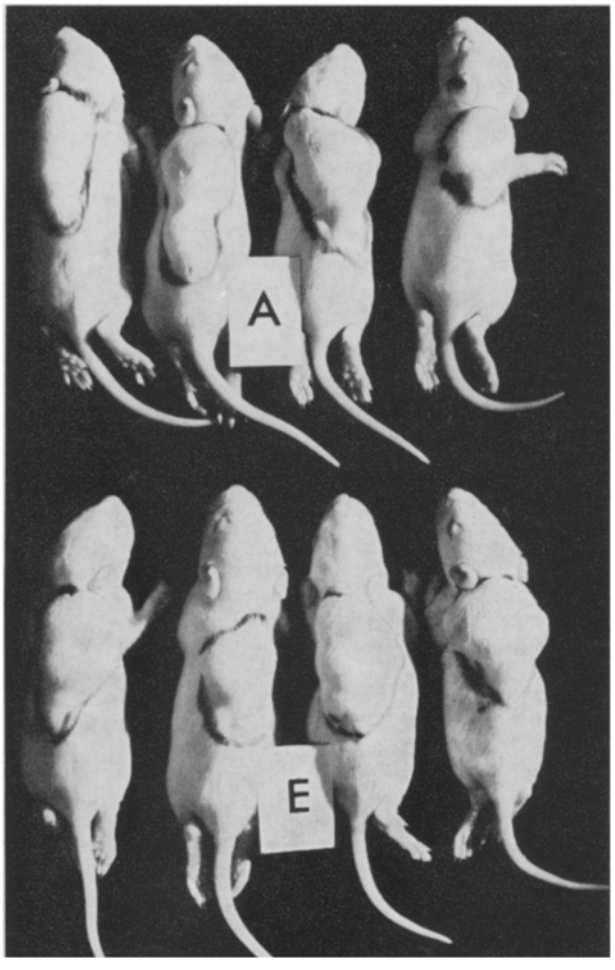

Fig. 4. Two groups of rats inoculated with tumour suspended in PGA and PGE solutions within 24 h of birth. Normal tumour growth 3 weeks after inoculation. No difference in size between the PGA and the PGE group.

beginning on the 6th day[6,8]. The second phase is the effector phase[8]. Sufficient PG must be available at the graft site to promote the diapedesis and migration of monocytes in both phases and at the same time, to counterbalance the PG antagonist secreted by tumour.

The above considerations obviously also hold true for the injection of PG into established i.e. 6-day or older tumour. Administration of PG would induce the initial phase on the 6th-7th day and the second effector phase on the 13th–14th day. By that time the tumour would have attained a diameter of at least 20 mm. The amounts of PG required to induce an effective mononuclear infiltration into tumour of that size, would have to be very large. Large amounts are likely to be poorly tolerated[15].

From these observations it appears that the PGs, particularly PG A2, are capable of inhibiting the implantation of tumour cells. They are not indicated as a treatment of established tumour.

Zusammenfassung. Allergische Reaktionen werden unter anderem durch Prostaglandin erzeugt. Krebsgewebe produzieren eine prostaglandinhemmende Substanz, und es wird nachgewiesen, dass grosse Dosen von Prostaglandin diese Substanz neutralisieren und eine allergische Reaktion um die Krebszellen hervorrufen, wodurch die Implantation der Tumorzellen verhindert wird.

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Niflumic Acid, Prototype of a Multiaction Antithrombotic Agent[1]

Antiaggregating agents, anticoagulants, and fibrinolytic enzymes each possess only one single antithrombotic property, thus interfering with just one phase of the intravascular clotting process. In this communication the interesting combination in principle of antithrombotic properties, of the antirheumatic drug, Niflumic acid (3-fluoromethyl-3-phenylamino-2-nicotinic acid)[2] is reported.

Methods. Enhancement of fibrinolytic activity induced by streptokinase and urokinase by Niflumic acid (Siegfried, Zofingen/Switzerland) was measured with the rotating standard clot[3]. This cylinder-shaped clot can be lysed on one end only, thus imitating a small human vessel which is completely occluded by a thrombus. The rate of its dissolution is read in microliters. The fibrinolytic activity exerted by Niflumic acid was assessed by determining the lowest molarity inducing fibrinolytic dissolution of a human hanging plasma clot[4] after 24 h incubation. Clots were suspended either in buffered saline pH 7.4 (BS) or human plasma which was heparinized (1 unit/ml) in order to prevent clotting caused by the thrombin released from the dissolving clot. Only plasma which dissolved within 12 h when clotted in presence of 7.5 units streptokinase was used. With the use of N_2H_4, the hanging clots were preincubated in BS containing 2.5 m*M* N_2H_4 for 3 h before Niflumic acid was added, and the mixture was then incubated for 24 h. The fibrin plates were made from a 2.5% solution of commercial human fibrinogen in BS. The fibrinolytic activity induced in rats by Niflumic acid was assessed with the micro-euglobulin lysis time[5], its effect on collagen-induced aggregation of platelets in plasma with the Dual Sample Aggregometer[6], and its inhibition of fibrinogen-

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Table I. Enhancement by Niflumic acid of the fibrinolytic activity of streptokinase and urokinase.

