TABLE II. Mobilization of Accumulated Carcass Cholesterol in Mice Fed Cholic and Hyodeoxycholic Acids, as a Function of Time.

Group	Carcass* total cholesterol, mg/g carcass				
	0 time	1 wk	2 wk	3 wk	4 wk
A. Normal—CFD†	2.45 ± .38	2.43 ± .19	2.66 ± .29	2.55 ± .36	2.55 ± .37
B. Regression—CFD	3.08 ± .43	3.05 ± .16	2.60 ± .19	2.48 ± .29	2.33 ± .49
D. *Idem* + 0.5% hyodeoxycholic acid	3.08 ± .43	3.11 ± .29	2.76 ± .15	2.68 ± .21	2.62 ± .20
E. " + 0.5% cholic acid + 0.5% hyodeoxycholic acid	3.08 ± .43	3.09 ± .14	2.68 ± .19	2.56 ± .37	2.78 ± .35

Statistical comparison of values:

Groups	0 time	1 wk	2 wk	3 wk	4 wk
A and B	$P < .01$	$P < .01$	$P = .64$	$P = .69$	$P = .32$
A and D	"	"	$P = .42$	$P = .45$	$P = .69$
A and E	"	"	$P = .90$	$P \cong 1.00$	$P = .24$

* without liver. † CFD = cholesterol-free diet. ± numbers are stand. dev.

of cholic acid effects by hyodeoxycholic acid suggests that the latter substance acts as an antimetabolite of cholic acid. Although general increases or decreases in metabolic rate would have similar effects on cholesterol mobilization, we have shown(4,7) that under our experimental conditions, the metabolic rate remains constant.

It was hoped that the effects of hyodeoxycholic acid in the mouse could be duplicated in other species, but in preliminary studies in the rat, this substance was ineffective at equivalent dosage levels.

Summary. The effects of cholic and hyodeoxycholic acids on mouse liver and carcass cholesterol mobilization were investigated. Cholic acid inhibited liver cholesterol mobilization, while hyodeoxycholic acid promoted a rapid elimination of accumulated liver cholesterol. Hyodeoxycholic acid reversed the inhibition caused by cholic acid. Neither of these substances effected the rate of carcass cholesterol mobilization.

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Aliphatic Amino Compounds as Inhibitors of Plasminogen Activation. (25585)

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The fibrinolytic system of man is the subject of increasing interest(1). While activators such as streptokinase have been studied extensively, inhibiting agents have received relatively little attention. ε-amino-n-caproic acid (ε-ACA) was first mentioned in this connection in 1957 by Japanese workers who described it as an anti-plasmin agent(2). It was shown subsequently by Ablondi *et al.*(3)

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and Alkjaersig *et al.*(4) that the primary action of this compound is to inhibit plasminogen activation. It is of interest that Müllertz (5) had reported earlier that lysine and ornithine are also inhibitors of plasminogen activation. A systematic study of aliphatic amino compounds seemed desirable. An *in vitro* system for studying plasminogen activation was utilized for this purpose.

Materials and methods. Amino compounds. The compounds tested were obtained from commercial sources. They were amino acids and diamines with a carbon chain of 3 to 8 C's: 1) 3 C's: β-alanine, 2) 4 C's: *DL*-α, *DL*-β and γ-aminobutyric acid and putrescine · 2 HCl, 3) 5 C's: Δ-aminovaleric acid · HCl, Δ-aminolevulinic acid · HCl (methyl ester), *L*-glutamic acid, ornithine · 2 HCl, (*L*+)-arginine · HCl and cadaverine · 2 HCl, 4) 6 C's: ε-aminocaproic acid (ε-ACA), *DL*-norleucine and *L*-lysine · HCl, and 5) 8 C's: ω-aminocaprylic acid. The ε-ACA used here was a commercial preparation from L. Light & Co. Ltd., Colnbrook, England. On high voltage electrophoresis it was homogeneous. *Constituents of in vitro systems*: Bovine fibrinogen with a coagulability of 97-98% was prepared according to Blombäck and Blombäck(6). The thrombin used was thrombin Roche (Topostasin). A preparation of human plasminogen + proactivator was obtained from Kabi, Stockholm. It had been prepared by Kline's modification of Christensen and Smith's procedure from human plasma Fraction III and contained 0.2 casein unit/mg. Human plasmin was a preparation of spontaneously activated human plasminogen with 0.2 casein unit/mg. A highly purified streptokinase preparation containing 500 units/μg nitrogen was supplied also by Kabi, Stockholm. *System for testing inhibition of plasminogen activation.* The inhibitory effect of ε-ACA and related substances on plasminogen activation by streptokinase was tested in a clot system at pH 7.4 and 37°C. Each tube contained the following components in their order of addition: 0.1 ml plasminogen solution (0.1 mg/ml 0.9% NaCl) + 0.5 ml Tris buffer of pH 7.4 and ionic strength 0.15 containing a sufficient amount of the compound to be tested to give final concentrations shown in Table I + 0.2 ml of 0.5% bovine fibrinogen in Tris buffer + 0.1 ml streptokinase solution (50 units/ml 0.9% NaCl) + 0.1 ml thrombin solution (20 N.I.H. units/ml 0.9% NaCl). Lysis time was determined from the time streptokinase was added. In absence of inhibitors an average lysis time of 10 minutes was obtained. In absence of inhibitors and streptokinase no dissolution of the clot was obtained within 48 hours. *Anti-plasmin assay.* The anti-plasmin activity of the compounds was assayed by incubation with spontaneously activated human plasminogen and determining degree of inhibition produced. Plasmin aliquots (0.4 ml containing 1.6 casein units) were incubated with 0.1 ml of a 10^{-1} M solution of the compound for 10 minutes at 37°C. Each sample was then assayed for residual plasmin on heated fibrin plates (subjected to 85°C for 45 minutes) as described by Nilsson and Swedberg(7). The result was expressed as percentage inhibition of the original plasmin solution.

