Biochemical Pharmacology, 1964, Vol. 13, pp. 391–394. Pergamon Press Ltd., Printed in Great Britain.

A STUDY OF ELASTASE INHIBITORS OF PLANT ORIGIN

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(*Received* 27 *September* 1963; *accepted* 13 *November* 1963)

Abstract—Elastase inhibitors have been found in beans and potatoes and have been shown to be the most effective inhibitors of elastase known at the present time. Their elastase-inhibiting activity is pharmacologically shown *in vitro* and *in vivo*.

THE inhibitor of pancreatic elastase has been demonstrated in the animal body, i.e. in the blood serum and in the pancreas (Baló and Banga[2], Tolnay and Bagdy,[10] Loeven[5] etc.). It is also known that protease inhibitors can be isolated from plants (Kunitz,[4] Werle *et al.*,[11] Sohonie *et al.*,[8] Mansfeld *et al.*,[6] etc.). We have therefore investigated polypeptidic and proteic materials from beans and potatoes, for their ability to inhibit elastase. These substances inhibit not only the elastolysis but also the casein-splitting effect of elastase. The inhibitor from beans shows, in addition, a considerable anti-tryptic activity, while the inhibitor from potatoes inhibits trypsin to only a negligibly small extent.

This paper deals with pharmacological investigations concerning the anti-elastase action of these materials.

It is known that elastase is able to damage the elastic fibres of the vessel wall (Romhányi[7]). When strips of rabbit aorta are incubated for 30 min in a suitable buffer solution containing 10 or 20 elastase units/ml (EU/ml), one observes: oedematous thickening of the intima or of the whole aortic wall, britteling of the elastic fibers and elastolysis. These changes did not occur if inhibitors were added to the system (Figs. 1–4).

In other experiments, the carotid arteries of dogs under Numal anaesthesia were prepared as an isolated circulatory system to study the effects of perfusion with various solutions *in vivo* by registering before and after perfusion the oscillogram of these carotis sections. As shown in Fig. 5, the oscillogram remained normal after perfusion with physiological saline; however, if 20 EU/ml are added to the solution, a significant widening is observed. The elastase-inhibiting effect is demonstrated on the other carotid artery of the same dog, where the perfusion is carried out with the saline containing the same amount of elastase plus inhibitor.

Borsy *et al.*[3] reported that elastase causes similar contractions of isolated rat uterus as trypsin. These contractions were not induced by elastase in the presence of our inhibitors. As shown in Fig. 6, the addition of two inhibitor units (IU) reduced to less than 50 per cent the contraction induced by 5 EU. This means a stronger inhibition than that measured biochemically on substrates such as elastin or casein. The serotonin-induced contractions were not antagonized by significantly greater amounts of these inhibitors, which demonstrates their specificity.

In recent experiments dealing with the *in-vivo effects of elastase*, paw-oedema was observed in rats after intraplantar administration of the enzyme.[9] After injection of 20 EU in 0·1 ml of saline, the size of the paw increases by 70–80 per cent, reaching its maximum after 1 hr. If the inhibitors are administered intraperitoneally, half an hour earlier than the oedema exciting agent, the experimental data show (Table 1) that:

1. The substances studied influence not only the oedema-producing effect of elastase, but that of dextran as well.
2. There is no close relationship between the amount of inhibitor administered and the oedema-reducing effect produced.

It is therefore concluded that their effects are the consequence rather of an anti-inflammatory than of a specific anti-elastase action.

TABLE 1. INHIBITION OF PAW-OEDEMA OF RATS INDUCED BY INTRAPLANTAR INJECTION OF 0·1 ml OF ELASTASE (200 EU/ml) AND DEXTRAN (3%).

Substances investigated	Dosages, mg/kg i.p.	Inhibition of paw oedema, %, induced by,	
		Elastase	Dextran
Bean inhibitor	50	28	35
[200 IU/mg]	100	43	60
Potato inhibitor	100	25	3
[54 IU/mg]	200	50	27

Studying the toxic effect of elastase, Borsy *et al.*[3] have observed that mice, after being injected with large amounts of this enzyme, die within a few minutes, showing severe symptoms of lung bleeding. The weight of the hemorrhagic and oedematous lungs was significantly greater than that of the normals and the destruction of the elastic elements could be demonstrated histologically. We observed recently, however, that such an *in-vivo* elastolysis is not elicited by all elastase preparations, and shows no close correlation with dosage.[9] As shown in Fig. 7, our purest elastase preparation (purissimum [1]), administered at the same dosage as a less purified one (purum), is much less active from that point of view. This is tentatively ascribed to the lack, in the purissimum elastase, of elastomucoproteinase fraction necessary for the *in-vivo* elastolysis.

