Estimation of Total and Active Antithrombin III Before and After Open Heart Operations

THESIS

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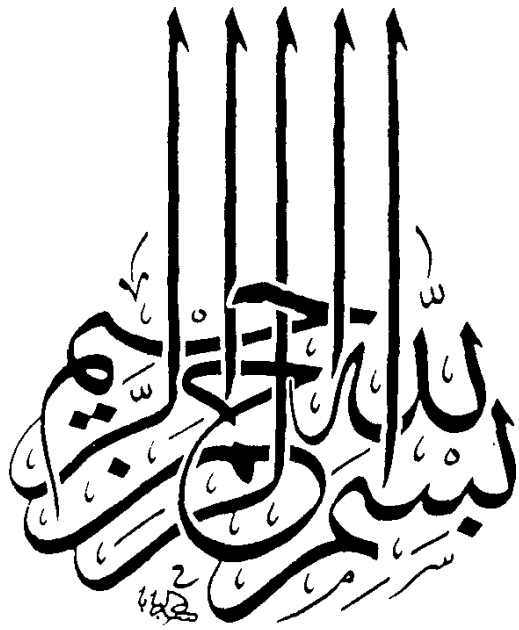

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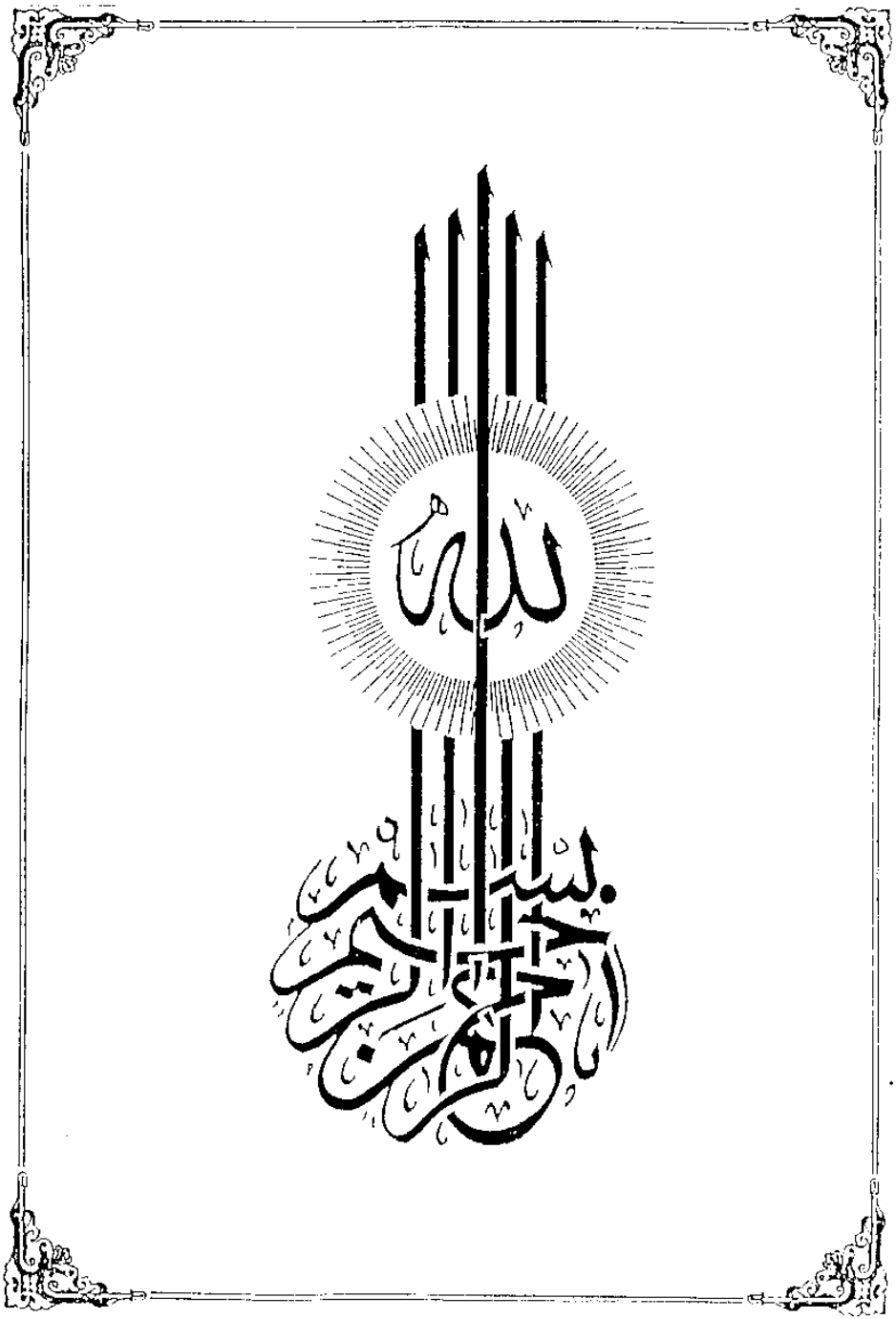