Results. Activity of the various compounds, in order of potency as inhibitors of plasminogen activation, is shown in Table I. ε-ACA, the reference compound, was most potent. Only one other substance, Δ-aminovaleric acid, showed activity at a concentration of 10^{-3} M. Δ-aminolevulinic acid and ω-aminocaprylic acid had significant activity at 5×10^{-3} M. γ-aminobutyric acid and lysine at 10^{-2} M and putrescine, cadaverine and ornithine only at 5×10^{-2} M. Even at the latter concentration, no inhibitory activity was found with the other compounds tested.

None of the substances were anti-plasmin agents as measured by the procedure described under methods.

Discussion. The results indicate that aliphatic amino acids with a carbon chain length of 4 to 8 are inhibitors of plasminogen activation, maximal activity residing in the 6-carbon compound, ε-ACA. That the terminal position for the amino group is critical is shown by the fact that whereas γ-aminobutyric acid has significant activity, α and β-aminobutyric acid do not. Furthermore, ε-ACA is a potent inhibitor whereas α-aminocaproic acid (norleucine) is inactive. A function for the carboxyl

TABLE I. Inhibitors of Plasminogen Activation *In Vitro*.

Inhibitor	Concentration and lysis times*				
	5×10^{-2}M †	10^{-2}M	5×10^{-3}M	10^{-3}M	10^{-4}M
ε-Aminocaproic acid		>20 hr	ca 4 hr	44	16
Δ-Aminovaleric "	>20 hr	ca 3 hr		27	15
Δ-Aminolevulinic "		ca 4 hr	52	16	11
ω-Aminocaprylic "	>20 hr	55	24	11	12
γ-Aminobutyric "	81	25		12	10
Lysine	80	24		13	12
Putrescine	45	12		10	9
Cadaverine	36	11		10	10
Ornithine	34	16		10	9

* Unlabelled values represent clot lysis times in min.; each value represents avg of at least 4 determinations in duplicate. Avg of 52 control determinations was 10 min. with range of 7-16 min.

† No prolongation of lysis time was found at 5×10^{-2}M with the following: β-alanine, α- and β-aminobutyric acid, glutamic acid, arginine, and norleucine.

group as well is shown by the fact that addition of an amino group in the α-positions of ε-ACA (α, ε-diaminocaproic acid or lysine) and Δ-aminovaleric acid (α, Δ-diaminovaleric acid or ornithine) results in considerable reduction of activity. Also, replacement of the carboxyl groups of the 4 and 5 carbon amino acids with amino groups (the diamines, putrescine and cadaverine) results in lessened activity.

ε-ACA appears to be a promising agent for clinical trial in fibrinolysis associated with various diseases or with use of streptokinase for treatment of thromboembolic episodes. Preliminary clinical studies in this laboratory confirm this impression. In addition to possible use of such compounds as therapeutic agents, the question arises whether any of them may play a role in physiologic or pathologic fibrinolysis and thrombosis. The 2 most potent substances, ε-ACA and Δ-aminovaleric acid have not been found in animal tissues. However, the latter compound has been reported by Fosdick and Piez(8) to be present in saliva of patients with periodontitis, presumably as a product of bacterial putrefaction. Local production of this inhibitor of fibrinolysis might account in part for the indolent characteristics of this disease. It is unlikely that naturally-occurring compounds such as Δ-aminolevulinic acid and γ-aminobutyric acid occur in a sufficient concentration in any tissue to exert an inhibiting effect.

Conclusions. Several aliphatic amino compounds are inhibitors of activation of plasminogen by streptokinase *in vitro*. The most potent of these are amino acids with a carbon chain length of 4 to 8 and with the amino group in the omega position. The significance of these findings is discussed.

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