Part II

MECHANISM OF THE COAGULATION OF BLOOD BY PROTEOLYTIC ENZYMES
Part II

Introduction

Many natural and artificial coagulants can coagulate blood; some of them, like thrombin, can directly convert fibrinogen to fibrin, while others transform prothrombin to thrombin. Amongst the natural and artificial coagulants, various snake venoms, certain proteolytic enzymes such as trypsin and papain and also ninhydrin, alloxan and salicylaldehyde (65) and naphthaquinone (66) are included.

The venoms have a profound effect on blood coagulation. Venoms of Notesthes scutatus, Bothrops atrox, Bothrops Jararaca act like a-platelet system in converting prothrombin into thrombin and this has been observed by Eagle (67), Hanut (68), on the other hand, observed that Bothrops atrox venom can also coagulate fibrinogen, converting directly into fibrin and calcium \((\text{Ca}^{++})\) hastened this activity. Hargreaves (69) found that Bothrops Jararaca can coagulate fibrinogen only and has no action on prothrombin. Macfarlane and Barhett (70) and others (27, 28) showed that Russell’s viper venom has a thromboplastin like action and require calcium \((\text{Ca}^{++})\) for its significant effect. Eagle (67), however, found that venoms of Crotalus horridus, Crotalus terrificus and of Bothrops nummifer have a thrombin like action, as they can directly convert fibrinogen to fibrin and do not require calcium \((\text{Ca}^{++})\).

The action of the venoms was supposed by Eagle to be due to the proteolytic activity of venoms themselves. Janszky (71)
determined the ratios of the clotting and the proteolytic activities of different snake venoms and suggested (72) that these two properties are identical.

Taborda and Taborda (75), on the other hand, differed from the above view. They found that the clotting and proteolytic properties of certain venoms are destroyed by heating them at two different temperatures and therefore concluded these properties to be different. Deutsch and Diniz (74) also suggested that the enzymes of snake venoms responsible for the clotting of fibrinogen and the proteolysis of haemoglobin have separate entities.

Douglas and Colebrook (75) and also Heard (76) found that trypsin has got the property to coagulate blood. Eagle and Harris (77) investigated the action of trypsin and found that it was capable of converting prothrombin to thrombin even in absence of calcium (Ca++) . Ferguson and Erickson (78) showed that trypsin mobilized an active thromboplastin from some inactive precursor and they considered that proteolytic factor in the plasma has a similar role in normal coagulation. Eagle and Harris (77) also noted that papain can coagulate plasma by converting fibrinogen directly to fibrin. That, these non-specific clotting agents such as different snake venoms and also proteolytic enzymes produce clots of similar network structure if not identical, under electron microscope, has been described by Laki (79). But Janszky (80), showed that some individual variation definitely exists in their actions at the molecular level.
The conversion of fibrinogen to fibrin by thrombin has been shown to be a proteolytic reaction by Lorand and Middlebrook (81) and Bettleheim and Bailey (82). This view has further been corroborated by Sherry and Troll (83) who have shown that thrombin can hydrolyse synthetic substrates and therefore acts as a proteolytic enzyme. Ferguson, Travis and Gerheim (34), found tryptic activity in commercial tissue thromboplastin preparations and concluded on the basis of their investigations that the thromboplastic activity of these substances might be due to their proteolytic activity.

These findings opened up the question whether thromboplastic activity of venom or tissue thromboplastin is due to the proteolytic enzymes present in them or (the clotting property might be due to the presence of a separate factor). Dyckerhof and Giganto (84) claimed the possibility of the presence of a coagulating principle, phya-thrombin (as he termed), in the enzyme papain and suggested that the clotting factor of papain is different from its proteolytic factor. Experiments have, therefore, been carried out to determine whether the clotting of plasma observed by the proteolytic enzymes such as trypsin and papain is due to their proteolytic property or some other clotting factor present in them are involved. The following experiments have been devised to elucidate the mechanism of the clotting reactions by these two enzymes.

1. The effect of calcium (Ca++) on the proteolytic and clotting activities of trypsin and papain.
2. The determination of pH-optima for clotting and proteolytic activities of papain and trypsin.

3. The effect of different inhibitors such as heparin, versene and hirudin on the proteolytic and clotting activities of trypsin and papain.

4. The determination of the inactivation temperatures of trypsin and papain for their clotting and proteolytic activities.