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CONTENTS

INTRODUCTION AND AIM OF WORK

I N T R O D U C T I O N

It has been postulated and generally accepted that coagulation occurs in a cybernetic manner with fibrin deposition and subsequent lysis occuring as a continuous process. The manifestations of normal hemostasis versus increased fibrin deposition (thrombus) or increased fibrinolysis (haemorrhage) depends upon a delicate balance between the procoagulant system and associated inhibitors as well as the fibrinolytic system and its associated inhibitors. Primary inhibitors of the procoagulant and fibrinolytic systems are comprised of anti-thrombin III, alpha$_2$ - macroglobulin, alpha$_1$ - antitrypsin, C1 esterase inhibitor and alpha$_2$ - antiplasmin **(Mosesson, 1974, and Irwin et al., 1975).**

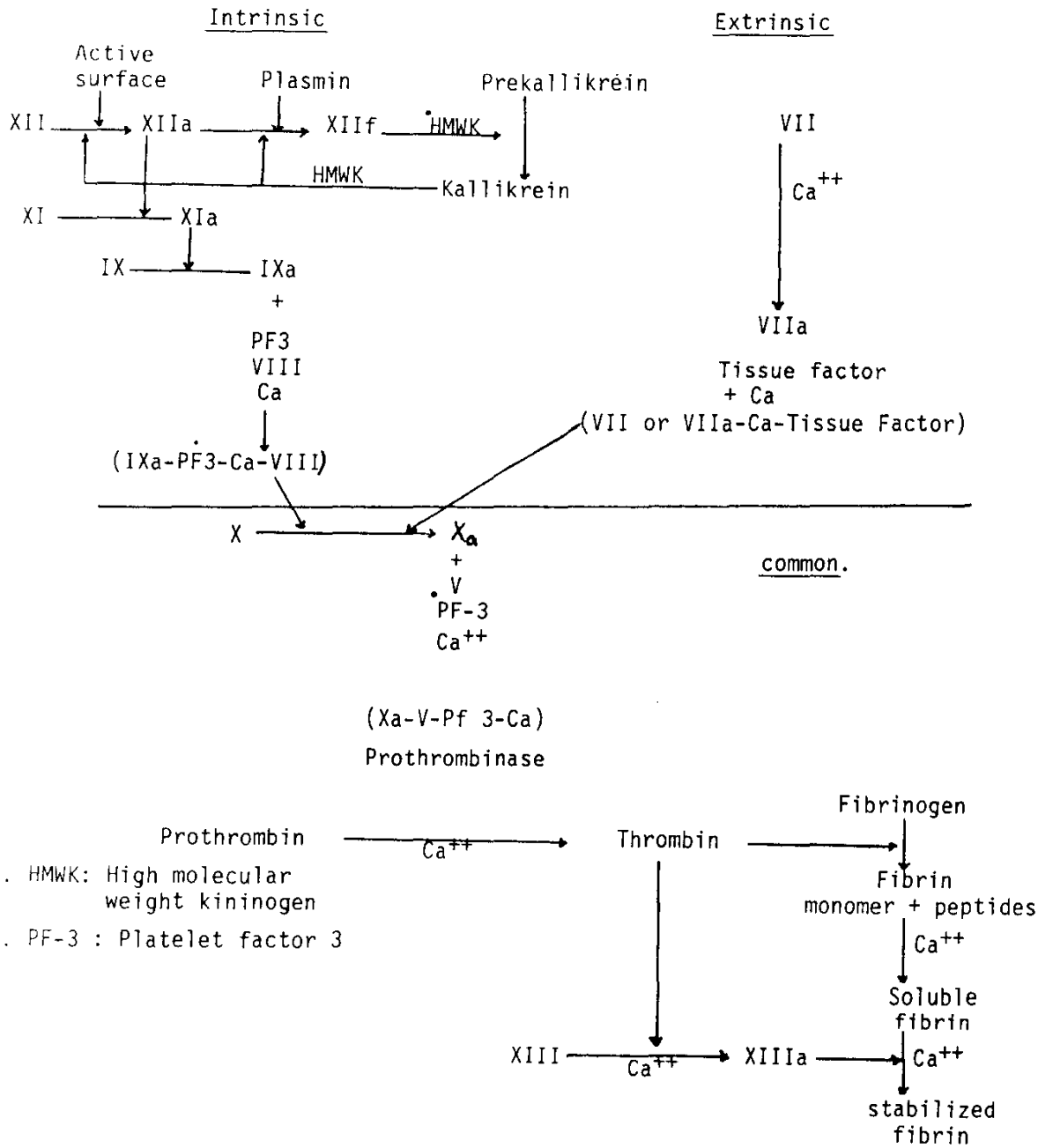
Anti-thrombin III (AT III), has been shown to inhibit the following serine proteases: (blood coagulaton factors).

. Thrombin **(Abildgaard et al., 1968).**

. Factor Xa **(Kurachi et al,. 1976).**

. Factor IXa **(Rosenberg et al., 1975).**

. Factor XIa **(Damus et al., 1973).**



. HMWK: High molecular weight kininogen

. PF-3 : Platelet factor 3

Coagulation cascade:

Modified by **Wintrobe et al. (1981)** from **Macfarlane (1966) & Davie and Ratnoff (1964)**, cascades.

- Factor XIIa and Factor XII_f **(Stead et al., 1976).**
