

pectinase concentration at the doses so far considered was examined to know the effects of doses lower than 0.5 Mrad on enzymatic activity. In Figure 2 the percentages of enzymatic inactivation at  $1.5 \times 10^{-3} M$  concentration as function of 1, 10, 50, 200, 500 and 1,000 Krad doses are reported.

At the dose of 1 Krad, the enzyme is still sensitive and such sensitivity increases exponentially until 200 Krad, tending, afterwards, to asymptote.

These results, showing the sensitivity of the enzyme also at low doses, seem to be promising for the application of ionizing radiations to fruit preservation, and the work can be considered as a preliminary research dealing with the inactivation of *in vivo* pectinase<sup>7</sup>.

**Riassunto.** È stata misurata la radiosensibilità dell'enzima Pectinasi a più concentrazioni e a varie dosi di irraggiamento. Si descrive inoltre un nuovo metodo per determinarne l'attività.

P. PANSOLLI

*Laboratorio Applicazioni in Agricoltura del C.N.E.N.,  
Centro Studi Nucleari Casaccia,  
I-00060- Roma (Italy), 5 December 1969.*

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### Concerning Collagen and Elastin in Aortas of Nine Different Species

A large number of studies related to the elastin and collagen content of human or animal aortic tissue have been using histological techniques, but only relatively few quantitative chemical studies have been accomplished<sup>1-5</sup>. It could be mentioned that HARKNESS et al.<sup>5</sup> studied the relative distribution of elastin and collagen in dog aorta from the arch to the lower abdominal segment. A similar study using aortas of goat, sheep, pig and human was conducted by GRANT<sup>6,7</sup>.

In our study, elastin hydroxyproline (elastin HP) content and collagen hydroxyproline (collagen HP) content in aortas of 9 different healthy species were determined. In 5 of these species aortas were separated into abdominal and thoracic segments prior to the hydroxyproline determination.

The elastin HP and collagen HP were also determined in aortas of a New Zealand albino rabbit subjected to an atherogenic diet for 0, 3 and 7 weeks respectively. It was hoped that this experimental study, on one hand, would shed some light on the aortic elastin and collagen content in 8 different species of mammals not examined previously but often employed as experimental animals in cardiovascular research; on the other hand it was hoped that this study could relate changes in elastin HP and collagen HP in rabbit aorta to the atherosclerotic involvement of aorta during development of dietary atherogenesis.

**Materials and methods.** Nine different animal species (of mixed sexes) were employed in this study. Aortas of mice, rats, golden hamsters, guinea-pigs, cats, dogs and rhesus monkeys were obtained from Pel Freez Biological Inc., Rogers, Arkansas. Bovine aortas were obtained through Dr. I. LIKAR from a commercial slaughter-house, Worcester (Mass.). Rabbits (New Zealand, albino) were obtained from Gloucester Rabbitry, Gloucester (Rhode Island). Three groups of rabbits (10 rabbits each) were fed an atherogenic diet (Purina rabbit chow fortified with 1% cholesterol) for 0, 3 and 7 weeks. At the end of these periods, rabbits were sacrificed, aortas were separated into thoracic and abdominal segments and the areas of the atherosclerotic lesions were determined planimetrically.

Further experimental procedures were identical in all investigated species. Aortas were stripped of their adventitial layers, separated into abdominal and thoracic sections in the rabbit, cat, dog, monkey and cow (and into intima and media in case of bovine aorta), dehydrated in several changes of acetone and dried in vacuum oven (temperature < 50 °C). Portions of dry tissue (5–15 mg)

were accurately weighed and homogenized in 15 ml of distilled water; collagen and elastin were separated and hydroxyproline content determined in both fractions. The experimental procedure was essentially the same as recently described by GRANT<sup>7</sup>. All results are expressed as milligrams of hydroxyproline in collagen or elastin fractions, respectively, per gram of dry tissue. Ten mouse aortas were combined per sample.

Table I. Determination of collagen hydroxyproline and elastin hydroxyproline in aortas of 9 species of animals

Species	No. of animals	Hydroxyproline (mg/g dry tissue)	
		Collagen fraction	Elastin fraction
Mouse	50	17.9 ± 0.7 <sup>a</sup>	3.1 ± 0.3
Rat	20	26.5 ± 1.2	4.4 ± 0.6
Hamster	10	22.4 ± 1.9	7.4 ± 0.5
Guinea-pig	20	16.2 ± 1.1	3.3 ± 0.4
Thoracic part	10	18.0 ± 1.9	5.5 ± 0.6
Rabbit			
Abdominal part	10	25.8 ± 7.2	4.9 ± 0.7
Thoracic part		23.5 ± 0.9	4.3 ± 0.2
Cat	10	31.9 ± 1.3	4.1 ± 0.4
Abdominal part			
Thoracic part	10	19.5 ± 2.0	4.4 ± 1.0
Dog			
Abdominal part	10	34.9 ± 2.3	3.8 ± 0.5
Thoracic intima		20.6 ± 2.0	6.3 ± 0.8
Thoracic media	10	22.9 ± 1.5	6.0 ± 0.5
Cow			
Abdominal intima	10	27.7 ± 3.8	6.5 ± 0.7
Abdominal media		38.6 ± 4.4	5.4 ± 0.5
Thoracic part	10	25.1 ± 0.8	4.7 ± 0.3
Monkey			
Abdominal part	10	30.6 ± 1.1	4.3 ± 0.2

<sup>a</sup> Mean ± S.D.

