EFFECT OF SEX HORMONES ON FACTOR XIII ACTIVITY

Thesis

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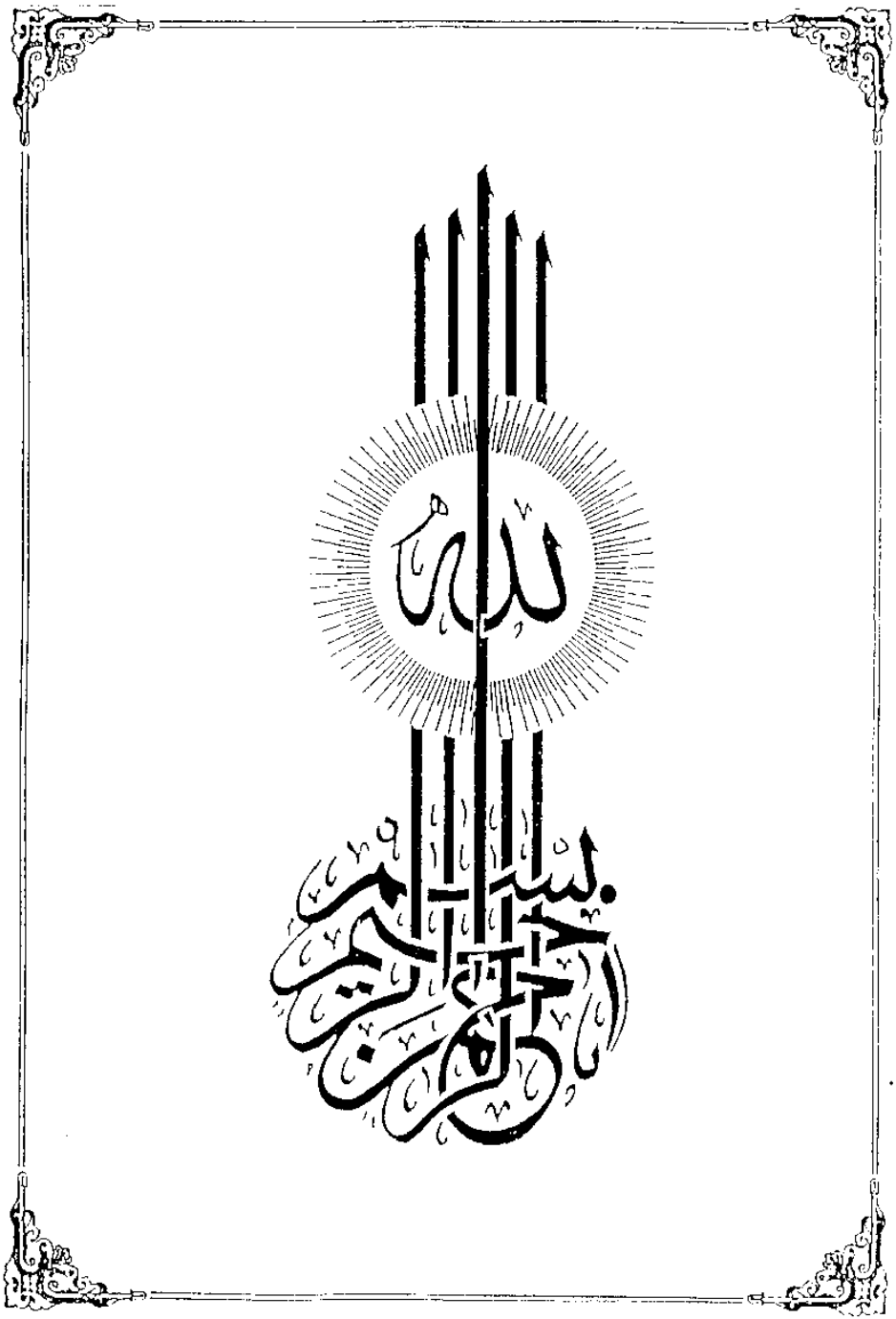

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INTRODUCTION

In the final steps of blood coagulation, fibrin, which is formed as a result of action of thrombin on the plasma protein fibrinogen, polymerizes and aggregates to form a macroscopic firm insoluble network the fibrin clot. The formation of this firm strong fibrin clot requires the presance of a specific clotting factor, known as fibrin-stabilizing- factor, FSF (Factor XIII).

