A METHOD OF INCREASING THE PURITY OF SOLUBLE ELASTIN OBTAINED BY PARTIAL HYDROLYSIS FROM AORTIC TISSUE OF VARIOUS SPECIES

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SUMMARY

Elastin, a scleroprotein of the connective tissue, is insoluble in water, saline or all non-hydrolytic solvents. One method of obtaining soluble derivatives is partial hydrolysis with oxalic acid. Elastins obtained from the aortic tissue of various species were hydrolyzed 5 times and 75 minutes as a modification of Partridge's method of partial hydrolysis, where 60-minute boiling with 0.25 M oxalic acid at 100 °C is repeated 4 times. Cellulose acetate, SDS-polyacrylamide gel electrophoresis and high performance liquid chromatography were used to identify the molecular mass and to control the purity of the soluble derivatives. These studies show that all soluble elastin derivative samples obtained with this method have two protein bands with molecular masses of 71,500 and 58.500 daltons which is closer to the molecular mass of tropoelastin. We believe that under these conditions of hydrolysis the purity of soluble elastin can be increased.

Key Words : Aorta; soluble elastin; acid hydrolysis

INTRODUCTION

Elastin fibers of the connective tissue are composed of two morphologically distinguishable components : fibrillar and amorphous. Chemically, these two components correspond to two different classes of proteins. The amorphous component, referred to as elastin, is responsible for the elastic properties of the tissue; and the fibrillar component is composed of structural glycoproteins named also as "microfibrillar glycoproteins" (1).

Elastin, the insoluble extracellular scleroprotein, is the three - dimensional covalently cross - linked polymer of soluble elastin precursor, tropoelastin (2). Thus, a proper procedure is required to obtain solubilized elastin having a molecular mass and other structural properties as close to tropoelastin as possible. One of the most common methods used for this purpose is Partridge's method (3), where 0.25M oxalic acid is used as hydrolytic solvent. Here, elastin is boiled in this solvent 4 times, each lasting for 60 minutes. The soluble fraction is essentially composed of two groups of peptides : α - elastin with molecular mass of 60,000 - 84,000 daltons and β - elastin, of 5,500 daltons. Tropoelastin, the soluble precursor of elastin, has a molecular mass of 72,000 daltons (4); therefore, α - elastin is frequently used for biochemical studies.

Based on Partridge's method of partial hydrolysis, we aimed to continue the procedure until complete dissolution of elastin, expecting to get a more pure soluble α - elastin.

MATERIALS AND METHODS

Tissue samples for obtaining elastin were removed from 10 healthy bovine and 10 lambs' thoracic aortas. Thoracic aortas of 15 human subjects (age range 20 - 40; mean 30) with no history of hypertension were taken within 25 hours after death. Special attention was paid to see that they had no atheromatous plaques macroscopically.

After immediate washing of the tissues with normal saline to remove the blood, they were thinly sliced and blended. Acetone and ether was used for defatting. Elastin was optained by the method described by Banga et al (5).

İ) Hydrolysis of elastins by oxalic acid treatment:
10 g of elastin was suspended in 75 ml of 0.25 M oxalic acid. The suspension was boiled at 100 °C for 75 minutes in a round - bottomed flask provided with an air condenser. After this period, the suspension was cooled down with ice and the supernatant filtered. The

precipitate was taken in 50 ml of 0.25 M oxalic acid and the above procedure was repeated five times. There was no further precipitation after the fifth boiling process, in all samples. The filtered solutions were collected and centrifuged for 10 minutes at 2000 rpm. The clear - yellow supernatant was dialyzed at 4 °C for 24 hours against distilled water in the cellophane dialysis membrane [Sigma D - 9652 (21 mm diameter; to block proteins with MW ≥ 12,000 Da)]. The dialyzate was then lyophilized. This hydrolyzed produce of elastin called soluble elastin derivative (SED) was dissolved in normal saline. Neither leaving in the tube, nor centrifugation caused precipitation in this yellow colored solution.

ii) Control of purity and determination of molecular weight :

Cellulose acetate electrophoresis : 5 µl of samples were put into each well containing 60 - 80 mg/ml of SED, and sera with 5 protein fractions were used as controls (Helena Laboratories, Texas USA). Electrophoresis was performed under 180 volts at pH 8.8 for 15 minutes after center application. Coloration of the cellulose acetate plates was done using Ponceau S and decoloration, 5 % acetic acid solution. Bands of proteins were evaluated at 525 nm wavelength with a F4 slit. The results were given in % of total protein, as well as in g/dl.

