vation of Gitlin (personal communication) that intravenous administration of heparin caused much less clearing activity in the serum of patients with secondary hyperlipemia due to nephrosis, than in normal individuals. He had found that increasing the albumin concentration in these patients who had low serum albumin levels, had no direct effect on degree of clearing. In view of our observation of inadequate clearing in patients with idiopathic hyperlipemia who have normal serum albumin levels, it seems that low values for serum albumin are not a major factor in producing inhibition of lipemia clearing.

Our finding of inhibition of clearing by serum of patients with primary as well as secondary hyperlipemia suggests the possibility of a common basis for the high blood lipid levels in these disorders.

Summary. 1. Serum from patients with primary and secondary hyperlipemia was found to inhibit the clearing activity present in normal serum after intravenous administration of heparin. 2. The degree of inhibition exerted by the hyperlipemic sera varied among the patients, but at appropriate concentrations of hyperlipemic serum complete inhibition could be attained in every case. 3. Intravenous administration of heparin caused considerably less clearing activity in hyperlipemic patients than in the control subjects.

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Anti-AcG: Specific Circulating Inhibitor of the Labile Clotting Factor.* (23293)

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With recent knowledge that a number of protein factors normally participate in the complex mechanisms of blood clotting, it is understandable that inhibitors might be developed, in the body, to act as specific antibodies against one or other of these protein factors. For instance, anti-AHF or anti-PTC have been demonstrated in occasional hemophiliacs or Christmas disease patients, respectively, particularly after transfusions (1). The present data are from the case of an elderly white male who developed hematuria following an uneventful gall-bladder operation. This bleeding tendency seemed to get worse after transfusions. The following tests establish the presence in his blood of a powerful inhibitor, specific for AcG (syn. (pro)accelerin, labile factor, factor V, etc.).

Methods are established routines(2), with the modifications indicated. A summary of the routine test findings is shown in Table I (see discussion). The significance of the several abnormal test results will emerge as the experimental analysis proceeds.

Prothrombin time tests. A major initial discovery was the prolongation of the plasma prothrombin time test (*cf.* Quick, 3). This was true also in mixtures of equal vols, of patient's (Y) and normal (N) plasma, immediately suggesting an inhibitor, since normal plasma might be expected to correct the test if it were merely a matter of factor deficiency. Partial, but not complete, correction of the prothrombin time could be obtained by (a) dilution of Y, (b) addition of AcG ($BaCO_3$-

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TABLE I. Tests of Patient's Hemostatic and Clotting Mechanisms.

Tourniquet test	Neg.
Bleeding time test	Neg. (4½ ; 2 min.)
Platelet count/mm³	Norm. (358-382 × 10^3)
Whole blood clot.—time	65 min. (prolonged)
Clot retraction	Norm. (no lysis)
Fibrinogen	Norm.
Thrombin clot.—time	Norm.
Protamine titration	Neg.
Prothrombin time	55 sec. (prolonged)
Prothrombin assay	Norm. (122 ; 126%)
Proaccelerin assay	<1% : anti-AcG + (abnormal)
Proconvertin assay	80% : no anti-proc.
AHF	70% : no anti-AHF
PTC	Norm. : no anti-PTC
P.U.R.	Defic. prothr. consumption
P.T.T.	Abnorm. partial tpln. time
T.G.	Abnorm. tpln. generation

adsorbed beef serum), or (c) mixture of Y's plasma with that of either of his two daughters (in tests made on the last day of hospitalization, when the patient was improving clinically). The daughters' test times were perfectly normal. A beef serum proconvertin (*syn.* SPCA, stable factor, factor VII) did not correct the defect. The above data are illustrated in Table II, for tests made on two occasions (A,B).

Thromboplastin. The above test results were essentially similar with a variety of human or animal thromboplastins. On preincubation of plasma (Y) and thromboplastin, before adding the calcium, the clotting-times were further prolonged. This was not significantly changed by adding more thromboplastin, but markedly shorter clotting-times were obtained when AcG was supplied in the calcium solution. *Thromboplastin dilutions.* Modifying the "prothrombin time" test, by varying dilutions of thromboplastin, gave apparent differences between the plasmas of patient (Y) and a *fresh* normal (N), as shown in Table III, tests of 3-23-57. A similar difference, at first glance, appeared to be present in tests of 5-8-57, when 1:50 AcG was added to stored (4 weeks at –20°C) Y and to an *aged* (prothrombin time: >60″) normal plasma (A). However, increasing the strength of AcG added to Y diminished the difference and in the last series (1:1 AcG) the data were not significantly different from the (A) tests. This argues strongly against any true "antithromboplastin" in Y, but rather points to the very great significance of the AcG concentration in such test series.