The inhibitory effect of our plant extracts were investigated against the elastase purum causing lung elastolysis. Physiological sodium chloride solution or a known amount of the inhibitor was injected intravenously 1 min before the intravenous administration of 4000 EU/kg, a surely toxic dose. The following facts were observed (Fig. 8): The greater the amount of inhibitor, the lesser the toxicity of elastase, the number of deaths becoming smaller and death occuring after more protracted periods. A dosage of 20,000 IU/kg, i.e. 100 mg/kg, fully antagonizes the toxic effect of 4000 EU/kg, i.e. 400 mg/kg elastase, including the effect on the lungs (Fig. 9), in which organ, the elastic elements usually destroyed by elastase are then well preserved (Figs. 10–12).

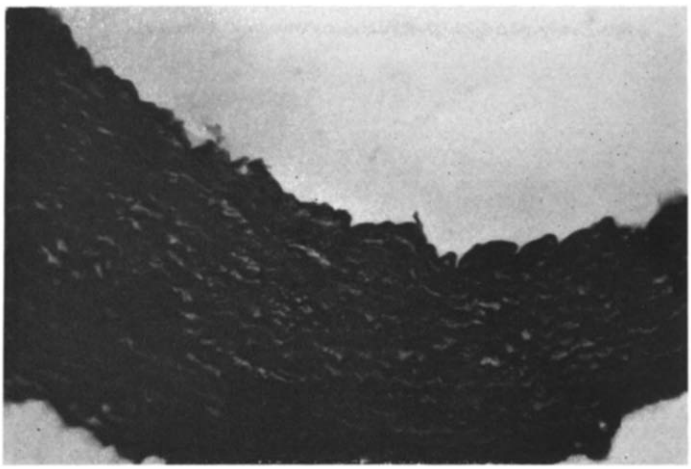

FIG. 1. Rabbit aorta after being incubated for 30 min at 37° in a Na_2CO_3-$NaHCO_3$-NaCl buffer solution (pH 8·9): without elastase.

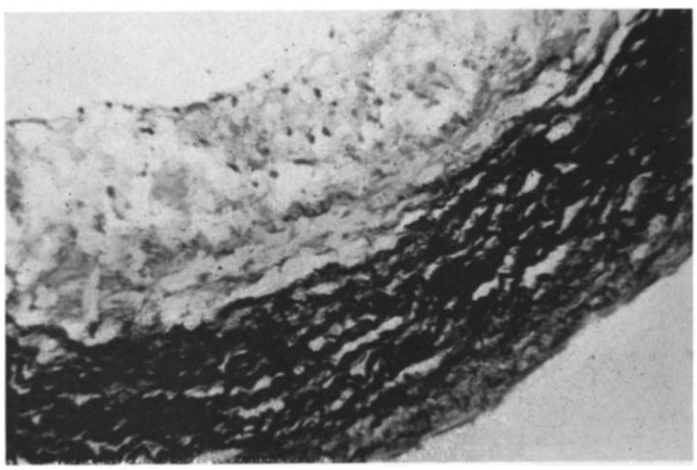

FIG. 2. As in Fig. 1., but containing elastase purum in a concentration of 10 EU/ml.*
* EU = elastolytic unit = 1 mg elastin dissolved in 30 min at 37°.

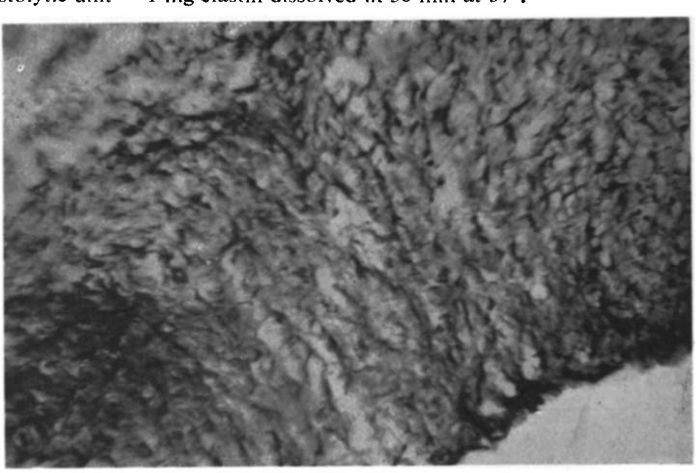

FIG. 3. As in Fig. 1. with 20 EU/ml elastase purum in the system.