In its absence, the clot will form, but it will be a weak, easily solubilized one.

Sex hormones have long been known to enhance blood coagulation and were found effective in haemostasis.They were reported to cause an increase of most of blood clotting factors, though decrease of others. Although studies regarding the effect of sex hormones on blood coagulation are conflicting, yet their procoagulant effect is doubt less.

Stability of the fibrin clot is of importance in various clinicopathological conditions. The study of factor XIII activity in animals treated by this group of drugs, namely sex hormones, as a part of their procoagulant effect, may prove of clinical value.

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REVIEW OF LITERATURE

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The phenomenon of blood coagulation entails three basic reactions, occuring in the following sequence (Seegers, 1969):

1. Formation of prothrombin activator (autoprothrombin C = F X a + thrombokinase) in response to rupture of the vessel or damage to the blood itself.

2. Formation of thrombin from prothrombin under the effect of the prothrombin activator.

3. Formation of fibrin clot. The thrombin acts as an enzyme that converts the plasma protein " fibrinogen" into fibrin threads that enmash red blood cells and plasma to form the clot itself .

This last reaction occurrs in steps, which include the release of two peptide fragments from the fibrinogen molecule, with formation of fibrin monomers. Fibrin monomers polymerize and aggregate to form soluble fibrin , which is then changed into insoluble fibrin clot(Husbey & Bang, 1971).

This final stage of blood coagulation, namely the formation of insoluble fibrin, is the direct result of cross-linking in the fibrin polymer by the action of a transpeptidating enzyme (Husbey, 1971) with fibrin to fibrin association by inter molecular γ - glutamyl-ε-lysine bridges (Lorand, 1972).

This very last step is under the influence of a plasma factor known as fibrin stabilizing factor (Factor- XIII), also known as Laki - Lorand factor, fibrinase or plasma transglutaminase.

HISTORICAL ASPECTS :-

Fibrin stabilizing factor (FSF) was first reported by Robbins (1944) who observed that a plasma factor promoted the formation of fibrin which was insoluble in dilute acids and alkalies.

Four years later, Laki and Lorand (1948) confirmed this finding and introduced the use of 5 M urea as a solubilizing agent. Since the recognition of this factor was due primarily to Laki and Lorand it became known as Laki-Lorand factor or L-L factor (Loewy & Edsall, 1954).

Few years later, Lorand and Jacobsen (1958) could isolate this factor and purify it.

Loewy and his co-workers (1961_a , 1961_b , 1961_c 1961_d)further isolated it and were able to purify it 8000-fold over plasma . They demonstrated that the action of FSF was enzymatic, and in view of its enzymatic nature, they proposed the name fibrinase for this factor (Loewy et al, 1961_a).

Lorand and Konishi (1964) showed that an inactive precursor of FSF existed in the plasma which could be activated by thrombin and calcium,

The inactive precureor has been officially recognized and named Factor XIII .

Recently, FSF has been shown to catalyze a transpeptidation reaction between lysine and glutamine residues, which leads to the formation of ε (γ- glutamyl - lysine) cross-linke between fibrin monomers to produce urea insoluble fibrin (Matacic & Loewy, 1968) , therefore its active form (F $XIII_a$) became established as transglutaminase (Esnouf, 1972).

Origin and distribution of factor XIII :-

The tissue of origin of FSF is unknown. Its concentration is decreased in the plasma of some patients with hepatic disease and in animals poisoned with carbon tetrachloride, suggesting that the liver may participate in its synthesis (Gerhold, et al , 1966) .

Factor XIII is present in plasma, but is apparently adsorbed to fibrin and therefore absent from serum(Lorand & Dickenman 1955). It was found to occur in tissues (Tyler and Lack, 1964) and also in platelets (Buluk, 1955; Nachman, 1965; Kiesselbach and Wagner, 1966).

Platelets are indeed a rich source of FSF. If the number of circulating platelets are arbitrarily set at 400,000/mm^3 , then the ratio of the total amount of plasma FSF to total platelet FSF is about 5:1. Thus , a centrifuged platelet button represents a concentrated source of FSF,which is also relatively free of plasma proteins (Kiesselbach and Wagner, 1966).

Factor XIII has also been found to be present in the placenta (Schwartz et al, 1973).

