changed 3 times during this period. Inorganic phosphorus was measured according to TAUSSKI and SHORR[13], proteins were determined by measuring absorbancy at 260 and 280 nm. The 120,000 × *g* supernatant, where most of the enzymatic activity reside in the soluble fraction, was used for the experiments. The reaction mixture contained: 25 μmoles *Tris* at pH 8.8; 2.5 μmoles ATP; 2 μmoles $CaCl_2$; 100 μmoles NaCl; 0.2 ml dialized enzyme fraction. Volume was brought to a total of 1.0 ml with double distilled water. The reaction mixture was incubated for 30 min at 30 °C and the reaction stopped by addition of 0.2 ml cold TCA 50%. Experiments were conducted in 3–4 replications and repeated at least 3 times.

Results of one of the experiments are presented in the Table. As can be seen, the additions of NaCl to the growth medium resulted in the stimulation of soluble ATPase activity of bean and corn roots, but induced an inhibition of this activity in *Suaeda* and *Atriplex* roots.

The addition of 100 μmoles of sodium chloride to the enzymatic reaction mixture revealed that the activity of the ATPase in the supernatant of bean and corn roots was stimulated (Na^+ activated), while the activity of this enzyme in the supernatant of *Suaeda* and *Atriplex* roots was inhibited.

Evidently, the results of these experiments suggest that a basic qualitative difference in the responses to Na exists between the ATPase systems in the 2 glycophytic and 2 halophytic species, a difference that might be of a general nature.

The effects of other cations on the ATPase activity of plants with different salt tolerances, are now being investigated[14].

Zusammenfassung. Die Wurzeln zweier Glycophyten (*Phaseolus vulgaris., Zea Mays*) und zweier Halophyten (*Suaeda monoica, Atriplex Halimus*), angezogen auf NaCl-freier und NaCl-haltiger Nährlösung, wurden auf ihre ATPase-Aktivität untersucht. Die Anwesenheit von NaCl in der Nährlösung sowie im Reaktionsgemisch aus aufbereitetem Wurzelhomogenat und Cofaktoren bewirkt in Glycophyten eine Förderung und in Halophyten eine Hemmung der ATPase-Aktivität.

C. T. HOROVITZ and Y. WAISEL

Department of Botany, Tel-Aviv University, Tel-Aviv (Israel), 2 March 1970.

[13] H. H. TAUSSKY and E. SHORR, J. biol. Chem. *202*, 675 (1953).

[14] Acknowledgement. Investigation supported by a grant from USDA under No. P. L. 480.

An Elastase Inhibitor from Canine Mandibular Gland

Pancreatic elastase (endopeptidase EC 3.4.4.7) is unique among proteolytic enzymes of animal tissue in that it hydrolyzes elastin very quickly, liberating approximately 130 moles of NH_2-terminal residues per 100,000 g of substrate[1].

The destruction of intrapancreatic vessels observed in experimental[2–4] as well as in human[5] acute pancreatitis includes the dissolution of elastic tissue by pancreatic elastase. The equivocal results obtained with the protease inhibitor from bovine lung[6] in the treatment of acute pancreatitis may be explained, at least partially, by the fact that this polypeptide inhibits elastolytic activity only slightly. A 2500-fold excess (weight for weight) of this inhibitor is needed to reduce elastase activity by 50%. The preparation of a potent elastase inhibitor and its use in acute pancreatitis, alone or in combination with the beef lung polypeptide, might prove of therapeutic benefit. In the course of previous studies[7] it was noted that canine mandibular tissue extracts exhibited significant inhibitory activity on pancreatic elastase in vitro. The present study was undertaken for the isolation and characterization of the mandibular elastase inhibitor.

Extraction and purification. Freshly dissected dog submandibular glands were homogenized for 3 min at 4 °C with distilled water and the homogenate was centrifuged at 44,000 *g*. The clear supernatant was subjected to ultrafiltration in a $^1/_4$ inch Visking tubing at 4 °C, and the ultrafiltrate was concentrated to a small volume. The concentrate was applied to a Sephadex G-50 (fine) column and the column was developed with distilled water. The effluent was tested for inhibitory activity and the active fractions were pooled and concentrated. Rechromatography of a small amount of concentrate on a Sephadex G-50 column which had been calibrated with NADP, oxytocin, trasylol, ribonuclease and trypsin indicated a molecular weight of 12,000 for the submandibular inhibitor. 100 μl portions of inhibitor concentrate were applied to strips of Whatman No. 1 paper and subjected to electrophoresis in 0.05 *M* formic acid at 2.5 mA/strip for 2.5 h.