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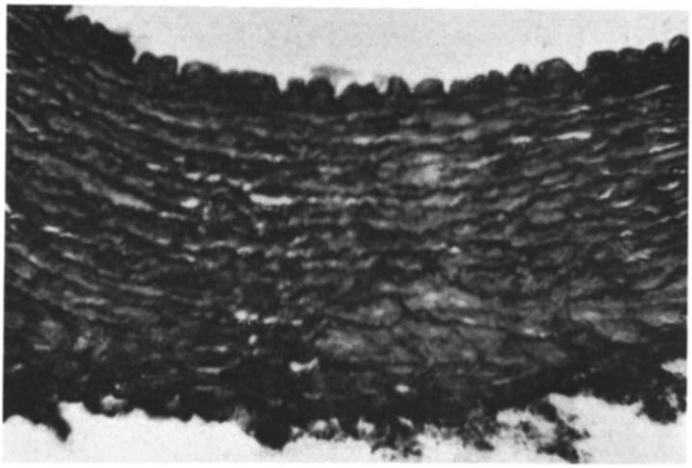

FIG. 4. As in Fig. 1. with 10 EU/ml elastase purum and 10 IU/ml† bean inhibitor added.
† IU inhibitor unit = 1 elastolytic unit inhibited.

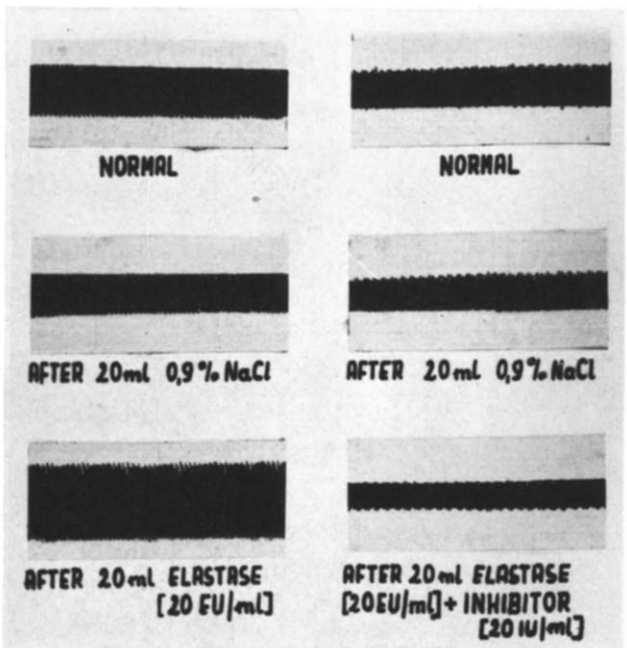

FIG. 5. Carotid arteries oscilograms of anaesthetized dog perfused separately *in situ*. The perfusion lasted 15 min, followed by blood streaming through the carotis again. The blood pressure, measured in the femoral artery, was found to be unchanged.