SDS - polyacrylamide gel electrophoresis (6) : Samples were diluted 1 : 10 with 20 mM Tris buffer, pH 6.8 to obtain a final concentration of 0.17 mgl soluble elastin. Denaturation was performed for 5 minutes at 100 °C. Running gel concentration was 10% and stacking gel concentration, 4.5%. Electrophoresis was performed in Pharmacia/Fine Chemical apparatus, starting with 25 mA at 42 V; after the passage of samples to the running gel, the potential was increased to 112 V and the current to 50 mA. 50 µl samples were applied and electrophoresis was terminated at 6 hours 30 minutes. As control, 7 proteins having 66,000, 45,000, 36,000, 29,000, 24,000, 20,100 and 14,200 daltons molecular masses were used as Molecular Mass Identifications Standard (Sigma MW - SDS - 70 L Stocks No SDS - 7). Staining for protein was performed using Coomasie blue [0.2% in ethanol/10% acetic acid (1 : 1)]. Decoloration was effectuated in a mixture of 95% etanol, 10% acetic acid, and distilled water (200 : 150 : 150).

High performance liquid chromatography (HPLC) (7) : Control of the purity of the samples was performed with HPLC under the below mentioned conditions :

Column	:	TSK 3000 SW G gel permeability. LKB, Sweden.
Flow rate	:	0.5 ml/min.
Buffer	:	200 mM NaCl, 50 mM Tris, pH 6.8 (filtered and degassed)
Recording chart speed	:	0.5 cm/min
Filter	:	278 nm
Graphical amplitude	:	0.1 OD
Samples	:	SED samples were prepared with the buffer solution to have a concentration of 4 µg/ml, and a volume of 150 µl was injected.

RESULTS

Cellulose acetate electrophoresis revealed the SED samples to contain a single homogenous band of protein with mobility corresponding somewhere between α_2 and β globulin fractions of human serum (Fig 1).

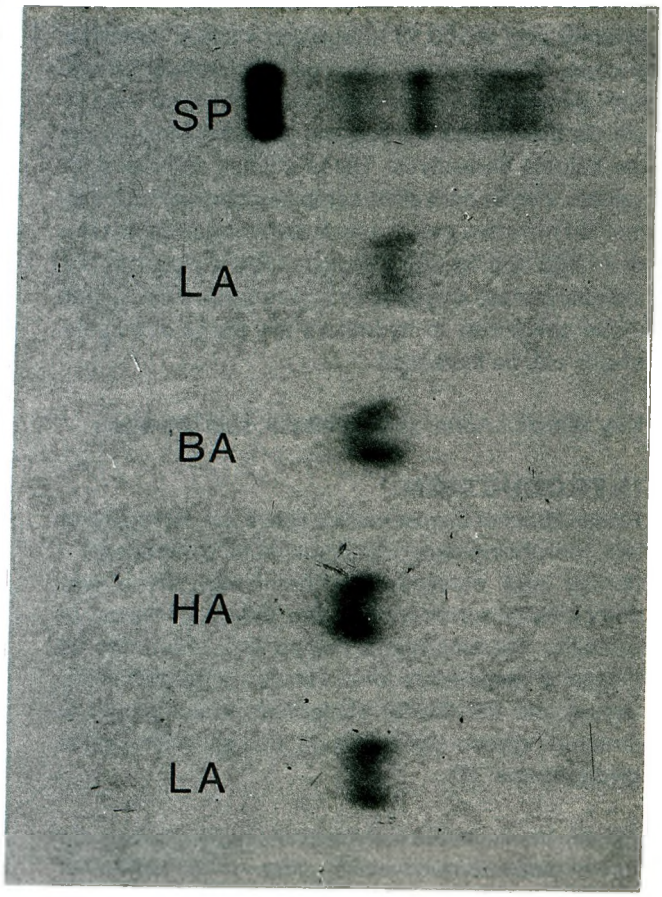

FIG.1: A method of increasing the purity of soluble elastin obtained by partial hydrolysis from aortic tissue of various species.

Two homogenous protein bands with molecular weight of 58,500 and 71,500 daltons were obtained with the SDS - polyacrylamide gel electrophoresis from the SED samples (Figs 2 and 3).

HPLC studies showed the samples to give a peak of proteins heavier than 100,000 daltons with 5 ml of eluent volume. All samples also gave a protein peak at 11.2 ml of eluent volume (Figs 4,5,6).

