Synthetic Activators of Fibrinolysis which Possess Anticoagulant Property

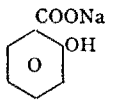
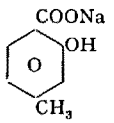
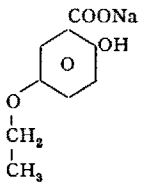
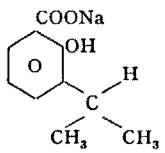
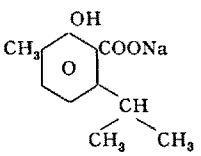
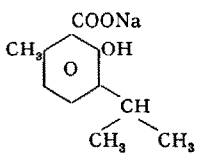
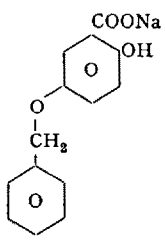
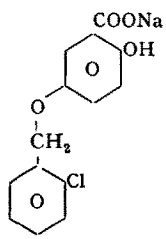
During the search for better thrombolytic agents it was observed that certain synthetic organic compounds when dissolved and incubated with human plasma resulted in a marked fibrinolytic and caseolytic activity[1]. The fibrinolytic activity generated in the presence of these compounds is quite stable in contrast to that induced by enzymes. This was, at least partially, explained by the destruction of antiplasmin[2]. There is a clear-cut relationship between the chemical structure of the compounds and their fibrinolysis-inducing ability[1,3,4]. One essential requirement for a synthetic compound for producing fibrinolytic activity appears to be a large asymmetric anion carrying one hydrophilic side chain and certain hydrophobic substituents. It was found that some substituted salicylic acid derivatives are in the test tube quite active 'fibrinolysis-inducers'. For serial testing, a newly developed procedure was used[5]. The method is based on the ability of a solution of the compounds in buffered saline (pH 7.4) to lyse on incubation preformed clots obtained by recalcification of human citrated plasma. Table I shows that by systematic modification of substituents of the salicylic acid molecule increasingly more active compounds are derived. The modifications eventually produced a more than fifty-fold increase of activity as compared with the mother compound. It can be assumed that further research will yield compounds with higher activity and a therapeutic potentiality as thrombolytic agents.

The assessment of the fibrinolytic potentiality of synthetic organic compounds requires serial testing at various molarities, because there is an optimal range of concentration above or below which they do not induce fibrinolytic activity. *Ortho*-thymotic acid sodium salt illustrates this phenomenon. It has a range of fibrinolysis-inducing activity from 0.03 to 0.01 M for the majority of plasma clots. With some plasma clots the effective range may extend to 0.04 or drop to 0.008 M with others. *Ortho*-thymotic acid sodium salt also provides a striking example of the close relationship between structure and fibrinolysis-inducing ability; shifting of its hydroxyl group from *ortho*- to *para*-position produces a totally ineffective compound.

Plasma containing *o*-thymotic acid sodium salt at appropriate concentrations clots after recalcification and a structurally normal clot (thrombelastogram) results. These clots lyse on incubation.

Ortho-thymotic acid sodium salt exhibits well an interesting property which is common to all synthetic organic fibrinolysis-inducing compounds (so far observed): it exerts a concentration-dependent anticoagulant effect which varies to some extent from plasma to plasma. A representative anticoagulant and fibrinolysis experiment with *o*-thymotic acid sodium salt using the plasma of a healthy donor is shown in Table II. The concentration-dependent anticoagulant and fibrinolytic ef-

Table I. Fibrinolysis (clot lysis) by salicylic acid derivatives. Effect of modifications of substituents on activity

Compound structure	Name	Activity at 0.0 M	Clot dissolution after 24 h incubation
	salicylic acid Na	0.2	traces
	*p*-methylsalicylic acid Na	0.07	partial
	5-ethoxysalicylic acid Na[6]	0.08	complete
	3-isopropyl-salicylic acid Na[6]	0.02	complete
	2-hydroxy-*p*-cymene-3-carboxylic acid Na[6]	0.02	complete
	*o*-thymotic acid Na	0.01	complete
	5-benzyloxy-salicylic acid Na[6]	0.008	complete
	5-(2'-chloro benzyloxy)-salicylic acid Na[6]	0.004	complete

[1] K. N. von Kaulla, *Chemistry of Thrombolysis: Human Fibrinolytic Enzymes* (C. C. Thomas Publisher, Springfield 1963).
[2] K. N. von Kaulla, Thrombos. Diath. Haem. *9*, 220 (1963).
[3] K. N. von Kaulla, Arch. Biochem. Biophys. *96*, 4 (1962).
[4] K. N. von Kaulla, Thrombos. Diath. Haem. *7*, 404 (1962).
[5] K. N. von Kaulla, J. med. Chem. *8*, 164 (1965).
[6] These compounds were kindly provided by Dr. J. M. Sprague, Merck Sharp & Dohme Research Laboratories, West Point (Pa. USA).

fect is clear-cut. The plasma used was purposely not diluted by the recalcification technique used[7].

