INTRODUCTION

Fibrinopeptide A (FPA) is of particular interest, because it is released whenever thrombin converts fibrinogen to fibrin. Human FPA is a 16 amino acid peptide with a molecular weight of 1540, and it is cleaved from the N-terminal part of the Aα-chain of fibrinogen by the action of thrombin. Since the fibrinogen molecule has a dimer structure, two identical FPA molecules can be cleaved from each fibrinogen molecule.

PATHOPHYSIOLOGY

Any increased FPA concentration in plasma represents fibrin formation due to thrombin activity and thus activation of the coagulation. Since, with the measurement of FPA, a product of the last step of the activation of the coagulation is measured, the determination of FPA may give an overall evaluation of the activation of the coagulation cascade.

The measurement of FPA by radioimmunoassay has been introduced by Nossel and co-workers. Since that time the radioimmunoassay, and later the enzyme linked immunoassay, have been widely used, and they are considered by many authors as important and significant tests for the diagnosis of prethrombotic and thrombotic states.

The major diagnostic applications to date are the detection of acute deep venous thrombosis, disseminated intravascular coagulation, pulmonary thrombosis and lung embolism, angina and myocardial infarction, cerebral infarction, and for the monitoring of anticoagulant therapy. Quite a number of reports have become available on the activation or liberation of thrombin during fibrinolytic therapy of acute myocardial infarction.

The determination of FPA has also been used to follow activation of the
coagulation in cancer, during pregnancy, in diabetes, in liver cirrhosis, and during intake of oral contraceptives or oestrogen therapy. The activation of coagulation was also followed by measuring different activation markers such as FPA and others during physical exercise.

**METHODS OF ASSAY**

Since normal concentrations are around or below 2 ng/ml (< 1.3 nmol/L) and pathological values very often in the range of 3–20 ng/ml (2–13 nmol/L), the only sufficiently sensitive methods are radioimmunoassay (RIA) or enzyme-linked radioimmunoassay (ELISA). Nossel and co-workers were the first to develop a RIA for the determination of FPA. The antibodies used by Nossel became available to a broader community and therefore the number of publications studying thrombotic and prethrombotic activities in different diseases by means of FPA measurements increased enormously. Since 1971 several modifications of the original RIA of Nossel have been published (e.g. refs 60 and 61). Some of these modifications have been introduced into the RIA procedure recommended below.

Two commercial RIA kits have been available for many years. The RIA-mat FPA, originally manufactured by Mallinckrodt, then prepared and sold by Byk-Sangtec (Dietzenbach, Germany) was a ready to use kit with all reagents including the labeled Tyr-FPA; it has since been discontinued.

The other RIA is prepared by Imco. Imco supplies only three vials containing anti-FPA antiserum, FPA standard and Tyr-FPA for iodination with. The iodination of Tyr-FPA, all dilutions of the standards and the antiserum, as well as all the solutions necessary for the performance of the RIA, have to be prepared. It is at present not clear whether Imco will continue to supply the reagents.

In 1980 a solid-phase ELISA for the measurement of FPA was published. This ELISA test is commercially available as a complete kit, Asserachrom FPA, supplied by Diagnostica Stago. It is this assay which is now most frequently used for the determination of FPA. Unfortunately it has recently been discontinued.

The FPA-ELISA distributed by Diagnostica Stago was rather unique in its concept, and might not be easy to set up with reagents still available; it will therefore be discussed only very briefly.

Since the Mallinckrodt kit RIA kit is no longer commercially available, the methodological section below will preferentially deal with the RIA method as described by Imco. The reagents supplied by Imco can easily be replaced by reagents distributed by other companies. A list of companies supplying anti-FPA-antiserum, fibrinopeptide A as standard and Tyr-fibrinopeptide A for iodination is given at the end of this chapter.

**PRECAUTIONS**

Although the determination of FPA is an interesting method to document activation of coagulation with the formation of fibrin, the method has several...