♂ 44	A	.36	.37	.38	.38
L.R.	B	.49	.46	.46	.50
♀ 50	A	.49	.49	.51	.51
I.F.	B	.47	.50	.50	.46
♂ 64	A	.49	.51	.50	.49
T.H.	B	.44	.43	.39	.38
♂ 49	A	.47	.47	.46	.46
E.M.	B	.36	.33	.31	.29
♂ 62	A	.41	.39	.36	.33
D.H.	B	.35	.34	.33	.33
♀ 42	A	.32	.32	.31	.31
M.A.	B	.40	.36	.32	.30
♂ 70	A	.56	.54	.52	.50
	I.M. NaCl	.25	.26	.28	.28
	TEPP 1×10^{-1}	.41	.40	.40	.40
G.S.	B	.42	.40	.40	.37
♂ 65	A	.41	.41	.41	.40
	5% Sod. Taur.	.41	.41	.41	.41
J.S.	B	.65	.64	.63	.63
♂ 32	A	.66	.66	.66	.66
	5% Sod. Taur.	.58	.58	.58	.575
H.M.	B	.40	.39	.37	.37
♀ 35	A	.37	.38	.37	.36
H.K.	B	.29	.27	.24	.21
♂ 63	A	.35	.35	.34	.31
	TEPP 1×10^{-1}	.31	.31	.31	.30
	5% Protamine	.30	.30	.29	.26

B = before.
A = after.

TABLE III. Effect of Tri-Calcium Phosphate Adsorption on Endogenous Lipolytic Activity of Oxalated Human Fasting Plasma. Tubes Contained 1 ml Plasma Plus 0.2 ml Human Low Density Serum Lipoproteins.

Patient Sex age	$Ca_3(PO_4)_2$ Adsorption	Incubation time at 37°C — Unesterified fatty acids in mEq/l 0	15′	30′	60′	Rate of release/hr	Chol. esters 0	60′
M.E.	B	.2	.4	.6	.6	.8	235	263
♂ 36	A	.2	.4	.5	.5	.8	230	233
E.H.	B	.2	.4	1.7	1.9	3.0	185	185
♀ 41	A	.2	.4	.6	.6	.8	180	180
A.N.	B	.4	.4	.5	.6	.2	224	224
♂ 45	A	.4	.4	.4	.4	.0	197	196
A.K.	B	.3	.3	.3	.5	.2	198	200
♀ 50	A	.3	.3	.3	.3	.0	183	183
A.B.	B	.4	.5	.6	.6	.4	271	274
♀ 42	A	.4	.4	.4	.5	.1	214	213
D.S.	B	.3	.3	.3	.3	.0	227	227
♀ 42	A	.3	.3	.3	.3	.0	224	224
P.S.	B	.2	.2	.2	.2	.0	156	156
♀ 44	A	.2	.2	.2	.2	.0	142	142
S.B.	B	.3	.3	.3	.3	.12	185	193
♂ 71	A	.3	.3	.3	.3	.04	176	179
D.Y.	B	.7	.7	.7	.7	.0	166	166
♀ 47	A	.6	.6	.6	.6	.0	161	161
J.Z.	B	.3	.3	.3	.3	.0	168	169
♂ 26	A	.3	.3	.3	.3	.0	147	147
R.F.	B	.5	.5	.5	.5	.0	212	212
♀ 62	A	.5	.5	.5	.5	.0	176	176
S.H.	B	.5	.8	.9	1.0	1.2	220	249
♂ 47	A	.4	.5	.6	.7	.4	189	202
B.L.	B	.6	.9	.9	.9	1.2	184	190
♂ 32	A	.6	.5	.5	.5	.0	176	178
W.N.	B	.7	.7	.7	.7	.18	230	242
♂ 66	A	.7	.7	.7	.7	.0	226	226

B = before.
A = after.

Discussion. The finding that tricalcium phosphate gel adsorbs post-heparin factor but does not affect pancreatic lipase activity affords another useful method of distinguishing between these two lipolytic enzymes. The results of the gel adsorption of the endogenous plasma clearing and lipolytic activity thus strongly support previous studies(4) indicating its identity with heparin lipoprotein lipase.