DISCUSSION

Elastin, a scleroprotein, is the most important biomolecule that provides the elasticity of the connective tissue. Its elastomeric properties play a crucial role both in the structure and structure - function interactions.

After obtaining pure elastin, it needs to be solubilized for all analytical and spectroscopic studies. Elastin is insoluble in water, saline and all non hydrolytic solvents: but the soluble derivatives can be obtained by partial hydrolysis (8). Partridge et al. (3) obtained α - elastin with a molecular mass varying between 60,000 and 84,000 daltons and β-elastin of 5,500 daltons, by hydrolysis with 0.25 M oxalic acid at 100 °C for 60 minutes. Kornfeld et al [9] obtained peptides of 100,000 dalton molecular mass by 30 min. hydrolysis with ethanol KOH method. Increasing the hydrolysis periods yields elastin peptides with molecular masses smaller than 50,000 daltons (kappa - elastin). There are also other methods of hydrolysis: use of elastase or elastases - like proteases for degradation of elastin (1) and hydrolysis with concentrated alkali or acids (9-11). Alfa - elastin obtained by Partridge's hydrolysis method shows some important structural characteristics of the elastin precursor, tropoelastin. In this study, by changing the conditions of oxalic acid hydrolysis, we aimed to obtain a pure soluble derivate which is closer to the molecular mass of tropoelastin. In our study, control of purity and determination of molecular mass was achieved by three methods.

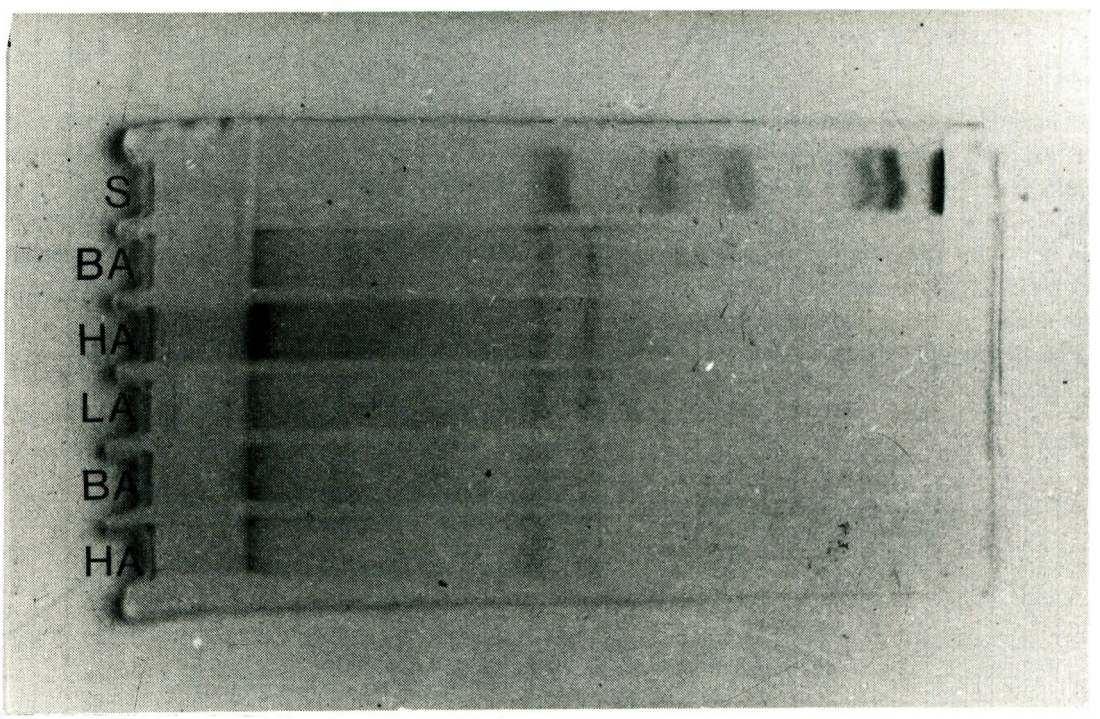

FIG.2: A method of increasing the purity of soluble elastin obtained by partial hydrolysis from aortic tissue of varios species.

The wide but single protein bands of the α- elastin samples from the aortic tissue of various species, obtained with cellulose acetate electrophoresis, correspond somewhere between α_2 and β globulin fractions of human serum proteins, suggesting that the electrical charge of SED samples are homogenous.

