The Role of Proconvertin and Stuart Factor in the Inactivation of Tissue Thromboplastin by Serum

It has been found in our previous studies[1] that the tissue thromboplastin inactivating capacity of native serum was significantly increased by partial adsorption with kaolin whereas it was lost by subsequent $BaSO_4$ adsorption. These results were in good agreement with HJORT's conception[2], who proved in careful experiments that the participation of factor VII complex—equivalent to his proconvertin reagent—is indispensable for the inactivation of tissue thromboplastin by serum inhibitor. At the same time, it became clear that every system with unadsorbed or partially adsorbed serum is unsuitable for the separate study of the inhibitor proper and of its cofactor contained in the factor VII complex as both these components participating in the process of inactivation react differently on various procedures carried out with serum or in pathologic states. For that reason, a simple system was elaborated for rough quantititive assay of the inhibitor proper, which represented the only variable[3]. This system was also suitable for the evaluation of the role of proconvertin (i.e. factor VII proper) and Stuart factor in the tissue thromboplastin inactivation by serum inhibitor.

Methods. As substrate, normal freshly prepared, platelet-poor, oxalated plasma was used. Normal native (i.e. untreated) serum was usually 24 h old. The normal adsorbed serum, practically devoid of all factor VII complex activity, was prepared by two-fold adsorption with kaolin (100 mg/ml) and additional adsorption with $BaSO_4$ (100 mg/ml). In the incubation system, $m/10$ $CaCl_2$ was used. Acetone-dried human brain extract (200 mg per 10 ml saline) with the activity of 13 ± 1 sec in the Quick test served as substrate for inactivation.

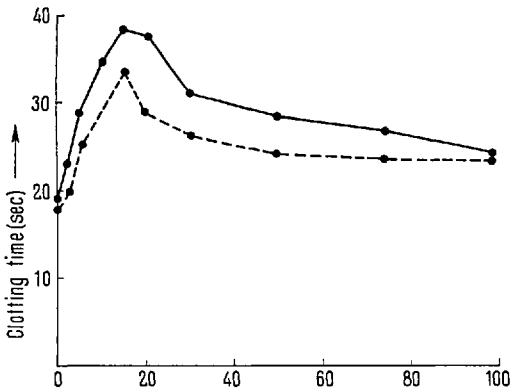
The testing system was similar to that described previously[1], but various inactivating mixtures instead of partially adsorbed serum were examined. In water bath at 37°C, two parts of the mixture tested were added to one part of $m/10$ $CaCl_2$, and finally one part of tissue thromboplastin was blown in. After 1, 30, and 60 min of incubation, a sample of 0·2 ml was taken and tested for its remaining thromboplastin activity on 0·2 ml substrate plasma. The sera deficient in proconvertin or Stuart factor respectively were kindly supplied to us by Dr. CAËN from Paris in lyophilized form. In addition to the control with normal native serum, the normal dry reconstituted serum was also used.

Results. Native serum contains both factor VII complex and the inhibitor. On gradually increasing the amount of this serum in the inactivating mixture with completely adsorbed serum, the thromboplastin inhibition, expressed by the prolongation of the clotting times after 1 h incubation, rises from zero to a maximum value which is reached at a level of 15% of native serum in the inactivating mixture (Fig.). A further increase above 20% leads, however, to a gradual decline in the thromboplastin inactivation. When the content of native serum is kept constant at 15%, and the 85% of adsorbed serum in the inactivating mixture is serially diluted in advance by veronal acetate buffer, a dilution curve can be obtained for the inhibitor proper. The properties of this inhibitor and its behaviour in various states are presented elsewhere[3-5]. The available amount of deficient sera being limited, no detailed quantitative analysis could be performed and these sera were substituted for normal native or dry, reconstituted serum in the inactivating mixture.

When proconvertin-deficient serum was used, no inactivation took place (Table I). As in patients with Stuart factor-deficiency, the content of proconvertin was moderately decreased (60% according to Dr. CAËN), 20% of this reconstituted serum and of normal redissolved serum (second control) was used instead of usual 15% of native serum in the inactivating mixture. Inspite of the virtual absence of Stuart factor, no substantial change in the inactivation was found (Table II).

Discussion. Present results confirm again the indispensable presence of proconvertin for tissue thromboplastin inactivation by a natural inhibitor, as was first postulated by HJORT[2]. In his proconvertin reagent, however, the Stuart factor was also present and the possibility of its involvement was disregarded. Nevertheless, the studies of PUDLÁK *et al.*[6] indicated that Stuart factor may have a special affinity for tissue thromboplastin. In view of our results, a formation of some complex involving both these components does not, however, seem necessary for the action of the inhibitor in contrast with the role of convertin, i.e. proconvertin-calcium-tissue thromboplastin complex which represents most probably the only form inactivable progressively by the natural inhibitor.

