Serum Elastase Inhibitor. Levels in Animal and Human Sera, including Selected Disease States.* (24822)

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The elastolytic enzyme of pancreas, elastase, can exert *in vitro* a powerful solubilizing action upon ordinarily highly insoluble scleroprotein elastin, and is the only known mammalian enzyme to have this effect. The role of the enzyme in the body is largely unknown. Animal and human sera possess an inhibitor for the enzyme(1,2,3). In the present study of inhibitor substance we shall present data concerning(1) certain variables involved in measuring the elastase-inhibitor level of serum, (2) localization of the inhibitor among serum fractions separated by starch-block electrophoresis, and (3) representative levels of the inhibitor in the sera of certain animal species, normal values for healthy humans of various age groups, and levels in selected disease states in the human.

Materials and methods. Elastase was prepared as previously described(3). The elastin substrate was made by treating ground defatted human aorta with 0.1 N NaOH at a temperature of 98°C for 45 minutes, according to the method of Lansing *et al.*(4). Unless otherwise stated, the inhibitor test system consisted of 20 mg of substrate, 4 ml of pH 8.0 veronal-acetate buffer containing a total of 0.8 mg of elastase, and 0.15 ml of the serum to be tested. The reagents were rapidly added in the order given. Controls consisted of the same quantity of substrate plus 4.15 ml of buffer containing a total of 0.8 mg of elastase. The tubes were incubated at 37°C for one hour. Percent inhibition was then determined by gravimetric data, using the formula:

$$\% \text{ inhibition} = \frac{\text{No. mg elastin solubilized in control} - \text{No. mg elastin solubilized in test}}{\text{No. mg elastin solubilized in control}} \times 100.$$

Serum fractions were obtained using starch-block electrophoresis with veronal-acetate buffer of pH 8.6. Fractions were eluted from each 0.5 cm of the block, and aliquots analyzed for protein content by Lowry's method (5): Inhibitor levels of representative fractions were then measured both by employing equal volumes of eluate and by employing aliquots adjusted to contain equal protein content. The original electrophoretic curve was then compared by visual inspection with these two types of inhibitor curve (Fig. 2).

Results. Inspection of Fig. 1 clearly indicates that per cent inhibition varies with time. A small portion of this variability might be explained by a mild thermolability of the inhibitor. Serum pre-heated to 37°C for 2 hours retained at least 75% of its inhibitory activity. Heating serum to 45°C for 20 minutes did not destroy its inhibitory effect, but at 55°C for the same time period a marked loss in activity was observed. The actual mechanism of the inhibition and the kinetics of the reaction cannot be assessed from data thus far collected. It may be said, however, that level of serum inhibitor is not influenced by pre-exposure of the serum to an excess of purified elastin.

Fig. 2 indicates that the inhibitor substance is located approximately at the $alpha_1$-globulin/albumin junction of the electrophoretic curve of serum, and is absent from other globulin fractions.

Inhibitor levels in the sera of certain animal species are specified in Table I. Time curves for dog serum showed a pattern qualitatively quite similar to that of Fig. 1 for human serum. For 21 human male sera mean value of elastase-inhibitor under the particular conditions of our experiments was 56.6% with a standard deviation of ± 10.5. Levels in females appeared slightly higher, with a mean value for 8 sera of 65.9%. No significant variation in inhibitor levels with age of the individuals from the newborn period to 68 years was detected. In Table II are presented in-

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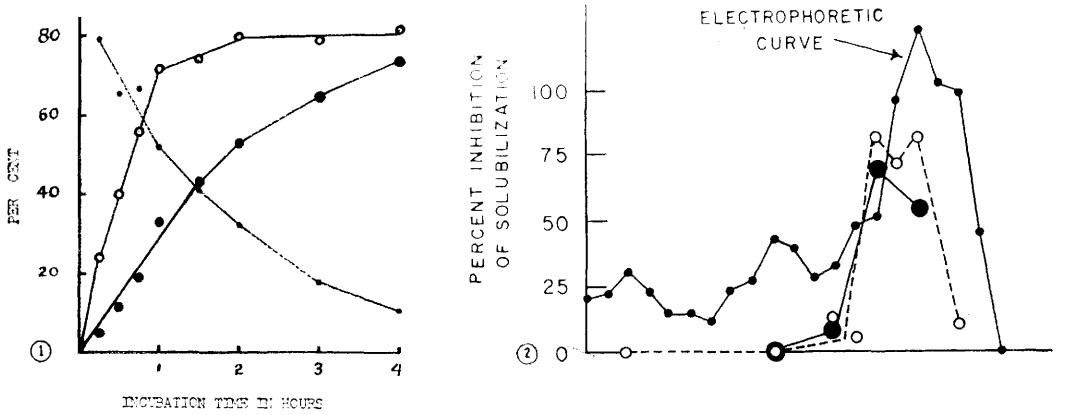

FIG. 1. Effect of time of incubation on measurement of elastase-inhibitor of human serum. For these curves the system comprised 20 mg elastin, 0.5 mg elastase, 4.0 ml buffer, and 0.1 ml of either serum or additional buffer. ○ = % elastin solubilized in control; ● = % elastin solubilized in presence of inhibitor (serum); • = % inhibition.