We found that after tricalcium phosphate adsorption endogenous heparin cannot be demonstrated in the supernatant plasma with octylamine precipitation methods(1). However, markedly decreased clearing and lipolytic activity may remain in some cases. This suggests that minor or unstable lipoprotein lipase activity may exist without heparin. Thus Robinson(8) found that removal of heparin from post-heparin plasma by a resin does not destroy the enzyme system but markedly reduces its stability. Korn(9) showed that a bacterial heparinase produced a partial (approximately 60%) inactivation of tissue lipoprotein lipase activity. His results indicated that heparin was an integral part of the molecule but that destruction of the former did not totally prevent lipolysis of lipoproteins by the remaining part of the enzyme. The effect of tricalcium phosphate adsorption is in accord with these findings.

The post-adsorptive plasma had less esteri-

fied cholesterol, and showed a diminution in the further esterification of cholesterol upon incubation, than the pre-adsorbed plasma. Although the meaning of this is unclear at this time, the apparent removal of some cholesterol esterase activity by tricalcium phosphate gel, a known heparin and prothrombin adsorbent, raises the possibility of some relationship between these substances. Unfortunately determinations of total cholesterol before and after adsorption were not done.

Summary. Tricalcium phosphate gel removes all or the major portion of post-heparin clearing factor activity from plasma but does not decrease the activity of human pancreatic lipase. It is therefore a useful agent in identifying heparin lipoprotein lipase. Endogenous plasma lipolytic activity is completely or nearly completely removed after gel adsorption in almost all subjects in whom it is present, affording further evidence of its similarity to or identity with post-heparin factor. Plasma cholesterol ester and cholesterol esterase is also apparently decreased in the post-adsorptive sample.

1. Nilsson, I. M., and Wenckert, A., *Acta Med. Scand.*, 1954, v150, Supp. 297.

2. Nikkila, E. A., and Pesola, R., *Acta Chem. Scand.*, 1956, v10, 144.

3. Engelberg, H., *Am. J. Physiol.*, 1955, v181, 309.

4. ———, *J. Biol. Chem.*, 1956, v222, 601.

5. Quick, A. J., *The Physiology and Pathology of Hemostasis,* Lea and Febiger, Philadelphia, 1951.

6. Borgstrom, B., *Acta Physiol. Scand.*, 1925, v25, 328.

7. Shore, B., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 73.

8. Robinson, D. S., *Biochem. J.*, 1956, v62, 18 P.

9. Korn, E. D., *Science,* 1956, v124, 489.

Received March 27, 1957. P.S.E.B.M., 1957, v95.

Paper Electrophoresis of Animal Hemoglobins. (23234)

GERALD P. RODNAN* AND FRANKLIN G. EBAUGH, JR.†
(Introduced by Jessica H. Lewis)
Department of Medicine, University of Pittsburgh and Nat. Inst. of Arthritis and Metabolic Diseases, N.I.H.

The discovery of an abnormal hemoglobin in sickle cell disease has stimulated intensive electrophoretic study of human hemoglobin (1-4). An interest in comparative aspects of erythrocyte turnover(5) led us to undertake electrophoretic characterization of hemoglobins of a variety of animal species. A wide spectrum of migration patterns was found, with some species demonstrating as many as 3 electrophoretically separable hemoglobins.

Materials and methods. Blood was obtained from active adult animals (and 3 calves) (Table I) by cardiac or venipuncture and either oxalate or heparin used as anticoagulant. (Penguin blood was taken from an animal severely ill with aspergillosis.) Human blood was drawn from several healthy adult males, from patients with sickle cell disease(6), and from an individual with hemoglobin C disease(7). Blood cells were washed 3 times with 0.9% NaCl, lysed with 2-3 volumes of distilled water, and centrifuged to separate out particulate (stromal) elements. 0.005-.020 ml samples of hemoglobin solutions (stored frozen until electrophoresis) were stripped on filter paper and placed in a Spinco electrophoresis cell(8) containing barbital buffer of .01 ionic strength, pH 9.2. Voltage was maintained constant at 270 volts for a 5 hour period of electrophoresis. Under these conditions the average current across each 3 cm wide paper strip was .5 m amp. Human A hemoglobin, and usually S and C hemoglobins as well, were run simultaneously in the same cell with animal hemoglobins. Human A hemoglobin

* Public Health Service Research Fellow of Nat. Heart Inst.

† Present Address: Hitchcock Clinic and Dartmouth Medical School, Hanover, N. H.