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SERUM ELASTASE INHIBITOR LEVELS IN EHLERS-DANLOS SYNDROME*

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Prior studies from this laboratory of the elastin-elastase-serum elastase inhibitor system disclosed, among other findings, a consistent 2- to 3-fold increase in the inhibitor in late pregnancy (Schneider, Walford, and Dignam, 1960). Other disease states, including arteriosclerosis, lupus erythematosus, dermatomyositis, severe liver disease, and nephrotic syndrome, showed mild but inconstant changes in inhibitor levels (Walford and Schneider, 1959). The regular elevation of elastase inhibitor in late pregnancy is of interest in view of the increased incidence of dissecting aortic aneurysm, which is primarily an elastic tissue disease, in pregnancy (Mandel, Evans, and Walford, 1954). Elastase inhibitor levels might thus reflect changes in the status of elastin or elastase in the body.

The investigation reported here was stimulated by the observation (Hall, Keech, Reed, Saxl, Tunbridge, and Wood, 1955) that the level of elastase inhibitor in a patient with Ehlers-Danlos syndrome was 50 to 100 times higher than normal. Such an elevation would be not only of theoretical and diagnostic importance for this disease state, but might afford a source wherefrom pure inhibitor could be isolated by appropriate techniques. Accordingly we have studied a group of seven patients with classical Ehlers-Danlos syndrome or certain features thereof. In addition to measurement of inhibition of elastolysis by elastase inhibitor, inhibition of casein proteolysis by the enzyme was determined.

Clinical Data

Case 1, a 7-year-old girl, had shown extreme elasticity of skin, marked hypermobility of all joints, and marked bruisability since the age of 6 months or earlier. Many cutaneous scars. No subcutaneous masses. No record of relatives (adopted child). Skin biopsy showed mild acanthosis and increase in dermal elastic tissue.

Case 2, a 42-year-old man, had shown hyperelastic skin and hypermobile fingers and wrists for many years. Positive tourniquet test. Bleeding work-up otherwise negative.

Case 3, a 28-year-old woman, had shown easy bruisability and hypermobility, especially genu recurvatum, since birth. Young daughter has slightly hypermobile joints.

Case 4, a 40-year-old woman, showed marked laxity of the skin of the right axilla and moderate laxity of the skin of elbows. Right shoulder dislocates as many as three times a day.

Case 5, a 45-year-old woman, had shown easy bruisability for 5 years. Her sister and one of her two children have hypermobile joints.

Case 6, a 35-year-old woman, showed hypermobility of fingers and slight elasticity of skin. Her mother had markedly lax skin and marked easy bruisability. No other positive family history.

Case 7, a 40-year-old woman, had shown lax skin and hypermobility of the elbows for many years.

Methods

Elastin was prepared by NaOH extraction of beef aorta as detailed elsewhere (Walford, Moyer, and Schneider, 1961). Elastase was obtained by CM-cellulose chromatography of powdered pork pancreas (Walford and Kickhöfen, 1962). Elastase inhibitor was measured by adding 0·15 ml. of a 1 : 5 dilution of test serum to 20 mg. elastin in a test tube, and rapidly adding 0·1 ml. elastase dissolved in 3·8 ml. pH 8·8 $NaHCO_3$-HCl buffer. Controls consisted of the same quantity of elastin and elastase with 3·95 ml. buffer. After incubation for 45 min. at room temperature, the tubes were centrifuged

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and the supernatants discarded. The remaining unsolubilized elastin was washed, dried overnight in the oven, and weighed. Percentage inhibition was determined by the formula mg. solubilized in control minus mg. solubilized in test divided by mg. solubilized in control multiplied by 100. Inhibition of proteolysis was determined as described previously (Walford and Kickhöfen, 1962), using 0·05 ml. serum. Two normal sera were run as controls for all the above tests.

Results and Discussion

The conditions outlined above have been found to be optimal for the measurement of serum elastase inhibitor levels. Under these conditions the percentage of inhibition is proportional to the amount of inhibitor present. Although not every one of the patients in this report represents absolutely typical examples of Ehlers-Danlos syndrome, several, especially Case 1, fulfil all the criteria for inclusion in this category (McKusick, 1960), and several others are strongly suggestive. As shown in the Table, we were unable to confirm the reported elevation of serum elastase inhibitor in Ehlers-Danlos syndrome. Sera from our patients displayed essentially normal values for the inhibitor with both elastolytic and proteolytic methods.

TABLE

INHIBITION OF ELASTOLYTIC AND PROTEOLYTIC ACTIVITIES OF ELASTASE BY ELASTASE INHIBITOR

Case No.	Per cent. Inhibition of Elastolysis	Per cent. Inhibition of Proteolysis
1	30	59
2	38	78
3	49	57
4	34	62
5	29	58
6	42	63
7	27	59
Control 1	46	87
Control 2	35	59

Summary

No abnormality of serum elastase inhibitor was present in a group of patients with varying stigmata of Ehlers-Danlos syndrome.

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Taux d'inhibiteur de l'élastase sérique dans le syndrome d'Ehlers-Danlos

RÉSUMÉ

Aucune anomalie de l'inhibiteur de l'élastase sérique ne fut trouvée dans un groupe de malades présentant de différents signes du syndrome d'Ehlers-Danlos.

Niveles del inhibitor de la elastasa sérica en el síndroma de Ehlers-Danlos

SUMARIO

No se encontró anomalía alguna en el inhibitor de la elastasa sérica en un grupo de enfermos con varios estigmas del síndrome de Ehlers-Danlos.