	Clot dissolved (µl)	Enhancement (%)
Niflumic acid (3 m*M* alone)	0	—
Streptokinase (5 U/ml alone)	14	—
Streptokinase + Niflumic acid (3 m*M*)	46	328
Urokinase (10 CTA U/ml alone)	12	—
Urokinase + Niflumic acid (3 m*M*)	60	500

Rotating standard clot method[3]. Readings after 24 h incubation at 37 °C.

induced aggregation of human erythrocytes by a sedimentation technique[7].

Results. The fibrinolytic activity of streptokinase and of urokinase is markedly enhanced as measured with rotating standard clot by a concentration of Niflumic acid which does not alone induce fibrinolysis. This is shown in Table I.

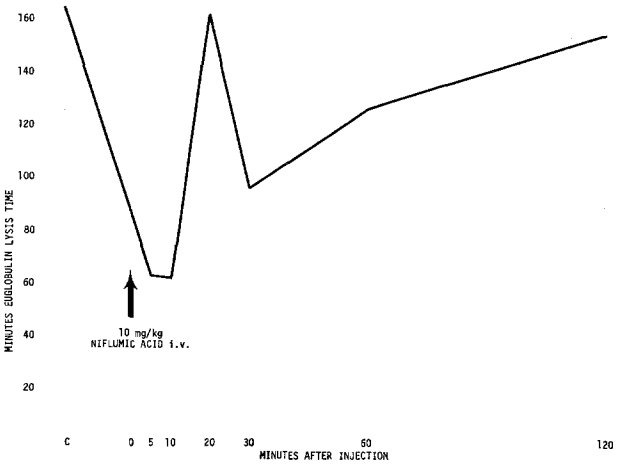

Fig. 1. Induction of fibrinolytic activity (shortening of the euglobulin lysis time) in a rat by the i.v. injection of 10 mg/kg Niflumic acid.

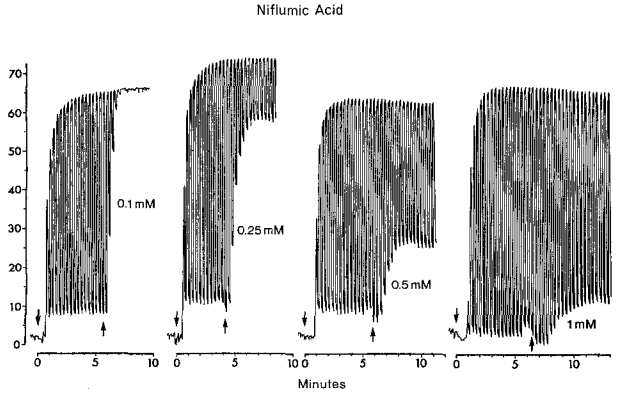

Fig. 2. Collagen-induced aggregation of human platelets in plasma. Antiaggregating effect of various concentrations (in m*M*) of Niflumic acid. Dual sample aggregometer[6]. The first arrow (↓) indicates collagen addition to control cuvets. The second arrow (↑) indicates collagen addition to test cuvets containing various concentrations of Niflumic acid. Note the progressive inhibition of aggregation with increasing concentration of Niflumic acid.

Niflumic acid alone in BS induced lysis of hanging plasma clots at 8 m*M*, and in presence of 2.5 m*M* hydrazine at 4 m*M*. N_2H_4 by itself does not induce fibrinolytic activity. Niflumic acid, in combination with N_2H_4, is in vitro much more powerful in dissolving preformed human plasma clots than streptokinase or urokinase are, as indicated by Table II. Preformed human plasma clots were suspended in 2.5 ml of samples of human plasma containing either urokinase, streptokinase, or the Niflumic acid-N_2H_4 combination. The hanging clot lysis was recorded after 24 h incubation. Also, after 6 h incubation, 0.03 ml of each plasma sample were applied to human fibrin plates which were read 18 h later. The plasma with streptokinase and urokinase exerted stronger fibrinolytic activity on the fibrin plates than did the plasma with Niflumic acid plus N_2H_4, but did not dissolve the performed hanging plasma clots. In contrast, the plasma containing Niflumic acid plus N_2H_4 lysed the preformed hanging plasma clot completely.

Niflumic acid also shortens the euglobulin lysis time after i.v. injection of 10 mg/kg into rats as shown in Figure 1: 5 min. after injection there is a marked shortening of the euglobulin in lysis time followed by a temporary rebound effect observed in all animals. The rebound effect can be eliminated by pretreatment with N_2H_4, a phenomenon under study. Although this fibrinolytic activity in vivo is short-lived, similar to that induced by i.v. adrenalin or nicotinic acid, it reveals that fibrinolytic activity is generated by Niflumic acid in vivo as well as in vitro.