TABLE II. Prothrombin Time Tests.

A. 3-25-57	
Normal (N) plasma	11.2 sec.
Patient (Y) "	54.2
Y + N plasma	52.4
Y + AcG	27.2
Y + proconvertin	57
B. 4-3-57	
Daughter (D_1) plasma	11 sec.
" (D_2) "	11.2
Patient (Y)	60.3
Y + saline	54.6
Y + D_1	24.6
Y + D_2	25.3

Assays for AcG and anti-AcG. AcG is routinely tested by the restoration of clotting in *aged* normal human plasma, which has lost most of its labile factor (test times: >60 sec.). The diluted test sample is added to this substrate, at 37°C, before the thromboplastin (human brain) and the Ca (0.02 M $CaCl_2$).

AcG. Compared with a fresh normal human plasma, patient Y assayed less than 1%. Further, when samples of (a) bovine serum AcG (above) or (b) fresh N plasma were mixed with Y, the AcG assay values were greatly reduced. This inhibitory effect did

TABLE III. Influence of Serial Dilutions of Thromboplastin (tpln.) on "Prothrombin Time" (Sec., at 37°C). *Test mixtures:* 0.1 ml plasma (or mixture with equal vol AcG, at strengths stated) + 0.1 ml tpln. (at dilution stated) + 0.1 ml 0.02 M $CaCl_2$. *Plasmas:* Y = patient; N = *fresh* normal; A = *aged* normal.

	Tpln. dilution	1/1	1/2	1/4	1/8	1/16	1/32
3-23-57:	Y	31.4″	52.8″	159.0″	360.0″	+	+
"	N	8.9″	11.5″	20.3″	27.0″	35.7″	55.2″
5- 8-57:	A + AcG 1:50	14.6″	16.0″	19.0″	21.4″	25.6″	33.6″
"	Y + " "	30.8″	38.6″	50.0″	64.4″	78.6″	102.0″
"	Y + " 1: 5	18.7″	22.6″	29.0″	34.4″	43.4″	56.0″
"	Y + " 1: 1	13.8″	15.6″	20.0″	25.2″	29.0″	36.0″

TABLE IV. Titer of Anti-AcG: % Inhibition.

	1:20	1:160	1:320
Citr. plasma	64	22	10
Oxal. "	69	25	14
$BaSO_4$ "	69	25	14
$(NH_4)_2SO_4$:			
25% sat., sup.	69	21	
25% sat., sed.	0	5	
33% " "	68	3	
50% " "	72	14	14

not require pre-incubation.

Anti-AcG. The inhibitor *titer* was determined from the reduction in AcG activity on adding serial dilutions of Y's plasma, serum, or fractions to a 1:10 dilution of the bovine AcG preparation. This "standard" AcG was reasonably stable, in an ice-bath, and assayed practically equivalent to fresh normal human plasma. Serial dilutions of the standard AcG served as *reference standard* for converting clotting-times into "percentages" of the original assay strength. Table IV shows typical assay (percentage) anti-AcG values in a preliminary series of fractionations of Y's plasma. The 1:20 dilutions illustrate the comparisons very clearly. Thus, (a) the original plasmas (1:20) gave 64% inhibition in a citrated and 69% inhibition in an oxalated sample. The oxalated plasma was used for the subsequent fractionations. (b) $BaSO_4$ adsorption failed to remove any anti-AcG, the full 69% inhibition being obtained with the supernatant. (c) After 25% saturation with $(NH_4)_2SO_4$, no inhibitor was found in the sediment, but the full amount (69% inhibition) was recovered in the 1:20 dil. supernatant. (d) At 33% saturation with $(NH_4)_2SO_4$, 68% inhibition was shown in the sediment, redissolved to original plasma volume. (e) At 50% saturation with $(NH_4)_2SO_4$, the redissolved sediment gave negligible further increase (72% inhibition). In all these tests, the inhibitory effect became minimal ($<15\%$) at a titer of 1:160 to 1:320. These preliminary studies suggest that the anti-AcG accompanies some *globulin* fraction. Serum electrophoresis showed certain globulin anomalies which are being analysed. Cryoglobulins were not demonstrable. Table V shows titer testing of the patient's serum, compared with his plasma. Actual test clotting-times are shown. These were very similar at each successive dilution, indicating that *all* the inhibitor in the plasma survived in the serum after clotting. The plasma and AcG preparation (asterisked*) were tested with saline: all others with standard AcG. *Normal* human serum did not alter the latter value (15.6 sec.), showing both its lack of AcG and of inhibitor.