FIG. 2. Localization of elastase-inhibitor activity in serum fractions obtained by starch-block electrophoresis. One curve (○- - -○) was obtained by testing equal volumes of eluate, another (●——●) by testing aliquots of different volume but of equal protein content.

hibitor levels in certain physiologic or pathologic conditions in the human. These include the third trimester of pregnancy, arteriosclerosis, liver injury (the criterion for selection here being a 4+ cephalin cholesterol flocculation), disseminated lupus erythematosus, dermatomyositis, and severe nephrosis. These diseases were selected as representing either greatly altered protein mtabolism, or conditions in which elastic tissue injury might conceivably be anticipated, with the idea that such changes might be reflected in altered serum elastase-inhibitor levels. Consistently high levels were obtained in pregnancy. There seemed to be a moderate increase in serum elastase-inhibitor in a number of the disease states investigated, but no particular pattern was evident.

Discussion. A comment is in order about the reproducibility of elastase-inhibitor determinations performed at the arbitrary incubation time of one hour. To test this point, 5 separate, single determinations were made over a 3-month interval on aliquots of the same sample of human serum. The values, in the order obtained, were 47.5, 50.9, 49.3, 46.1, and 45.7% inhibition. This seems very satisfactory concordance for a measurement of this type. It is true that the curves for per cent elastin solubilized in control and in presence of inhibitor (as illustrated in Fig. 1) may vary somewhat from day to day. The variation is not marked, however, and the 2 curves vary in a proportional manner. Hence the overall per cent inhibition remains reasonably constant in separate determinations done at

TABLE I. Representative Values for Elastase-Inhibitor in Sera of Normal Adult Animals. Values are given as % inhibition for 3 or more individuals of each species.

Species	% inhibition
Chicken	55.2, 63.7, 67.5
Rabbit	48.7, 53.8, 52.3
Guinea-pig	56.1, 63.0, 52.0
Rat	37.9, 55.1, 57.6, 59.2
Mouse	78.4, 82.0, 85.7, 92.2
Sheep	59.8, 60.9, 79.0
Dog	21.8, 26.6, 36.8
Monkey	40.4, 55.6, 59.0

TABLE II. Levels of Serum Elastase-Inhibitor in Third Trimester Pregnancy, and in Selected Disease States in the Human.

Condition	% inhibition
Pregnancy, third trimester	89.4, 91.7, 94.7, 96.0, 97.8, 98.6, 98.6, 99.0, 99.5
Disseminated L.E.	58.4, 62.4, 71.8, 81.3, 82.6
Dermatomyositis	24.1, 81.3, 81.8, 85.7, 86.4
Arteriosclerosis*	46.9, 51.0, 59.0, 61.5, 71.4, 95.3
Liver damage (4+ ceph. floc.)	35.9,† 69.2,† 79.3,† 88.5,‡ 89.3§
Nephrotic syndrome	69.6, 77.9, 94.2

* All patients less than 60 years old.
† Laennec's cirrhosis.
‡ Infectious hepatitis.
§ Paraproteinemia, type undetermined.

the same time intervals up to and including one hour. At 2 and 4 hours of incubation, however, the determination is not highly reproducible. It should also be remarked that certain adjustments must be made in the test system if different batches of elastase itself are used, since the enzyme preparation, while quite potent, is not pure. This can be done by using stored frozen serum as a standard, in accordance with the values cited above. All numerical data presented in this paper, however, were obtained by use of a single batch of the enzyme. In a number of experiments we found that addition of twice the amount of serum led approximately to a doubling of per cent inhibition, and correspondingly for other amounts. This accords with results published by Banga *et al.*(2) and indicates that per cent inhibition bears a quantitative relation to a serum factor or factors.