Collagen-induced aggregation of human thrombocytes in plasma is reduced or prevented by Niflumic acid as shown in Figure 2. Furthermore, Niflumic acid prevents fibrinogen-induced aggregation of human erythrocytes, as demonstrated by the following representative data: Erythrocyte sedimentation after 2 h (control): 4 mm, Ery + fibrinogen; 38 mm, Ery + fibrinogen + Niflumic acid, 0.01 m*M*; 40 mm, 0.05 m*M*; 8 mm, 0.1 m*M*; 2 mm.

Discussion: Many bulky non-symmetric organic anions induce fibrinolytic activity when added to human plasma in vitro[8] by reducing antiplasmin[9] and antiactivator-activity[10], endowing a protein fraction with plasminogen activator activity[11], and also inhibit to various degrees collagen-induced aggregation of human platelets[12].

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Table II. Preformed plasma clot dissolving versus fibrinolytic activity. Inefficiency of streptokinase and urokinase as compared to Niflumic acid plus N_2H_4

Hanging clot in plasma containing	Lysis of hanging plasma clot	Fibrin plate Lysis area mm^2
Streptokinase (100 U/ml)	none	156
Urokinase (250 CTA U/ml)	none	280
12 m*M* Niflumic acid + 2.5 m*M* N_2H_4	complete	139

For readings, see text.

Small concentrations of N_2H_4 enhance the fibrinolytic activity induced by synthetic compounds, in part by a further reduction of antiplasmin activity[13]. There are 2 types of synthetic compound-induced fibrinolytic activities: in vitro or 'chemically' induced[8] and in vivo or 'pharmacologically' induced[14]. The vasoactive drug, nicotinic acid, is an example of this 'pharmacological' type of compounds which is inactive in vitro. Niflumic acid, a trifluoro-methylphenyl amino derivative of nicotinic acid as shown in this communication, induces fibrinolytic activity by both the 'chemical'[15] and the 'pharmacological' pathway, thus demonstrating the possibility to design drugs inducing fibrinolysis by both mechanisms. Niflumic acid enhances the fibrinolytic activity of both streptokinase and urokinase. It is also of interest that fibrinolytically inactive concentrations of a congener of Niflumic acid, flufenamic acid, enhance very markedly in vitro the endogenous fibrinolytic activity of pig plasma, when fibrinolytic activity is generated in vivo by a liver bypass[16]. Furthermore, as shown with the model of hanging clot in plasma, Niflumic acid is a much more effective agent for dissolving preformed clots than streptokinase or urokinase. This difference appears to be due to the small molecular size of Niflumic acid, as compared to the large size of streptokinase and urokinase. This enables it to diffuse quickly into the clot, thus inducing fibrinolytic activity from within[17]. The fact that Niflumic acid possesses, in addition to fibrinolytic properties mentioned above, the ability to prevent platelet and also erythrocyte aggregation which play a role in thrombus formation[18], makes it an attractive prototype to be used as a stepping stone for the development of optimal multiaction antithrombotic agents.

Zusammenfassung. Niflumsäure, Prototyp eines mehrfach wirkenden antithrombotischen Medikamentes, induziert Fibrinolyse in vitro und in vivo, hemmt Erythrozyten- und Thrombozytenaggregation und induziert eine stärkere Fibrinolyse vorgebildeter Gerinnsel als Strepto- und Urokinase.

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Possible Cellular Localization of Cholecystokinin-Pancreozymin

A number of electron-microscopic studies were carried out on the duodenal mucosa, and a total of 7 types of endocrine cells have been identified. According to the latest classification[1], the cell types in question are the EC, S, EG, I, D, D_1 and G types. However, the hormonal product is known only with some of these types of endocrine cells. The enterochromaffin (EC) cells product is the 5-hydroxytryptamine[2], the G type cells product is the gastrin[3–5], the S type cells product is the secretin[6,7], the EG type cells produce the enteroglucagon[8] and the D_1 type cells produce the gastric inhibitory polypeptide[9]. In all the cell types enumerated, with the exception of the EC cells, the presence of the hormone in the cell was proved by means of specific antisera, via immunofluorescence.

The hormonal product of the rest of the cells (the D type cells and the I type cells) is not known. Theoretically probable are, beside other substances, first of all the motilin[10] and the cholecystokinin-pancreozymin (CCK-PZ)[11], it was on the duodenal mucosa that the 2 substances have been reliably determined by biochemical analysis. Application of antisera to identify the CCK-PZ in the cells carries with it a danger of non-specific reactions, as is indicated in works of VANNORDEN and PEARSE[12], who

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