Stability data. (a) *Temperature.* The anti-AcG proved remarkably stable at –20°, 4°, 22°, and 37°C. At 37°C, there was no change in titer after 48 hrs, and at 22°C (room temp.) it did not alter in ten days. At 60°C, there were only very minor fluctuations in test values over 1 hr. At 70°C, a progressive loss of inhibitor potency occurred, with practically complete inactivation in ½ hr. At boiling temperature, the anti-AcG was destroyed within 5 min. (b) *pH.* The inhibitor was stable for over 2 hrs between pH 5 and 10. It was slightly weakened at pH 4.0 and 11.5. At pH 2.5, about half its potency was lost in 2 hrs. These tests were performed by adding N/10 HCl or NaOH, using the glass electrode, and restoring to original pH (8.25) before testing, at 1:1 and 1:20 dilutions (accurate volumes). (c) *Fat solvents.* 5 min. shaking with an equal volume of either pure *ether* or *benzene* did not alter the inhibitor test values, whether determined immediately or when retested 24 hrs later.

Discussion. The routine tests, summarized in Table I, may be interpreted as indicating that the patient's blood is deficient in AcG because of the presence of a powerful inhibitor, specific for the labile clotting factor. Other possibilities were ruled out, thus: (a) *Prothrombin*: normal, assaying 122% and 126% by specific one-stage and two-stage

TABLE V. Clotting-Times: AcG Assays.

		Plasma	Serum
Norm.	1: 1	15.5*	(15.6)*
Pat.	1: 0.2	42.	44.4
	1: 2	26.	26.2
	1: 8	22.	22.4
	1: 64	17.	17.5
	1:256	16.6	16.8

* Saline (time in sec.).

tests, respectively. (b) *Proconvertin*: normal, at 80%, with no evidence of any anti-(pro) convertin. (c) The *plasma cofactors* for thromboplastin generation appeared normal, according to preliminary tests, using known hemophilic (AHF-deficient) or PTC-deficient plasmas as substrate and following the prothrombin utilization rate (P.U.R.) in recalcified systems, modified, in the present studies, by addition of AcG (bovine). In these systems, the patient's plasma corrected the substrate defect, and, when Y and N plasma mixtures were tested, there was no evidence of any anti-AHF or anti-PTC. However, *P.U.R.* on Y's plasma alone (without added AcG), on first examination of this case, was markedly deficient, with only 33% (instead of the normal>90%) prothrombin consumed in 1 hr at 37°C. Another test series, on the last day of hospitalization, gave a normal P.U.R. However, there was much more rapid prothrombin utilization when AcG was also added. (d) *Protamine titrations* were performed on recalcified Y's plasma mixed with serial dilutions of protamine sulfate. The saline control clotting-time, at 37°C, was prolonged to 21½ min. Protamine improved this to 13½ min., but was most effective at very minute concentrations (0.1-0.2 microgram/ml). This, therefore, is similar to most normal plasmas, except for the longer clotting-times throughout. Such a result cannot be taken to indicate the presence of any "heparin-like" factor. (e) The *abnormal* test findings were: 1) lack of AcG and presence of anti-AcG, 2) prolonged clotting-times of whole blood or recalcified plasma, 3) prolonged prothrombin time, 4) prolonged partial thromboplastin time (P.T.T.), *ref.*(4), a 1-stage test in which a brain cephalin preparation is substituted for the brain thromboplastin of the Quick test. 5) Abnormal thromboplastin generation (T.G.). The Biggs-Douglas(5) original test was modified by using brain cephalin instead of platelets. Both the $Al(OH)_3$ plasma and the serum of patient Y gave abnormal test results. When tests 4) and 5) were repeated on equal vol. mixtures of plasmas Y and such deficients as hemophilia (AHF-), Christmas disease (PTC-), Stuart, *ref.*(6), and aged (AcG-), the abnormalities persisted. 6) Abnormal prothrombin consumption (P.U.R.), see (c). The one explanatory common factor is lack of AcG in all these test systems. This lack is imposed, even on reagents originally containing AcG, when mixed with patient's plasma (or serum) supplying the powerful inhibitor. No longer can these test systems perform the purposes for which they are intended. This is both an important piece of evidence of the role of AcG in these systems, and of the disturbing effects of anti-AcG. One previous report(7) in the literature claims anti-AcG as the cause of a bleeding disorder. Many of the present data agree with findings on the German case, but there are a few discrepancies. Some of these may very well be quantitative, but others, such as our complete failure to inactivate the inhibitor with fat solvents, require further elucidation.

Summary. The above data demonstrate an exceptionally interesting and unusual case, namely, a specific inhibitor of AcG (labile factor), occurring in a human subject. Its presence satisfactorily explains the anomalies of a number of clotting tests and accounts for the clinical hemorrhagic syndrome.

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