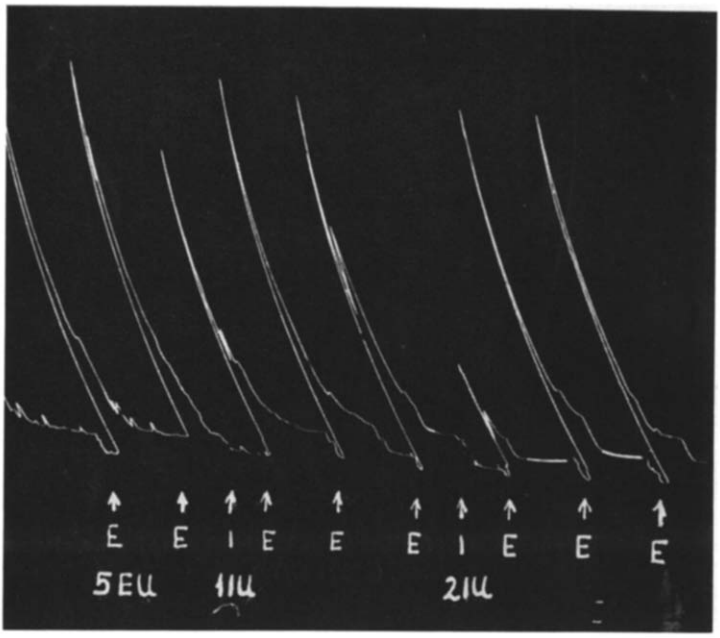

FIG. 6. Contractions of isolated rat uterus in response to elastase purissimum. 10-ml bath, 30°. E = elastase purissimum (100 EU/mg) added every 4 min. I = bean inhibitor (200 IU/mg) added 2 min before elastase.

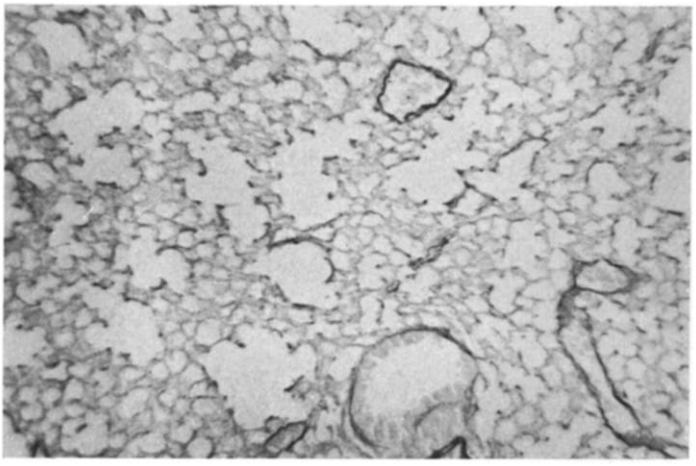

FIG. 10. Elastic elements of the lungs of control mice treated i.v. with 0·9% NaCl solution only.

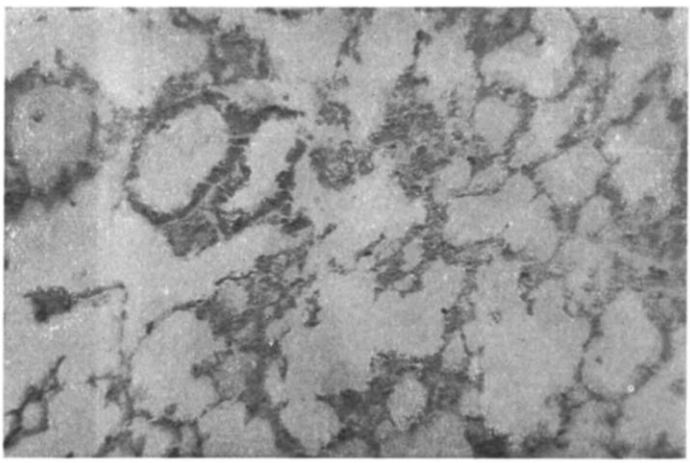

FIG. 11. Acute elastolysis in the lung of mice perished within 3 min after i.v. elastase purum treatment (4000 EU/kg).

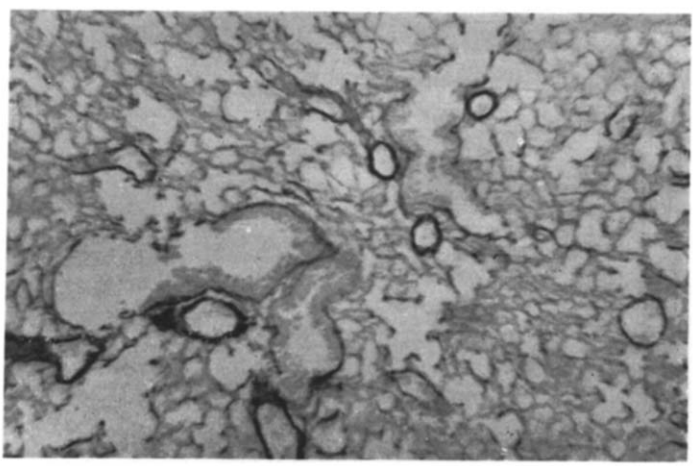

FIG. 12. The lung elastolysis caused by elastase purum is antagonized by i.v. pretreatment with the bean inhibitor (as in Fig. 9).