The molecular weights of SED samples obtained by Partridge's classical hydrolysis method are 62,000 and 80,000 daltons (12). In this study, all samples obtained with our proposed method revealed two protein bands with molecular masses of 58,500 and 71,500 daltons by SDS - polyacrylamide gel electrophoresis.

In the HPLC results, the protein peak obtained at 5 ml which is the protein with molecular mass higher than 100,000 daltons is thought to be an elastin aggregate. It is shown that due to its high apolar amino acid content elastin aggregates when it is heated (13). There is a wide variation of the ratio of the two peaks observed in all samples. Probably, this is caused by the differences in aggregation rates of the SED's obtained from the aortic tissue of different species.

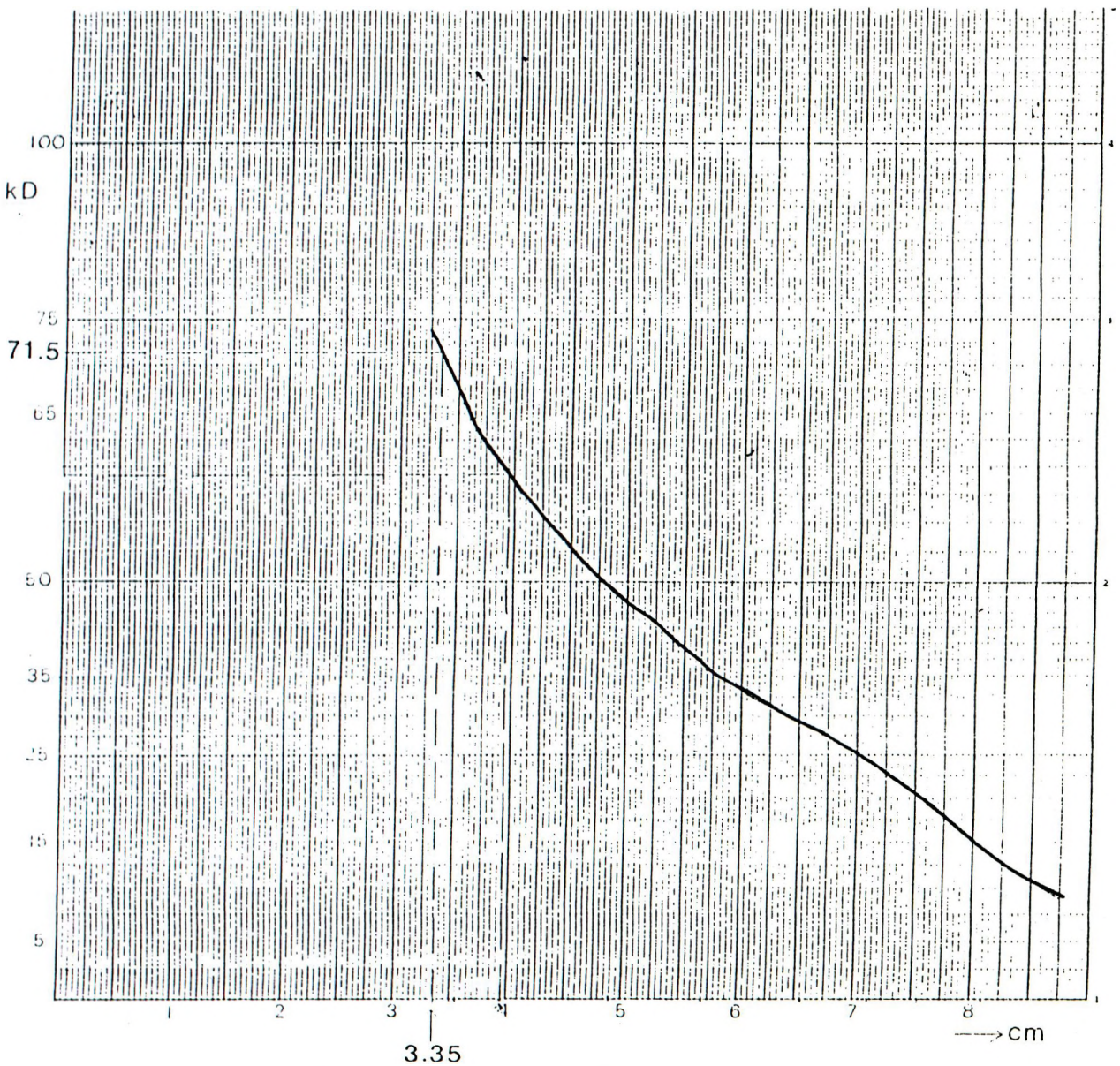

FIG.3: A method of increasing the purity of soluble elastin obtained by partial hydrolysis from aortic tissue of various species.