This assumption is to some extent in disagreement with observations describing normal inactivation in $BaSO_4$ adsorbed sera[7-9]. It seems, however, that no attempt has been made in these studies to determine the possible small residue of proconvertin after adsorption. According to the analysis of HJORT[2] and of ourselves[3], it is very probable that tissue thromboplastin, proconvertin, and inhibitor react in quantitative proportions. If diluted thromboplastin is used for inactivation, a small percentage of proconvertin may be sufficient to 'saturate' the



Percentage of native serum in the inactivating mixture (diluted with completely adsorbed serum)
Clotting times After 60 min incubation ————
After 30 min incubation - - - - - - -

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amount of thromboplastin present, since about 15% of factor VII complex sufficed for maximal inactivation of undiluted thromboplastin under our experimental conditions.

Tab. I

Inactivating mixture		Clotting times after incubation of		
		1 min	30 min	60 min
Normal native serum 15%	Normal adsorbed serum 85%	12·6″	30·0″	34·1″
Reconstituted normal serum 15%	Normal adsorbed serum 85%	12·7″	29·0″	31·2″
Proconvertin deficient serum 15%	Normal adsorbed serum 85%	15·3″	16·5″	16·6″

Tab. II

Inactivating mixture		Clotting times after incubation of		
		1 min	30 min	60 min
Normal native serum 15%	Normal adsorbed serum 85%	12·6″	26·8″	33·9″
Reconstituted normal serum 20%	Normal adsorbed serum 80%	11·8″	27·4″	34·6″
Stuart factor deficient serum 20%	Normal adsorbed serum 80%	11·6″	26·2″	32·6″

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Résumé

Le rôle de la proconvertine et du facteur Stuart dans l'inactivation de la thromboplastine tissulaire par l'inhibiteur sérique a été examiné dans un système adéquat. Après avoir reconfirmé l'indispensabilité du complexe facteur VII pour l'action de l'inhibiteur, il a été constaté que seule la proconvertine est nécessaire pour l'inactivation tandis que le facteur Stuart ne paraît pas participer à ce processus.

Biological Properties of Synthetic Bradykinin

In the course of synthetic work on polypeptides BOISSONNAS, GUTTMANN, and JAQUENOUD recently synthesized[1] a nonapeptide with the following structure: H-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg-OH.

A preliminary account of its biological activity was given in this journal[2] with the conclusion: 'Further work will demonstrate if it is identical with or closely similar to pure bradykinin'.

Active concentrations of different bradykinins on various biological structures.

	Synthetic bradykinin[5]	Natural bradykinin by the action of	
		Trypsin[6]	*Bothrops* venom[7]
Isolated guinea pig ileum	1 ng/ml	1 ng/ml	1 ng/ml
Isolated rat uterus	0.03 ng/ml	0.1 ng/ml	0.1 ng/ml
Blood pressure in the anaesthetized cat	500 ng/kg	400 ng/kg	30 ng/kg
Capillary permeability by intradermal injection in the guinea pig	1 ng	1 ng	10 ng

Since then ELLIOTT, LEWIS, and HORTON have, in view of the biological properties of this synthetic nonapeptide, reinvestigated their proposed structure for natural bradykinin[3] and, after new degradation studies, provided evidence[4] that natural bradykinin is a nonapeptide having the structure depicted above. Therefore, the previously reported synthetic nonapeptide[1, 2] is in fact synthetic bradykinin.

Further experimental work with this synthetic bradykinin by KONZETT and STÜRMER[5] has revealed that it behaves like pure natural bradykinin[6] in several tests. From a comparison of the quantitative data, some of which are given in the Table, it can be seen that the values for the activity of synthetic bradykinin and of natural pure bradykinin obtained from ox plasma by the action of trypsin are identical within the limits of biological variations. The conclusion therefore follows that, in its biological activity, synthetic bradykinin is identical with natural bradykinin obtained from ox plasma by the action of trypsin.

Data on the biological characteristics of pure natural bradykinin obtained from ox plasma by the action of *Bothrops jararaca* venom, which were reported by JAQUES and MEIER[7], have been included in the Table. They correspond reasonably well with the figures for the other natural bradykinin and for synthetic bradykinin. With regard to biological activity all three bradykinins behave similarly and cannot be distinguished from one another.

The synthesis of bradykinin opens up the possibility of using a pure and chemically defined polypeptide of high biological activity in medical research.

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