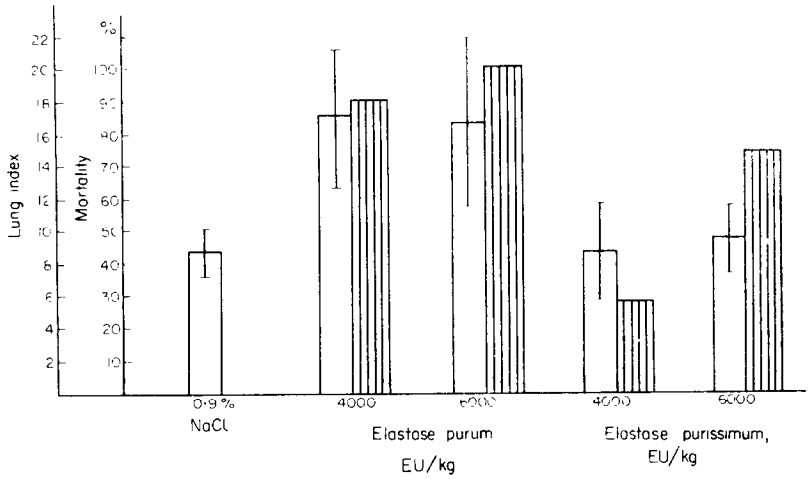

FIG. 7. Acute effects of two elastase preparations (elastase purum: 10 EU/mg, elastase purissimum: 100 EU/mg) on the lung index (= mg lung weight/g body weight) and mortality of mice after i.v. administration. The surviving animals were killed 30 min later. Open columns: lung index, shaded columns: mortality.

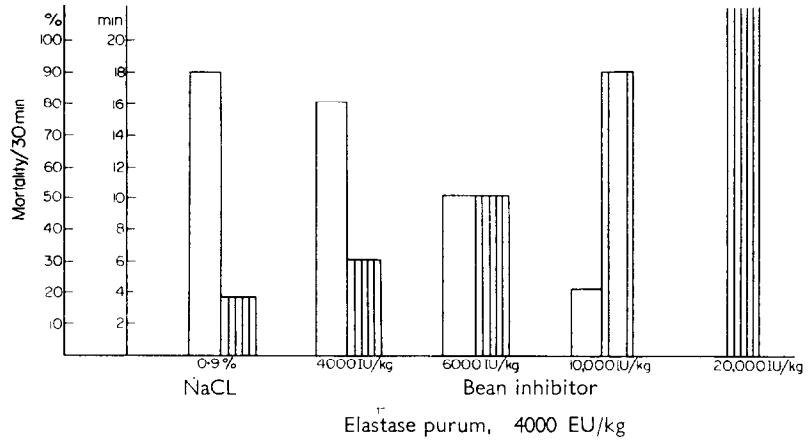

FIG. 8. Inhibition of acute i.v. toxic effect of elastase purum by i.v. pretreatment with the bean inhibitor. Mortality of the mice decreases and the time of their survival increases. Open columns: mortality, shaded columns: time of survival.

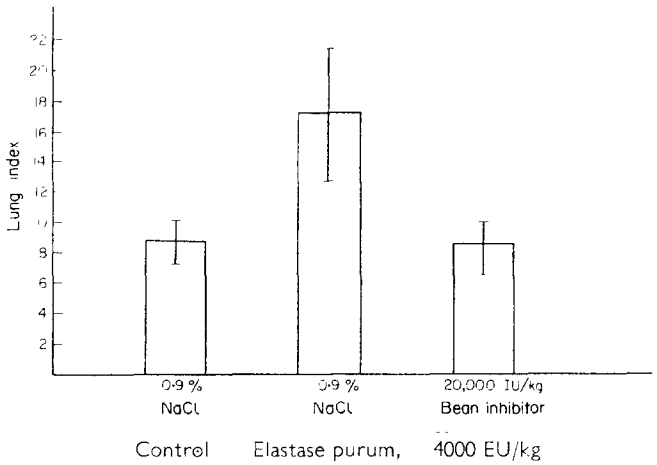

FIG. 9. As in Fig. 8: development of oedema and hemorrhage of lung banned.

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