Inhibitor activity was detected in a band about 4.5 cm on the cathodic side, which gave a peptide stain with chloroimide reagent and a pink color with Sakaguchi reagent. The eluates (2% v/v acetic acid) of the inhibitor fraction from 19 electrophoresis paper strips were concentrated to a small volume. A 15 μl portion of this concentrate was applied to a cellulose acetate strip and subjected to electrophoresis for 30 min at 3.7 mA/strip using a formic acid solution, 0.05 *M*, pH 3.6, as before. 2 closely associated but distinct bands appeared when the strip was stained with Amido Black 10B.

Extracts from submandibular glands of different species. Extracts of submandibular glands from man, beef and pig were prepared in an analogous fashion. The ultracentrifugates and ultrafiltrates were tested for inhibitory activity against elastase (10 μg), trypsin (5 μg), and chymotrypsin (10 μg). For purposes of comparison an

[1] S. M. PARTRIDGE and H. F. DAVIS, Biochem. J. *61*, 21 (1955).
[2] M. C. GEOKAS, D. RAYMOND MURPHY and R. D. MCKENNA, Arch. Path. *86*, 117 (1968).
[3] M. C. GEOKAS, Arch. Path. *86*, 127 (1968).
[4] M. C. GEOKAS, Arch. Path. *86*, 135 (1968).
[5] M. C. GEOKAS, H. RINDERKNECHT, V. SWANSON and B. J. HAVERBACK, Laboratory Invest. *19*, 235 (1968).
[6] I. TRAUTSCHOLD, I. WERLE and G. ZICKGRAF-RUDEL, Pharmacologist *16*, 59 (1967).
[7] M. C. GEOKAS, Ph. D. Thesis, McGill University (1966), p. 123.

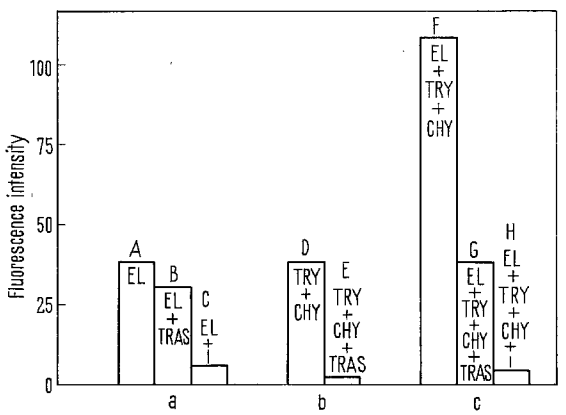
amount causing about 50% inhibition of bovine chymotrypsin was used of each extract. The results are given in the Table.

The assay of inhibitory activity was carried out with Remazolbrilliant Blue-hide as substrate as described previously[8]. Inhibitory activity against elastase was also tested with Remazolbrilliant Blue-Elastin as substrate[9]. All the enzymes used were obtained from Worthington Biochemical Corp., Freehold, New Jersey. Interesting species differences concerning the inhibitory capacity of the mandibular inhibitor are observed. The porcine inhibitor is inactive against trypsin and the beef inhibitor is inactive against elastase; whereas the canine and human inhibitors are active against trypsin, chymotrypsin and elastase.

