SHORT COMMUNICATIONS

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Studies on the elastolytic activity of serum

To prove the systemic presence of elastase (EC 3.4.4.7) it would be important to demonstrate its activity in blood plasma or serum. However, serum contains a large amount of elastase inhibitor[1-3] and the negative results obtained hitherto when assaying for elastolytic activity in serum have been attributed to this fact. Recently, HALL[4] has shown that the sensitivity of methods for measuring elastolysis is enhanced 23 times by using congo red-stained elastin as substrate instead of the biuret reaction with native elastin. This improvement makes it possible to demonstrate elastase in 5 μl of blood plasma in a final volume of 1 ml. The colour yield, expressed in terms of the absorbance at 495 mμ of liberated congo red, is linear in the range 0–5 μl plasma in a test volume of 1 ml. HALL[4] has emphasized that the elastase inhibitor in plasma does not influence the elastolysis of congo red-stained elastin.

It is known that elastin does not bind the congo red stain covalently and, because of this loose binding, in the presence of soluble proteins of large molecular weight the stain is liberated from the insoluble elastin and becomes visible. Fig. 1 shows the effect of different proteins on the liberated congo red expressed in terms

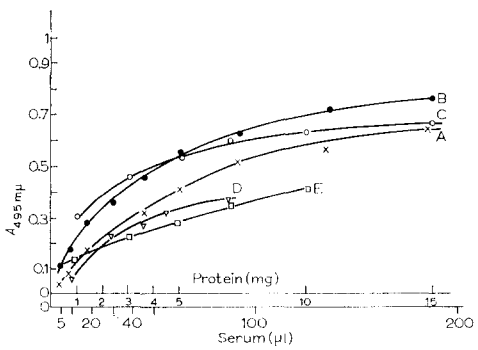

Fig. 1. Liberation of congo red stain by different proteins from 10 mg of congo red-stained elastin. A, serum protein; B, heated serum; C, gelatin; D, ovalbumin; E, casein. Abscissa, mg protein and μl serum per sample. Ordinate, absorbance at 495 mμ of liberated congo red stain.

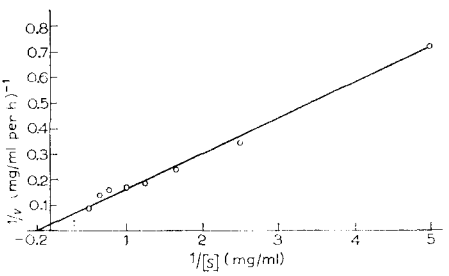

Fig. 2. Lineweaver–Burk plot for the action of gelatin (5 mg in the final test solution) on 10 mg of congo red-stained elastin.

of absorbance at 495 mμ. In Fig. 1, Curve A represents the congo red liberated by human serum. For the first three absorbance determinations, corresponding to 0.36, 0.72 and 1.44 mg serum protein (*i.e.* 5, 10 and 20 μl serum) in a final volume of 10 ml, the curve is linear with respect to the serum concentration but begins to flatten thereafter. According to D. A. HALL (personal communication) only this initial linear release of stain (up to 5 μl serum) can be looked upon as the result of enzymic action. In our experiments the absorbance of the colour released by this amount

under the experimental conditions used by HALL[4] was 0.035–0.040. Smaller absorbance values fall within the range of instrumental error using the Beckman spectrophotometer. Besides this, as the data of Curves D and E of Fig. 1 show, the amount of congo red liberated from the stained elastin by ovalbumin and casein was the same as that liberated by serum at identical protein concentrations. The heated serum (Curve B) and the gelatin (Curve C), tested at identical protein concentrations, gave higher absorbance values. On the basis of this, one can conclude that congo red-stained elastin is not suitable for determining whether or not native plasma or serum contains elastase.

