facing upward and serial frozen sections done. The sections were inserted into pre-weighed bottles containing 3 *M* GuCl. Each bottle contained 100 µm of thickness of the vascular wall. Large amounts of proteinase inhibitor were found in each of 10 such segments cut through the thickness of the arterial wall. Thus, unlike the plasminogen activator the proteinase inhibitor appears to be fairly uniformly distributed through the aortic wall.

Presence of trypsin inhibitory activity in selected canine connective tissues.

Tissues	Trypsin Inhibitory activity in supernate of dialyzed extract
Pulmonary Vein	++++
Cornea	++++
Aorta	++++
Articular cartilage	++++
Epiphyseal cartilage	+++
Knee joint ligament	+++
Tooth pulp	+++
Iris	++
Vena cava	++
Femoral periosteum	++
Trachea	++
Dentin	++
Foot tendon	++
Fascia	+
Heart valves	+
Pulmonary artery	+
Sclera	+
Skin	±
Vitreous humor	±
Lens	±

Neither the relationships between the material assayed here and other tissue cationic proteinase inhibitors nor the spectrum of enzymes it inhibits is known. However, the observation that a cationic proteinase inhibitor is widely distributed in normal connective tissues is of interest. It suggests that this inhibitor may play a role in regulating the metabolism of normal and diseased connective tissues. Preliminary data indicate that this proteinase inhibitor can inhibit the proteolytic activity of buffy coats of blood leukocytes.

We have recently isolated this proteinase inhibitor from cartilage and aorta by affinity chromatography. Current experiments aim at characterizing the molecule and determining the spectrum of enzymes which it inhibits.

Zusammenfassung. Extrakte verschiedener Bindegewebe wurden auf ihre Proteasehemmung hin elektrophoretisch untersucht. Alle Gewebe zeigten kationisch wandernde Aktivität und ihre Anwesenheit im zellfreien Dentin lässt den Schluss zu, dass das Material extrazellulär lokalisiert ist.

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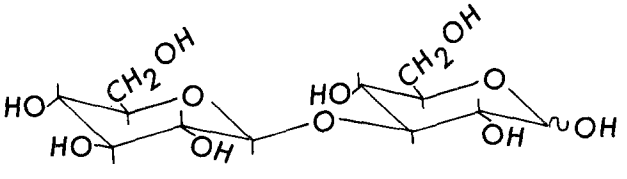
[10] We wish to thank Mrs. P. LISK and Mrs. H. E. GUENTHER for their skilled technical assistance. We are particularly grateful to Mrs. A. CROXEN for her assistance in the trypsin inhibitor assays, and to Mrs. M. GILBERT for preparation of the manuscript.

[11] This work was supported by the National Institute of Health, Grants No. AM-09132, HL-14968 and a Grant from the Chicago Heart Association.

Laminaranase Activity in the Crystalline Style of the Surf Clam (*Spissula solidissima*)

The crystalline style of the surf clam, *Spissula solidissima*, is an anatomical structure containing various enzymes[1] immobilized on a glycoprotein substrate. The function of the style, located in the stomach of bivalvia, is to triturate, with mortar and pestle-like action[1], particulate diatomaceous and algal food matter while at the same time initiating enzymic hydrolysis of carbohydrate polymers through both exo and endo action to ultimately produce glucose as an energy source. The term 'crystalline' is used to designate the occurrence of calcium oxalate crystals in the cortical layers of the style[2] which probably assist grinding of the food. The entire style may apparently dissolve and disappear under adverse conditions, such as inadequate food supply, only to again reform[3].



Laminarabiose

We have examined the stomach and intestines for various carbohydrase activities, and prepared an acetone precipitated powder, yield 0.7%, with the various carbohydrase activities shown in the Table. Afterwards we found that the style itself is the source of this activity, and could be prepared in 10% yield (60% of the style material on a dry basis), by triturating the style with a minimum of water in a mortar and pestle, centrifuging, and precipitating with an equal volume of cold acetone added to the supernatant. The acetone-dried friable, white powder, (N content 9.5%, glycose content 5.9% dry basis) was identical in turn-over number and activities to that shown for the previous material in the Table. Between 16 and 50 mg glucose can be generated from laminaran by one gram of the material per min, and we wish to announce that this seems to be the major carbohydrase activity of the style.

The preparation is stable indefinitely at 0° and is without loss of activity (turn-over numbers determined

[1] G. OWEN, in *Physiology of Mollusca*. II (Eds. K. M. WILBUR and C. M. YOUNGE (Academic Press, New York 1966).

[2] J. C. NELSON, Biol. Bull. *49*, 86 (1925).

[3] M. R. SCHMEER, Science *144*, 413 (1964).

from zero order reaction kinetics) for at least a fortnight at room temperature.

