Ginzel, Tschabitscher, and Wayand(5) and of Bowers(6) with o-methoxyphenylglycerol ether in the treatment of tetanus could be explained on the basis of inhibition of polysynaptic reflexes. These authors found that side reactions such as hemolysis, anemia and hemoglobinuria which are frequently produced by the i.v. injection of Myanesin were not produced by the i.v. administration of o-methoxyphenylglycerol ether, although the same well-known pharmacological effects on spinal reflexes described for Myanesin by Berger and Bradley(8) and by Henneman, Kaplan and Unna(9) could be obtained.

It can be stated with certainty that Myanesin is the more potent drug on a milligram per milligram basis, its potency being probably about 3 times that of Resyl. On the other hand the LD_{50} of Myanesin in rats is about 3 times lower than the LD_{50} of Resyl. Although higher doses of Resyl are required to obtain quantitatively equal effects as with Myanesin the therapeutic ratio of both drugs is about the same.

Summary. 1. Resyl (o-methoxyphenylglycerol ether) has been found to block effectively the transmission of impulses through the internuncial units of spinal reflex arcs in cats in doses of 75 mg/kg. 2. On a mg/mg basis Myanesin is about 3 times more active than Resyl, however, on the basis of the ratio of activity to toxicity Resyl and Myanesin appeared to be equally effective.

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Effect of Carboxylic and Amino Acids on Fibrinolysis Produced by Plasmin, Plasminogen Activator and Proteases.* (20870)

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It was reported recently that citrate increases the fibrinolysis induced by streptokinase-activated human globulin(1). This agent(2) and the globulin fraction isolated from spontaneously active human blood(3) were later found to be potent activators of plasminogen. Both preparations produce fibrinolysis chiefly by activation of the plasminogen present in the fibrin substrate. In the present paper bovine plasmin and a number of other proteases were compared with the plasminogen activators to determine whether the increased fibrinolysis depends on a modified enzymatic effect on fibrin or on activation of plasminogen in the substrate. A number of chemical compounds were studied in an effort to find a possible relationship between increased fibrinolysis and chemical structure.

Methods and materials. The following plasminogen activator preparations were used: *Streptokinase activated globulin ("S-activator")*: Normal human globulin was activated by streptokinase and subsequently lyophilized(4). Buffer solutions were prepared, in which the globulin concentration was 1/20 of the original concentration in serum. *Spontaneously active globulin ("Spontaneous activator")*: A lyophilized globulin fraction was prepared from spontaneously active blood obtained from human corpses after sudden anoxaemic death(5). Buffer solutions were

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TABLE I. Activity of Fibrinolytic Agents on Fibrin Containing Salts of Carboxylic and Amino Acids.

Agent	Preparation	Fibrin	Citrate	Tartrate	Succinate	Malonate	Oxalate	Lactate	L-Aspartic acid	Glycine	L-Lysine, 0.0062 M	L-Lysine, 0.0125 M
S-activator	I	A	220	200				104				
		B	232	159	180	127	146	99	108	91	120	
		C	170		144		100			90	100	
		E	255		186		156		156	112		50
	II	E	320		166		130		186	124		52
	Mean		239	180	167	127	144	102	150	104	110	51
Spontaneous activator	I	A	125	200								
		B	116	146	147	113	134	109	44	81	0	
		C	144		108		120			60	16	
		E	300		190		214		108	84		0
	II	E	295		240		180		86	58		0
	Mean		196	173	171	113	162	99	79	71	8	0
Bovine plasmin	I	C	106		96		112			84	52	
		E	112		114		108		108	92		64
	II	E	100		110		98		100	96		58
	Mean		106		107		106		104	91	52	61
Trypsin		A	120					108				
		B	127	116	127	107	104	102	100	106	105	
		C	124		94		64			96	56	
		D	100		88		88		80	100		116
		E	106		124		98		104	100		104
	Mean		115	116	108	107	89	105	95	101	81	110
Chymotrypsin		CDE	103×		99×		99×		95+	98+		103+
Aspergillus protease		CDE	100×		108×		90×		87+	120+		112+
B. subtilis protease		CDE	81×		95×		74×		93+	106+		110+