Table II. Determination of collagen hydroxyproline and elastin hydroxyproline in rabbit aortas during development of dietary atherogenesis

Duration of cholesterol diet (weeks)	Aortic atherosclerotic involvement of total area of aortic segment (%)		Hydroxyproline (mg/g of dry aortic tissue) in			
	Thoracic part	Abdominal part	Collagen fraction Thoracic part	Abdominal part	Elastin fraction Thoracic part	Abdominal part
0	0	0	18.0 ± 1.9 <sup>a</sup>	25.8 ± 7.2	5.5 ± 0.6	4.9 ± 0.7
3	2.3	2.0	17.6 ± 3.1	24.0 ± 4.6	4.6 ± 0.7	4.3 ± 0.6
7	14.2	15.0	23.2 ± 3.2	26.5 ± 3.4	5.5 ± 0.7	5.2 ± 0.6

<sup>a</sup> Mean ± S.D.

**Results and discussion.** Results of determinations of elastin HP and collagen HP in aortas of the 9 different species are presented in Table I. In rabbit, cat, dog, cow and monkey where thoracic and abdominal aortic segments were examined separately a significantly higher concentration of collagen HP was observed in the abdominal segment. In bovine aorta, somewhat higher concentration of collagen HP was observed in media than in intima; this situation was more pronounced in the abdominal segment than in the thoracic segment.

The lowest concentration of collagen HP was observed in guinea-pig and mouse; in the same 2 species the lowest concentration of elastin HP was noted. Some indication of the higher concentration of elastin HP in thoracic segment than in the abdominal segment was observed in all examined species.

In the GRANT'S<sup>6,7</sup> study the percentage of elastin present in the wall of the aortas of sheep, goat, pig and human decreased from the arch to the lower abdominal region, while the collagen content simultaneously increased. HARKNESS et al.<sup>5</sup> have found that in the dog, the elastin/collagen ratio was always lower in the abdominal segment than in the thoracic segment of the aorta. Our data on dog aorta agree with the data of HARKNESS<sup>5</sup> and our results on aortas of rabbit, cat, cow and monkey, where ratio of elastin HP to collagen HP was always higher in thoracic segment (Figure) further amplify this finding. This could imply that indeed the higher elastin HP to collagen HP ratio in thoracic segment could be a general characteristic of mammalian aortas. This could be perhaps related to the function of the aorta, especially the segment closer to the heart to provide the tissue with greater elastic properties

to effect a continuous blood flow from a discontinuous blood discharge by a heart.

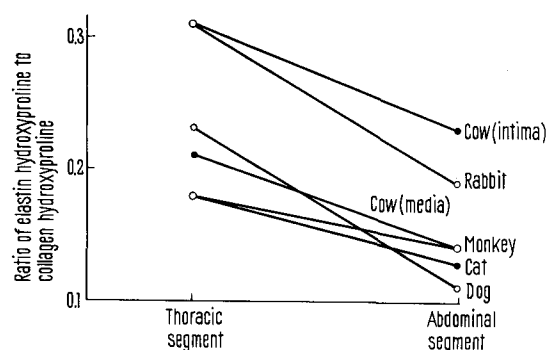
In rabbits (Table II) where the concentrations of collagen and elastin hydroxyproline were determined during the development of dietary atherogenesis, some indication of the decrease in elastin HP (more pronounced in thoracic segment) was observed after 3 weeks of cholesterol diet. After 7 weeks of the diet the concentration of elastin HP in both segments was virtually identical to the quantities observed in control animals. Some increase in collagen HP content in thoracic segment was observed after 7 weeks of cholesterol diet.

The indicated decrease in elastin HP after 3 weeks of cholesterol feeding could be perhaps related to the observed increase in aortic permeability toward egress of <sup>131</sup>I-albumin observed after 3 weeks but not after 7 weeks of cholesterol diet<sup>8</sup>. Plans are in progress to study morphological correlates of these biochemical changes<sup>9</sup>.

**Zusammenfassung.** Histochemische Untersuchungen am Elastin und Kollagen der Aorta bei Mäusen, Ratten, Goldhamstern, Meerschweinchen, Katzen, Hunden und Rhesusaffen ergeben quantitative Unterschiede hinsichtlich Elastinhydroxyprolin und Kollagenhydroxyprolin. Die Einflüsse einer cholesterinhaltigen Diät wurden kontrolliert.

V. STEFANOVICH

Department of Pathology, University Hospital, and Departments of Pathology and Biochemistry, Boston University Medical School, Boston (Massachusetts 02118, USA), 16 December 1969.



The ratio of elastin hydroxyproline to collagen hydroxyproline in thoracic and abdominal segments of the aorta in the normal rabbit, cow, monkey, cat and dog.

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