- Plasmin **(Rosenberg et al., 1975).**
- plasma Kallikrein **(Schapira et al. 1982).**
- Factor VIIa. **(Kondo and Kisiel, 1987).**

So, anti-thrombin III plays an important role in the inactivation of blood coagulation factors such as thrombin and factor Xa, and is essential for the anticoagulant effect of heparin **(Yin et al., 1971).** It plays a central role in the prevention of intravascular clotting as demonstrated by the correlation between a reductin of antithrombin activity and thromboembolic phenomena **(Von Kaulla and Von Kaulla, 1972).** Anti-thrombin III determinations have been shown useful for aiding in diagnosis of numerous thrombohemorrhagic disorders, including disseminated intravascular coagulation (DIC) deep venous thrombosis (DVT) and pulmonary embolus. **(Hedner and Nilsson, 1973).**

The modern cardiac surgery becomes possible with development of cardiopulmonary bypass (CPB) . CPB is a technique in which blood is diverted away from the heart and lungs into a machine that substitutes for the ventilatory and pumping functions of these organs. The major components of CPB system are venous catheters, blood reservoir, pump, oxygenator , heat exchanger , filter and arterial catheter. This system also involves two

cardiotomy sucker systems and a venting system for the left ventricle **(Edmunds et al., 1978).**