The only known elastase inhibitors of mammalian origin include an inhibitor found in low concentrations in pancreatic tissue extracts[10], and the α_1-antitrypsin and α_{2M}-globulin inhibitors of serum[11,12]. The canine mandibular elastase inhibitor which occurs in high concentration in mandibular gland tissue appears to be unique because of its high potency against all 3 endopeptidases, trypsin, chymotrypsin and elastase. Moreover, this inhibitor is effective against both the specific elastolytic and the non-specific proteolytic activity of elastase. In an attempt to simulate the in vivo conditions that might exist in pancreatic tissue in acute pancreatitis the following experiments were carried out: The beef lung inhibitor and the canine mandibular inhibitor were each used against elastase alone (Figure bar group a) and against a mixture of trypsin, α-chymotrypsin and elastase (bar group c). Whereas the beef lung inhibitor reduced trypsin and chymotrypsin activity to zero (bar group b), it was ineffective in reducing elastase activity in a mixture of trypsin, chymotrypsin and elastase (bar G). The canine inhibitor on the other hand suppressed 96% of the combined activity of the 3 enzymes (bar H). The potentiating effect of trypsin and chymotrypsin on the elastase/elastin system in vitro (illustrated by Figure, F) has recently been studied in some detail[13]. It has been shown experimentally that small amounts of elastase are capable of rendering the net-like structure of elastin susceptible to destruction by trypsin and chymotrypsin. Such a concept might explain the small amount of elastase secreted as a digestive enzyme into the intestine; mainly to render the elastic fibers of food susceptible to the more abundant proteases, trypsin and chymotrypsin. It is conceivable that a similar process may be operative in the pancreas during the development of necrotizing pancreatitis. The destruction of elastic tissue of vessel walls might be effected by elastase action, augmented by trypsin and chymotrypsin, whereas the digestion of other proteinaceous elements would be subject to the additive action of the same proteases. It appears therefore that the canine mandibular inhibitor alone or the porcine inhibitor combined with the beef lung polypeptide might be very suitable for therapeutic experimentation in suppressing the autodigestive processes in acute pancreatitis. Work is now in progress concerning further purification and characterization of the mandibular inhibitor. It is possible that the elastase inhibitor described here may be identical with the trypsin-chymotrypsin inhibitor isolated by TRAUTSCHOLD et al.[14] from the same tissue[15].

Species	ml of Inhibitor		% Inhibition of		
	Ultracentrifugate	Ultrafiltrate	Elastase[a] (10 μg)	Trypsin (5 μg)	Chymotrypsin (10 μg)
Pig	0.045		40.7	0	59
Beef	0.05		0	10.5	48.5
Dog	0.009		17.7	50.4	50.3
Man	0.3		14.6	45.4	45.4
Pig		0.01	49.1	0	56
Beef		3.0	–	–	12.3
Dog		0.1	14	39.2	51.2
Man		0.3	–	4.1	–

[a] Determination with Remazolbrilliant Blue-Elastin.



Assays were carried out with 20 mg fluorescein-labeled elastin in 0.05 *M* bicine buffer, pH 8.8 containing Triton X-100 (1:4000); total volume 4 ml. Incubation at 37 °C for 60 min with continuous shaking. All experiments were subjected to pre-incubation at 37 °C for 10 min prior to addition of substrate. A) 2 μg elastase/4 ml incubation mixture. B) Same as A) + 1000 KIU Trasylol. C) Same as A) + 1 ml canine mandibular inhibitor (ultrafiltrate concentrate). D) 125 μg trypsin + 125 μg chymotrypsin. E) Same as D) + 1000 KIU Trasylol. F) 2 μg elastase 125 μg trypsin + 125 μg chymotrypsin. G) Same as F) + 1000 KIU Trasylol. H) Same as F) + 1 ml canine mandibular inhibitor (ultrafiltrate concentrate).

Zusammenfassung. Ein Inhibitor der Enzyms-Elastase wird beschrieben und dessen biologische Bedeutung sowie die eventuelle therapeutische Verwendung diskutiert.

M. C. GEOKAS, P. SILVERMAN and H. RINDERKNECHT

University of Southern California, School of Medicine, Los Angeles (California 90033, USA), 17 March 1970.

[8] H. RINDERKNECHT, M. C. GEOKAS and B. J. HAVERBACK, Clin. chim. Acta *21*, 197 (1968).
[9] H. RINDERKNECHT, M. C. GEOKAS, P. SILVERMAN, Y. LILLARD and B. J. HAVERBACK, Clin. chim. Acta *19*, 327 (1968).
[10] W. A. LOEVEN, Acta physiol. pharmac. néerl. *10*, 228 (1962).
[11] F. KUEPPERS and A. G. BEARN, Proc. Soc. exp. Biol. Med. *121*, 1207 (1966).
[12] J. S. BAUMSTARK, Arch. Biochem. Biophys. *118*, 619 (1967).
[13] H. RINDERKNECHT, P. SILVERMAN, B. J. HAVERBACK and M. C. GEOKAS, Clin. chim. Acta, in press (1970).
[14] I. TRAUTSCHOLD, E. WERLE, H. HAENDLE and H. J. SEBENING, Physiol. Chem. *332*, 328 (1963).
[15] Acknowledgment. This work was supported by USPHS Grants No. AM-04683-07 and No. AM-08293-04.