Fig. 2 illustrates the kinetics of the effect of gelatin on congo red-stained elastin plotted according to Linewaever and Burk. As can be seen, the graph is linear and the calculated K_m values (mg/ml) are between 6.6 and 6.8 which corresponds to the data for "plasma elastase" of HALL[4]. The v_{max} (mg/ml per h) is about 50 for gelatin and 57 for plasma, calculated on the basis of HALL's results[4]. This proves that the kinetic parameters calculated from the liberation of stain from congo red-stained elastin are identical for both plasma and gelatin.

When the high molecular weight proteins were digested by trypsin and elastase, the resulting peptides—having lower molecular weights in comparison with the proteins—did not reproduce on congo red-stained elastin the effects of the proteins. Corresponding quantities of peptides showed 1/10 to 1/15 of the values found with the parent proteins (on the basis of $A_{495\ m\mu}$).

According to NAUGHTON AND SANGER[5], pancreatic elastase, like trypsin, is inhibited by diisopropylfluorophosphate (DFP). The liberation of congo red by serum from congo red-stained elastin is not influenced by DFP. This means that the effect of serum or plasma on the congo red-stained elastin differs from that of elastase.

The resorcinol–fuchsin and orcinol (Merck) stains are bound specifically and very strongly to elastin. In the presence of dissolved proteins practically no stain is liberated from the resorcinol–fuchsin-stained or orcinol-stained elastin. Table I presents results on the effects of gelatin and serum on the resorcinol–fuchsin-stained and on the orcinol-stained elastins, using different elastin:orcinol ratios in the staining techniques[6]. Since serum taken in the same proportion as gelatin gave somewhat

TABLE I

EFFECT OF PROTEINS ON THE LIBERATION OF STAIN FROM RESORCINOL–FUCHSIN- AND ORCINOL-STAINED ELASTINS

The experimental mixture containing 20 mg stained elastin in 10 ml buffer (pH 8.6) was incubated for 30 min at 37° in the presence of the given quantity of gelatin or serum. Absorbance was read against the blank.

Proteins	*Preparations*	$A_{560\ m\mu}$	$A_{580\ m\mu}$
Gelatin 10 mg	Resorcinol–fuchsin-stained elastin	0.010	
	Elastin:orcinol (1: 2.5)		0.005
	Elastin:orcinol (1: 5.0)		0.007
	Elastin:orcinol (1:10.0)		0.010
Serum 15 μl	Resorcinol–fuchsin-stained elastin	0.030	
	Elastin:orcinol (1: 2.5)		0.020
	Elastin:orcinol (1: 5.0)		0.025
	Elastin:orcinol (1:10.0)		0.030

higher absorbance values, it cannot be excluded that the serum has a slight elastolytic effect (see Table I).

To decide this question we have performed gravimetric measurements with congo red-stained and unstained elastin prepared according to PARTRIDGE *et al.*[7]. According to the method of HALL[4], in the presence of 20 μl serum, the quantity of dissolved elastin would be 3.2 mg as calculated from the congo red stain liberated during the incubation. In contrast with this, in the case of both congo red-stained and unstained elastin, the quantity of dissolved elastin found gravimetrically in several experiments (and also controls) was not more than 0.8–0.9 mg. This means that the colour yield does not represent only elastin protein that is solubilized but also stain that is otherwise liberated from the congo red-stained elastin.

Our conclusion is that congo red-stained elastin is not a suitable substance for the determination of elastase in serum or plasma. The effect of serum which was exhibited on orcinol-stained elastin or unstained elastin and measured colorimetrically or gravimetrically is not convincing, because it is too slight and lies within the limit of experimental error.

LOEVEN[8], using Cohn's fractionation method for plasma proteins and further purification methods, found a substance which was free of any elastase inhibitor and showed a synergistic effect on the activity of elastase. WALFORD AND SOPHER[9] have found in the serum of pregnant women, after separation of elastase inhibitor, a fraction which augmented the elastolytic activity of elastase. It seems that serum or plasma may contain some enzymic activity which influences the degradation of elastin. However, to be able to prove this, the plasma fraction must be obtained free of elastase inhibitor.

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