Because elastase is known to have potent proteolytic activity against a wide variety of substrates(6), the question arises whether the inhibitor is indeed a specific substance, or whether the inhibition is merely a phenomenon of general protein interference. Evidence seems to favor the former view and may be set forth as follows. In separate experiments we found that casein—which is rapidly and extensively hydrolyzed by elastase—has only a negligible inhibitory effect upon elastolytic activity of the enzyme. At the same time, the serum elastase-inhibitor has no measurable inhibitory effect upon casein-proteolytic activity of the enzyme(3). Secondly the inhibitory activity of serum is located in a specific area of the electrophoretic curve as illustrated in Fig. 2. Thirdly, we found no relation between serum elastase inhibitor activity and total protein content of 7 human serum specimens with total protein values ranging from 3.6 to 7.9 g per 100 ml. Paper electrophoretic protein patterns were also available for these 7 specimens. An attempt was made to correlate per cent elastase inhibition with absolute values for $alpha_1$-globulin and/or albumin in these sera, in view of our results with eluates from starch-block electrophoresis (Fig. 2). No strict correlation obtained, although the 2 highest inhibitor levels were shown by the 2 sera with highest $alpha_1$-globulin fractions, and the lowest inhibitor level by the serum with the lowest $alpha_1$-globulin fraction. While the inhibitor resides in the $alpha_1$-globulin/albumin portion of serum, it is not simply proportional to the absolute quantity of either of these whole fractions.

The level of elastase-inhibitor in normal human serum appeared to be independent of age. Individuals tended to maintain the same inhibitor levels when examined on successive occasions. Inhibitor level did not vary significantly with ingestion of food, including fat-rich meals. Consistently high values were obtained in sera from the third trimester of pregnancy (Table II). This may be of some interest in view of the increased incidence of dissecting aortic aneurysm among those females less than 40 years of age who are pregnant(7). In this age group dissecting aneurysm is particularly associated with elastic tissue lesions. The serum elastase-inhibitor appeared moderately elevated in most instances of all the pathologic states studied. No explanation exists at present for the occational low or very high values obtained (Table II). We were not able to confirm the existence of abnormally low values for elastase-inhibitor in sera of patients with arteriosclerosis, as reported by Balo and Banga(1). Our values for animal sera (Table I) ought not to be taken for more than they are worth, since time curves and other studies were not done except for man and the dog. The animal data are merely meant to suggest that a wide variety of animal sera also contain elastase-inhibitor, and probably in a range broadly comparable to human serum.

Summary. A study of certain variables involved in measuring elastase-inhibitor activity of animal and human serum is presented, with particular emphasis upon time of incubation of the system. The inhibitor is shown to reside at the $alpha_1$-globulin/albumin junction of electrophoretically fractionated serum, but is not itself directly proportional to absolute values of either of these fractions. Inhibitor level in humans is probably independent of age. Mean value for males is about 56.6% under the experimental conditions outlined in

this report. Value for females is somewhat higher. Representative levels for sera of chicken, rabbit, guinea-pig, rat, mouse, sheep, dog, and monkey are given. Inhibitor is greatly elevated in sera of human females during the third trimester of pregnancy. In a number of selected disease states the inhibitor was found usually to be moderately elevated, although no consistent pattern was noted. The inhibitor was not diminished in sera of patients with arteriosclerosis.

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Alpha-2 Lipoprotein in Man and Its Relation to Myocardial Infarction.*† (24823)

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In studies on human serum lipoproteins using starch zone electrophoresis, the lipoproteins, as measured by cholesterol distribution, are readily separated into 3 main fractions (1,2). Fig. 1-A shows a cholesterol distribution curve obtained from a normal young male subject together with corresponding protein distribution curve. In healthy elderly individuals a similar curve is obtained as shown in Fig. 1-B, with a decrease in alpha-1 cholesterol percentage of approximately 1/3 and a corresponding increase in beta cholesterol, with no change in alpha-2 cholesterol percentage. Among elderly individuals with moderate or advanced atherosclerosis, a common type of curve is shown in Fig. 1-C, with a higher percentage of cholesterol in the beta fraction. Similar results have been noted by other workers(3,4,5) using paper electrophoresis in which only alpha and beta fractions are usually reported. In 24% of our male subjects there were lipoprotein curves with increased percentage of cholesterol in the alpha-2 fraction. Recently E. B. Smith(6) using paper electrophoresis noted a component which migrated between alpha and beta fractions (designated pre-beta fraction by this author) which appeared associated with presence of recent myocardial infarction in the patient. Schettler and his coworkers(7) using starch electrophoresis also mentioned that changes in the lipoprotein pattern may occur with myocardial infarction. We were therefore interested in determining the incidence of such diseased states among subjects with increased alpha-2 cholesterol.

Methods. Our subjects were patients of one of the authors (WBK). Lipoprotein determinations were made on selected patients visiting the physician for periodic check-ups. Ages varied from 35 to 83 years, most between 50 and 70. Three hundred male patients, taken in order, were used. The subjects were all of better than average economic status and presumably all had adequate dietary intake which was probably relatively high in fats. All blood samples were obtained in the morning with subjects in basal state and lipoprotein determinations carried out by the method used earlier(2).

Results. Patients were divided into groups on the basis of percentage of cholesterol found in the alpha-2 fraction. Thirty-eight, or

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