I and II represent different preparations of active agents. Final concentration of added compounds was 0.0125 M. Different batches of fibrinogen (ABCDE) were used to prepare the fibrin plates and one series of experiments (batch B: 2-3) was performed with each batch. For the 3 last proteases only means of values for 3 (×) and 2 (+) batches of fibrinogen are given (max. dev. 15%).

prepared in which the globulin concentration was one-half of the original concentration in serum. The following proteases were used: *Bovine plasmin.* A spontaneously active preparation of bovine globulin(6). *Trypsin.* A 0.05% solution of bovine crystalline trypsin (Armour, 50% $MgSO_4$) in N/400 HCl, diluted 1:25 with buffer before use(4). *Chymotrypsin.* A 0.1% solution of crystalline chymotrypsin (Armour, 50% $MgSO_4$), diluted 1:15 with buffer before use. *Aspergillus protease*(7) 0.02% in buffer. *B. subtilis protease*(7) 0.05% in buffer. *Barbital buffer.* 0.1 M; pH 7.6. Fibrinolytic activity was estimated by the fibrin plate method(4,8) and the active agents were used in concentrations which produced normal fibrinolytic activities of the same magnitude. Commercial samples of high purity were used of: sodium citrate, sodium succinate, sodium oxalate, sodium sulfate, sodium fluoride, sodium-potassium tartrate, lactic acid, malonic acid, L-aspartic acid, glycine, dihydrochlorides of L-lysine and L-ornithine, glycerol, glucose, lactose, ethanolamine, and pyridine. Neutralized solutions of these compounds were added to freshly prepared(8) fibrinogen solutions. Equal amounts of buffer were added and the mixtures were diluted with saline to a concentration of 0.1% fibrinogen, an ionic strength of 0.15(8), a pH between 7.55 and 7.60 (controlled by a glass electrode), and a concentration of added compounds of 0.0125 M. The final solutions were used to prepare fibrin plates. The activities found on plates with fibrin containing added compounds were con-

verted into percentages of the activity on plates containing normal fibrin by interpolation on dilution curves obtained with the appropriate active agents(4). The standard deviation on a single estimation is 11%(9). Mean values of duplicate estimations (each consisting of 3 replicates(4)) were used.

Results. The activity of the plasminogen activator preparations and plasmin was not significantly influenced by a calcium precipitant (sodium fluoride, 5 experiments), a divalent inorganic anion (sodium sulfate, 6 experiments), and some organic hydroxyl compounds (glucose, 5 experiments; lactose, 3 experiments, and glycerol, 3 experiments).

The effects of a number of organic acids are summarized in Table I. The enhancing and inhibitory effects were pronounced only in experiments using *activator preparations* and the effects seemed to depend on the number of carboxyl and amino groups. Lactate (amonocarboxylic acid) was without effect. All dicarboxylic acids produced a considerable increase in the activity of the activator preparations, and citrate (atricarboxylic acid) was most effective. However, the spontaneous activator produced more variable results than the S-activator. The difference between the 2 activator preparations was especially pronounced in the experiments with amino acids. The activity of S-activator was enhanced by aspartic acid (amonoaminodicarboxylic acid) and unaffected by glycine (amonoaminomonocarboxylic acid) while the activity of the spontaneous activator was somewhat decreased in both instances. Lysine (adiaminomonocarboxylic acid), in 0.0062 and 0.0125 molar, concentrations, produced respectively a strong and a complete inhibition of the spontaneous activator, while the S-activator was only moderately inhibited by the stronger concentration. Identical results were observed in one series of experiments with L-ornithine (0.0062 M). Some amines (ethanolamine and pyridine) did not significantly influence the active agents.