Appropriate anticoagulation, induced by heparin is a fundamental requisite for heart surgery with CPB. Variation above or below an optimal range may cause severe complications. Intraoperative thrombosis or excessive bleeding occur occasionally during cardio-vascular operations **(Ponari et al., 1979).**

The anti-coagulant effect is measured by the activated clotting time (ACT) of whole blood **(Hattersley et al., 1966).** In **1975, Bull et al.** recommended a therapeutic range for ACT of 400 to 600 seconds during CPB. The lower limit was based on the clinical observation that blood clots do not occur in the extracorporeal circuit if the ACT is greater than 300 seconds.

The criteria for adequate anticoagulation included prevention of the consumption of clotting factors, the formation of microscopic deposits of fibrin in the extracorporeal circuit, and of the appearance of fibrin monemer in the plasma. This fibrin is known to be a sensitive indicator of activaton of the coagulation process. **(Kisker et al., 1977).**

So, activatd clotting time (ACT) should be at least 400 seconds, or four times normal,all over the duration of

the bypass in order to prevent coagulation and consumption. Exclusing coincident coagulopathies, the only major anticoagulant effect present after bypass is heparin effect which is completely reversible with protamine **(Young et al., 1978).**

The anticoagulant effect of a given dose of heparin varies greatly among patients. There is no correlation between the half-life of heparin (L ½) and the dosage of heparin administered **(Effeney et al., 1981).**

At the end of bypass the residual heparin must be neutralized by protamine to achieve effective haemostasis. Ten minutes following the administration of the initial dose of protamine sulphate an assay for residual plasma heparin is performed. The thrombin clotting time of the patient's plasma are determined in the presence of increasing amounts of added protamine sulphate. The quantity of protamine sulphate required to obtain the minimum thrombin time is used to estimate additional protamine sulphate requirements **(Hawksley, 1966).**

Heparin rebound is defined as the resurgence of anticoagulant activity in a heparinized patients whose blood had been thought to be adequately neutralized with protamine **(Hampers et al., 1966).** Heparin converts the

antithrombin III from a progressive inhibitor to an immediate inhibitor of various serine proteases (Rosenberg and Damus, 1973).

If the heparin is removed from heparin-antithrombin complex by the addition of protamine, the antithrombin III reverts to be a progressive inhibitor (Kitani et al., 1980).

Heparin + AT III [Heparin-AT IIIa] $\xrightarrow{\text{Excess Protamine}}$ Heparin - protamine + AT III.

By the low of mass action, the heparin-protamine complex remains stable only in the presence of an excess of protamine. If this excess is not maintained, then heparin can be freed from its complex with protamine and the equilibrium is shifted back to reactivation of the antithrombin (Bachmann et al., 1975). Although heparin rebound is not usually seen when a significant excess of protamine is used in the neutralization of heparin. There has been a hesitency on the part of surgeons to give too much protamine to a patient based on the idea that protamine itself can act as an anticoagulant (Anderson et al., 1979). However, protamine is a very poor anticoagulant, and it would take an excess far and above that which is usually given to affect hemostasis (Ellison et al., 1981).

When excess protamine (4.5 : 1) is used to neutralize the amount of heparin ordinary given during CPB procedures, the complexes that are formed between heparin and protamine are quite large and have capacity of activating anti-thrombin III. Thus, the incidence of heparin rebound after CPB procedures should be greatly reduced if a moderate excess of protamine is used in the neutralization of heparin at least (1.5 : 1). **(Shanberge et al., 1987).**

Aim Of Work:

Our aim of work is to measure the total concentration of antithrombin III and the active antithrombin III levels before and after cardiopulmonary bypass to compare between them and to detect the effect of heparin on it. Also, we shall study the effect of perfusion time on the level of antithrombin III concentration and activity.

Evaluation of the two methods of estimation of antithrombin III will be done.

REVIEW OF LITERATURE