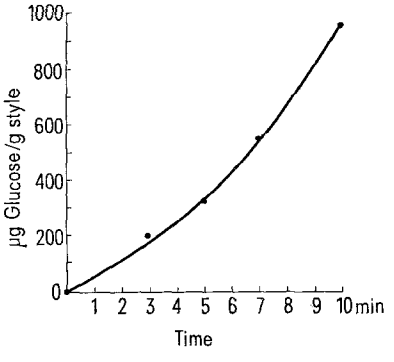
Laminaran is an algal carbohydrate polymer energy reserve which has as the major repeating structural unit the disaccharide laminarabiose (3-*O*-β-D-glucopyranosyl D-glucose). Glucans with (1 → 3), β-D-glucoidic linkages are found in some higher plants, e.g., barley and oats[4].

The major style carbohydrase activity, that of a (1 → 3)-β-D-gluconase, seems to be similar to laminaranases of bacterial origin[4]. In addition to the compounds shown in the Table, the material is inactive on sucrose, lactose, and guar gum. The amylase activity is chloride dependent, and the degree of polymerization required for action on (1 → 3)-β-D-glucans must be greater than two.

Action of the preparation on laminaran has a broad (4.5–5.5) pH optimum and temperature optimum at 37 °C. The laminaranase activity is not enhanced by chloride, iodide, fluoride, bromide and magnesium salts, nor by oxalate, nor calcium. It is precipitated by ammonium sulfate at concentrations greater than 25% saturation.

Carbohydrase activities of the crystalline style of the surf clam

Substrate	Glucosidic linkage	Relative turn-over number
Laminaran	β1,3	100
Cellulose	β1,4	3
Starch	α1,4; α1,6	2
Starch + 0.02 *M* NaCl	α1,4; α1,6	72
Gentiobiose	β1,6	1
Cellobiose	β1,4	7
Maltose	α1,4	0
Maltose + 0.02 *M* NaCl	α1,4	6
Laminarabiose	β1,3	1
Laminaratriose	β1,3	47
Laminaratetraose	β1,3	68
Laminarahexaose	β1,3	57
Nigeran	α1,3; α1,4	0.3
Pullulan	α1,4; α1,6	0



Generation of glucose from laminaran by the crystalline style of the surf clam.

We were able to 'mobilize' the enzyme by inserting a small metal rod into a portion of the style and spinning it slowly on a magnetic stirrer in a 0.06% solution of laminaran. An example of the results obtained is shown in the Figure. After about 10 min, however, the style itself begins to dissolve. The enzyme has also been successfully immobilized on glass. In a manner similar to the way the style of bivalves is able to lyse algae and bacteria, our preparations are able to lyse yeast cells. The cell population is reduced by 50% and then activity ceases.

In view of the demonstrated inhibitory effect of clam extracts on the growth of the sarcoma 180 tumor in Swiss albino mice, the inhibition of Krebs 2 carcinoma, and the carcinolytic activity on the human HeLa cell line by SCHMEER[3,5], we now take the opportunity to point out the similarity between this suspected anticarcinomic principle, or 'mercenene' of clams and our 'laminaranase' preparations. Both preparations are glycoproteins and are inactivated by boiling and therefore both may be enzymic in nature. Both are precipitated from aqueous solutions saturated with greater than 25% ammonium sulfate, and can be precipitated from solution by acetone. Activity apparently varies with the season, or conditions corresponding to the formation or dissolution of the style by the clam.

Zusammenfassung. Isolierung und Charakterisierung eines Enzyms aus *Spissula solidissima*, welches die Fähigkeit besitzt, gewisse Polysaccharide spezifisch zu spalten.

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[4] G. O. ASPINALL, *Polysaccharides* (Pergamon Press, Oxford 1970).

[5] M. R. SCHMEER, D. HORTON and A. TANIMURA, Life Sci. *5*, 1169 (1966).

[6] The laminaran and its component sugars were prepared by one of us (B.A.L.) in the laboratories of the late Professor FRED SMITH at the University of Minnesota.

[7] We thank W. E. GUILD for laboratory assistance, the Shelter Island Oyster Company, Long Island, N.Y. for samples, and the Sea Grant Advisory Service for their help. This study was supported in part by a Sea Grant from the National Oceanic and Atmospheric Administration, U.S. Department of Commerce.

Extraction and Production of Renin and Angiotensin II Across Several Vascular Beds in Dogs[1]

In the present study we investigated the role of the lungs, the splanchnic region with exclusion of the spleen, the spleen and the liver in the inactivation or production of renin and angiotensin II by measuring plasma renin concentration (PRC) and plasma angiotensin II (PA II) in their efferent and afferent vessels. The study was performed on 4 intact dogs and on 7 dogs after exclusion of the liver, which is known to be a major site of renin[2–5]- and angiotensin II[6–8]-inactivation. In this study the words extraction, inactivation and production do not refer to specific mechanisms.

Materials and methods. 11 mongrel dogs, with an average weight of 24.9 ± 4.8 (S.D.) kg were anesthetized with i.v. sodium pentobarbitone 30 mg/kg body weight.