The activities of the *proteases* were generally not significantly influenced. Bovine plasmin and *B. subtilis* protease were moderately inhibited by lysine and di- and tricarboxylic acids respectively. The results of trypsin experiments on different batches of fibrin varied much more than those obtained in similar experiments with other proteases. A slight increase in the activity of trypsin was produced by citrate.

The difference observed in experiments with active agents from human blood and with bovine plasmin strongly supports the assumption that these substances are different entities, *i.e.*, plasminogen activators and plasmin proper(2,3). Only the activity of plasminogen activators was enhanced by carboxylic acids. The large variations in the activity of trypsin might be caused by a varying susceptibility of the different batches of fibrin to the direct fibrinolytic and to the plasminogen activating potency of this enzyme(7,10). The reaction between bovine plasmin and the fibrin substrate was unaffected by carboxylic acids. These results suggest that the increased fibrinolysis induced by carboxylic acids depends on the activation of the plasminogen in the fibrin substrate.

The final (enhancing or inhibitory) effect appeared to depend on the number of carboxylic and amino groups in the organic acid. Ginsburg, de Vries and Katchalski found that lysis of human plasma clots (induced by addition of streptokinase, chloroform or menstrual blood) was inhibited by polylysine, polyornithine and polyarginine. The monomers were ineffective under their experimental conditions(11). Polyaspartic acid obviated the antifibrinolytic effect of these basic amino-acid polymers *in vitro*(11) and prevented their toxic manifestations *in vivo*(12).

Summary. 1. Fibrinolysis produced by activation of plasminogen in bovine fibrin by plasminogen activator preparations from human blood was increased considerably by di- and tri-carboxylic acids and decreased by diaminomonocarboxylic acids. 2. Fibrinolytic activity of a number of proteases was generally not significantly influenced. Bovine plasmin was moderately inhibited by lysine.

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Blocking Effect of Ethyl Chloride Spray on Cardiac Pain Induced by Ergonovine.* (20871)

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Under suitable conditions ethyl chloride spray has proved effective in blocking the somatic component of cardiac pain when this occurs in acute myocardial infarction, angina pectoris and the shoulder-hand syndrome associated with coronary insufficiency(1-5). In initial trials by one of the authors (I.S.) ethyl chloride spray also relieved the angina induced by ergonovine in patients with effort angina and a normal electrocardiogram (Stein ergonovine test(6-8)). In such subjects with pre-existing coronary insufficiency, the intravenous injection of ergonovine induces electrocardiographic changes and/or pain which matches in character and location that induced by exertion; nitroglycerin promptly stops the pain and reverses the changes in the S-T segment and T-wave. The purpose of this investigation was to study more systematically the blocking effect of ethyl chloride spray in such patients with impaired coronary circulation and a positive Stein test.

Materials and methods. Thirty-six tests were done on 7 patients (44 to 70 years, 5 males and 2 females) who had a history of angina of effort and a normal resting 12-lead electrocardiogram. Three patients had moderate hypertension. One had a history of old myocardial infarction. None had congestive failure. At each test, the exact location of pain induced by walking was noted. A control blood pressure, pulse rate and resting electrocardiogram were taken. Leads I, II, III, aVr and V_4 were recorded on a direct-writing 2-channel Viso-cardiette. While Lead V_4 alone or Leads II and V_4 were being recorded, a colorless solution containing U.S.P. ergonovine maleate, 0.2 mg per ml of distilled water, was injected intravenously at the rate of 1 ml per minute. Injection was stopped when either pain or electrocardiographic changes appeared. Leads I, II, III, aVr and V_4 were repeated and blood pressure and pulse rate again determined about 1, 5 and 10 minutes after the end of the injection. When the test became positive, a sublingual dose of nitroglycerin (0.6 mg) was administered to terminate pain and/or electrocardiographic changes. This was usually given 6 to 8 minutes after the ergonovine, but occasionally only 2 minutes afterward if chest pain was intense. Electrocardiograms were taken until record became normal, usually 2 to 5 minutes after the nitroglycerin. At the first trial no more than 0.2 mg of ergonovine was injected. If 0.2 mg did not produce a positive effect, the

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