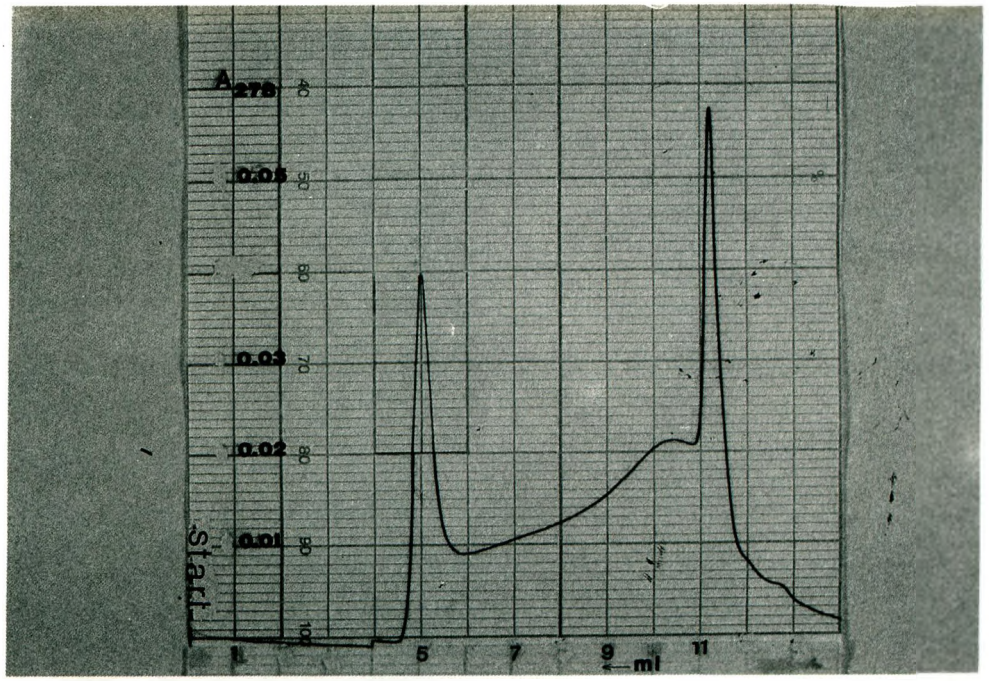

FIG.4: A method of increasing the purity of soluble elastin obtained by partial hydrolysis from aortic tissue of various species.

The molecular mass that we have observed for SED with our proposed hydrolysis method is 71,500 daltons, which is closer to the molecular mass of tropoelastin (72,000 daltons) than the soluble elastins obtained using the other methods (3, 9, 14, 15) for soluble elastin derivatives (Table I).

Nearly identical molecular weights obtained for elastins form different tissues, suggest that our hydrolytic conditions, specially the duration, are appropriate for obtaining homogenous soluble elastin derivatives. Thus reproductibility has also been increased. In addition, molecular mass determination being an important indicator of purity, our molecular mass data support strongly the above mentioned results on control of purity.

TABLE I. Methods for preparation of soluble elastin derivatives

Method	Reference
Alkaline hydrolysis	Kornfeld - Poullain (9)
Acid hydrolysis	Partridge (3)
Enzymatic hydrolysis (elastases)	Bode (14)