Synthetic organic compounds which exhibit both fibrinolytic and anticoagulant activity are bound to have therapeutic potentialities and call for further investigation. Their mechanism of action is unknown except to say that it cannot be demonstrated that the compounds directly activate purified human plasminogen, and yet an activation of the fibrinolytic system of human plasma occurs. The compounds are poorly active or non-active in bovine, cat, dog, rabbit, or rat plasma. As a working hypothesis, it is assumed that the fibrinolysis-inducing synthetic compounds inhibit (or neutralize) an antiactivator of the plasminogen system. They are furthermore capable of inhibiting the clotting process at a yet undetermined level[8].

Table II. Anticoagulant and clot-dissolving properties of *o*-thymotic acid sodium salt

mg compound per ml plasma	Mol	Recalcification time (min)	Clot dissolution after 24 h incubation
4.3	0.02	65	complete
3.87	0.018	19	complete
3.44	0.016	10	complete
3.01	0.014	6	complete
2.58	0.012	4.30	partial
2.15	0.1	4	none
1.72	0.008	4	none

Zusammenfassung. Derivate von Salicylsäure aktivieren im Reagenzglas das fibrinolytische System des menschlichen Plasmas und üben eine gerinnungshemmende Wirkung aus. Durch planmässige Veränderungen der Substituenten des Salicylsäuremoleküls gelang es, in zunehmendem Masse Verbindungen mit steigender fibrinolytischer Aktivität aufzufinden. Die gerinnungshemmende und fibrinolytische Wirkung wird am Beispiel der *ortho*-Thymotinsäure erläutert.

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[7] K. N. VON KAULLA, J. Thorac. Surg. *36*, 857 (1958).

[8] The work reported here was supported by the American Heart Association and in part by the Idaho and the Wyoming Heart Associations.

Cardiac Mitochondrial $NADH_2$-Cytochrome c Reductase and Cytochrome Oxidase after Cardiopulmonary By-Pass in Dog[1]

During and after extracorporeal circulation procedures, cardiac mitochondrial succinate dehydrogenase activity[2], and oxygen consumption rate measured by the differences between coronary arterial and venous oxygen content of blood[3] are decreased. Also, certain changes in the physicochemical properties of the actin-myosin system are observed following heart-lung by-pass procedures[4] in the dog. However, the possibilities of metabolic derangements in cardiac sarcosomes are numerous[5]. Thus, it is necessary to investigate the possible changes of mitochondrial function at the NAD-$NADH_2$ to cytochrome c, and at cytochrome c to oxygen. The cardiac mitochondrial $NADH_2$-cytochrome c reductase and cytochrome oxidase activities are studied[6,7] after partial heart-lung by-pass.

Methods. A method of cardiac mitochondrial extraction is reported elsewhere[8]. The details of isovolemic and normothermic partial cardiopulmonary by-pass perfusion procedures have also been reported previously[4]. A total of 15 dogs of both sexes, weighing 14 to 20 kg each, are anesthetized with chloralose (80 mg/kg). Of these, 10 dogs are used as the experimental group. Cardiac mitochondrial cytochrome oxidase is measured according to a modified method of SMITH[7]. The rate of oxidation of $NADH_2$ by cytochrome c (probably mediated by the $NADH_2$-cytochrome c reductase of MAHLER et al.[6]) is studied by a macro-oxygen electrode (Clark type), a specially designed magnetic stirring reaction chamber contained within a temperature-controlled water bath (a total capacity of 300 ml), and recorded on a Beckman 160 Physiological Gas Analyzer (Palo Alto, Calif. USA). The water in the bath is circulated by a Haake thermoregulator and pump. The reaction chamber contains $0.05\,M$ phosphate buffer, pH 7.4, $2 \cdot 10^{-5}\,M$ cytochrome c, and $1.2 \cdot 10^{-3}\,M$ $NADH_2$ in a final volume of 4.4 ml, equilibrated for 10 min at 25°C. The reaction is started by adding 0.1 ml of mitochondrial suspension (protein concentration of 2.08 to 3.4 mg per ml, pH 7.4, in $0.17\,M$ phosphate buffer and $0.25\,M$ sucrose solutions), and the oxidation rate of $NADH_2$ is measured for 10 min[9].

Results and discussion. The present study suggests that the cardiac mitochondrial cytochrome oxidase and the rate of $NADH_2$ oxidation by cytochrome c (probably

[1] This study was supported by Research Grant HE-09016 from the National Institutes of Health, U.S. Public Health Service.

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[3] C. DENNIS, D. P. HALL, J. R. MORENO, and Å. SENNING, Circulation Res. *10*, 298 (1962).

[4] Y. W. CHO and P. M. GALLETTI, J. thorac. cardiovasc. Surg. *47*, 628 (1964).

[5] T. P. SINGER, Ann. N.Y. Acad. Sci. *72*, 480 (1959).

[6] H. R. MAHLER, in *Methods of Enzymology* (Ed., S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 2, p. 688.

[7] L. SMITH, in *Methods of Enzymology* (Ed., S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 2, p. 735.

[8] Y. W. CHO, J. THEOGARAJ, M. M. AVIADO JR., and S. BELLET, Arch. int. Pharmacodyn. *155*, 225 (1965).

[9] E. G. BALL and O. COOPER, Proc. Nat. Acad. Sci. *43*, 357 (1957).