Preliminary Notes

The elasticity-increasing property of elastomucoproteinase

Pancreatic elastase is a proteolytic enzyme which dissolves elastin specifically[1-3]. Other proteolytic enzymes of the pancreas, trypsin and chymotrypsin, are without elastolytic activity[4,5]. Elastomucoproteinase described by us[1] shows also no elastolytic activity on isolated elastin but dissolves a mucoprotein from bovine ligamentum nuchae and from human aorta which on the basis of the Molisch-Szára reaction differs from the mucoprotein dissolved from native collagen.

We succeeded in separating elastomucoproteinase from collagen mucoproteinase preparatively as well as through paper electrophoresis. The elastolytic activity of elastomucoproteinase on different substrates was found to be negligible compared with elastase (Table I).

TABLE I

ELASTOLYTIC ACTIVITY OF ELASTOMUCOPROTEINASE AND ELASTASE

Substrate	*E.U./mg**	
	elastomuco-proteinase	*elastase*
Bovine ligamentum nuchae (powdered)	4	80
Elastin from ligamentum nuchae**	4	130
Elastin from bovine aorta**	4	130

* Elastolytic units[2].
** Elastins were prepared by the method of PARTRIDGE AND DAVIS[3]

Since elastomucoproteinase does not digest either elastin or collagen but dissolves a mucoid, one can suggest that this dissolved mucoid may stabilize the elastic fibres.

The effect of elastomucoproteinase was therefore investigated upon the tensile properties of ligamentum nuchae, of human aorta and carotid artery. Load–extension curves were measured before and after treatment by the enzyme.

Strips were cut from ligamentum nuchae parallel and across the carotid artery corresponding to the direction of the fibres and the work necessary to stretch the material to 30 % extension was determined[6] in Ringer solution. Enzyme treatment was carried out for 3 h at 37° at pH 7.4, in 0.025 *M* veronal–acetate buffer, at an enzyme concentration of 0.1 mg/ml. Suitable control experiments were carried out without elastomucoproteinase and with crystalline trypsin. The effect of enzyme is given by WOOD'S[6] parameter $R = (W_2 - W_1)/W_1$ in which W_1, W_2 represent the work required for 30 % extension before and after enzyme treatment. Hysteresis was not a factor, since the load-extension curve was also measured before enzyme treatment.

The value of R using ligamentum nuchae was found to be constant, —0.20, if the wet weight of the strips did not exceed 25–35 mg. The R value is negative which denotes that the strip has been weakened by the treatment, *i.e.* the stability of the

fiber decreased. In experiments with human carotid artery the age of the subject and the pathologic conditions influenced a great deal the effect of the elastomucoproteinase. In carotid arteries from human cadavers in which the internal layer was covered with atheromas the strip became more extensible, *i.e.* more elastic, after enzyme treatment as compared with the carotid from healthy and young individuals, *i.e.* the negative value of R increased in individuals with atherosclerosis. Investigations were performed with 36 human carotid arteries and in Table II values of R belonging to different age groups are exemplified.

TABLE II

EFFECT OF ELASTOMUCOPROTEINASE ON HUMAN CAROTID ARTERIES

Age of subject (years)	*Condition*	R	
		Control: buffer only	*elastomucoproteinase*
19	normal	0.0	— 0.20
30	normal	0.0	— 0.21
44	slight alteration	0.0	— 0.35
70	atherosclerosis	0.0	— 0.45
73	atherosclerosis	0.0	— 0.50
93	atherosclerosis	0.0	— 0.55

Several workers[7–9] have been engaged in the study of the changes of elastic extensibility in relation to atherosclerosis. It was generally observed that with age and with arterio- or atherosclerosis the elastic extensibility of blood vessels was greatly decreased. Our experiments confirm this observation. However, following the effect of elastomucoproteinase the decreased extensibility of the aged arteries improved and their elastic extensibility was regained.

Trypsin had no effect whatever on the tensile properties of blood vessels.

The mucoprotein dissolved by elastomucoproteinase contains hexuronic acid, hexosamine and neuraminic acid and differs from the mucoid$_2$ isolated from bovine Achilles tendon with the aid of collagen-mucoproteinase[10]. The mucoid$_2$ was shown to be a stabilizing factor of collagen fibres[11] and consisted mainly of neutral heteropolysaccharides.

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