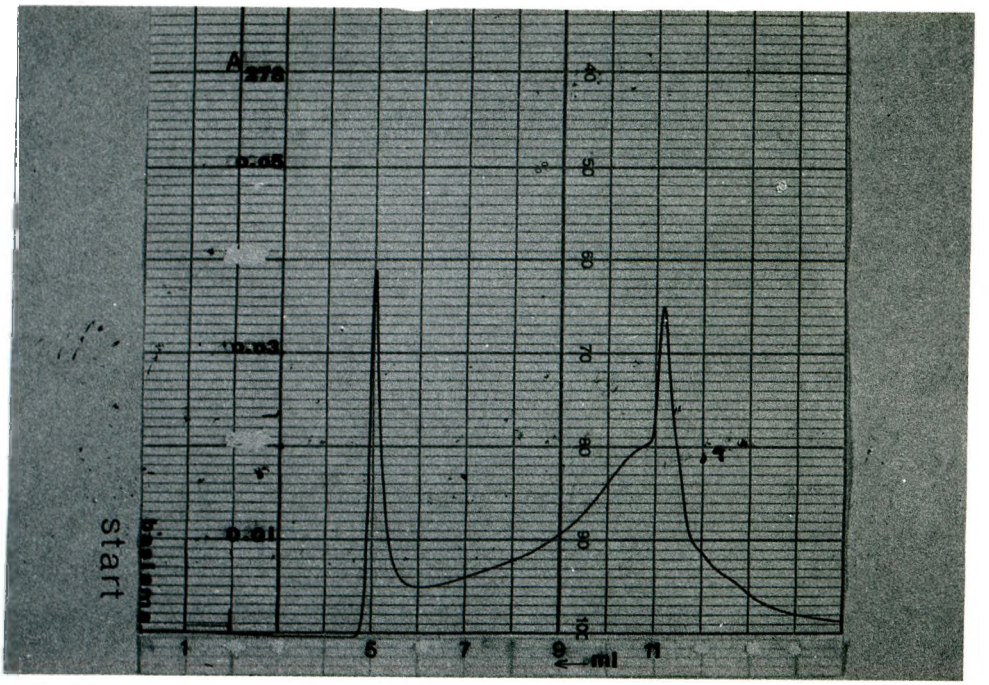

FIG.5: A method of increasing the purity of soluble elastin obtained by partial hydrolysis from aortic tissue of various species.

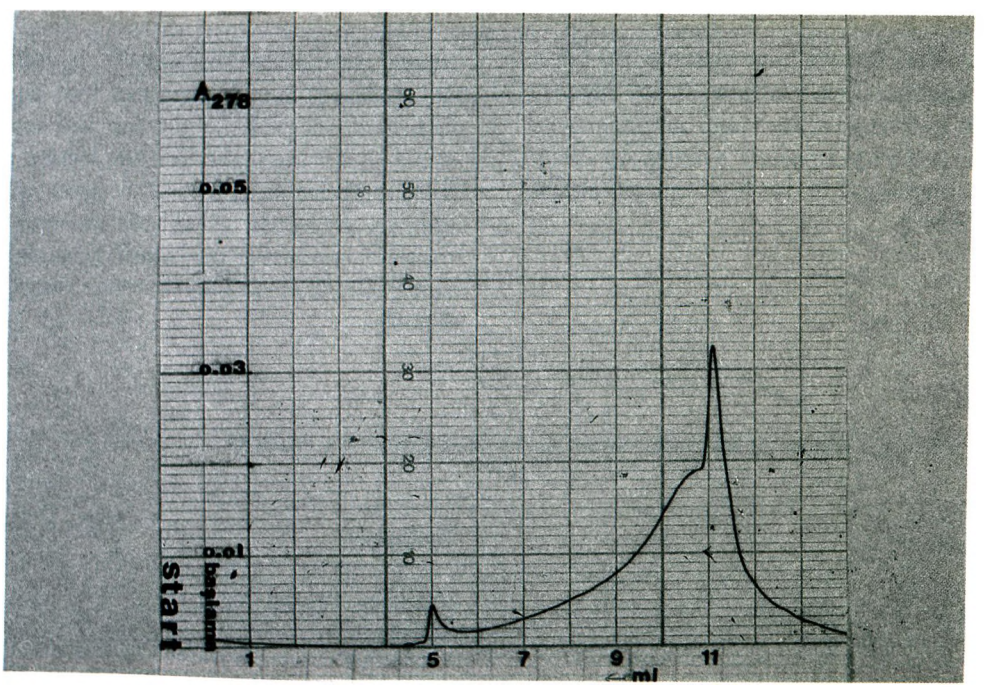

FIG.6: A method of increasing the purity of soluble elastin obtained by partial hydrolysis from aortic tissue of various species.

Our proposed method of obtaining a sufficiently pure and a high-molecular-mass soluble elastin is a modification of the oxalic acid hydrolysis procedure of Partridge. Our conditions of hydrolysis make possible the complete dissolution of elastin, at the same time yielding a soluble derivative very close to tropoelastin, the soluble precursor of elastin, in molecular mass. In conslusion, it can be stated that the soluble elastin thus obtained is suitable for biochemical as well as immunological studies.

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