Biochemical Characteristics :-

Factor XIII isolated from human plasma has a molecular weight of 350,000 and can be dissociated into subunits, the process enhanced by a variety of conditions such as dilution, low pH and heat (Loewy et al. 1961_b) . The two different types of subunits of plasma FSF, the "a" and "b" chains, have a molecular weight of about 75,000 and 88,000 respectively (Schwartz et al, 1971) . On the other hand , platelet factor XIII has a molecular weight of 146,000 , it gives a single type subunits, the "a" chains, with a molecular weight of about 75,000 (Schwartz et al, 1971).

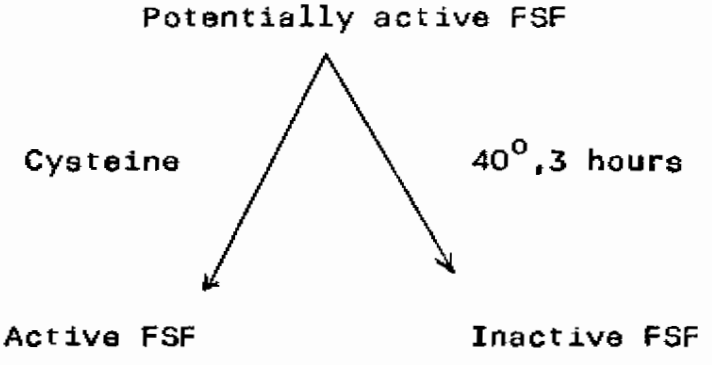
In fact the "a" chains of platelet and plasma FSF are identical,not only in molecular weight , but also in gel electrophoresis and in amino acid composition. The subunit structure of plasma factor XIII was claimed to be a_2 b_2 while that of platelets is a_2(Schwartz et al, 1971).

Mc Donagh et al (1978) stated that factor XIII , circulating in plasma as non-covalently associated tetrameric zymogen polypeptide (a_2 b_2), has the enzymatic activity residing in the "a" chains,while the "b" chains have a carrier or protective function for the zymogen.

At the same time, Lewis et al (1978) reported that factor XIII from normal plasma is composed of two antigenically distinct subunits,which they called A and S , while normal serum contains only free S units which do not support stabilization of purified fibrin clot .

Sulfhydryl groups are essential for the activity of factor XIII (Lorand and Jacobsen, 1958) and cysteine stabilizes the purified enzyme (Loewy et al, 1961_b).

In fact Lorand and Jacobsen (1958) denied the previous suggestion of Loewy and Edsall (1954) that cysteine could substitute for FSF, and concluded that it was only capable of activating " potentially active form" of FSF. They proposed the following scheme to illustrate what they called various forms of FSF:



The inactive form of FSF was found to increase on storage as well as heating (Kiesselbach and Wagner, 1966).

Factor XIII does not dialyze out from plasma (Lorand, 1950).

Kiesselbach and Wagner (1966) found that the circulating level of FSF is not dependent upon the fibrinogen level because when studied by immunologic methods, afibrinogenemic patients were found to have traces of fibrinogen in their plasma.These afibrinogenemic patients had less than 2 mg/100 ml of plasma fibrinogen and normal amounts of plasma FSF.

The haemostatically effective level of F XIII is about 5 % (Walls & Losowsky, 1968) . In prophylactic transfusion, F XIII as low as 0.5 % may be sufficient to prevent spontaneous bleeding.

The plasma half life of factor XIII has been estimated from 3 - 7 days to as long as 12 days (Miloszewski and Losowiski, 1970).

F XIII is stable and is present in fresh, frozen, stored reconstituted dried plasma. The long half-life, the stability of factor XIII in plasma products and the low level required for haemostasis provide good possibilities for prophylaxis.

Role of FSF in the formation of insoluble fibrin clot :

Fibrinogen under the enzymatic influence of thrombin can give rise to two major types of clot structure (Lorand, 1950) :

(1) A "fibrin clot" obtained when purified fibrinogen is mixed with purified thrombin in either the presence or the absence of calcium. Such a clot can be reversibly dispersed in 5 M urea or in one-per-cent monochloro-acetic acid, From this, it is concluded that the gel net-work is held together by inter molecular hydrogen bonds between some of the side chains of the fibrin monomers.

(2) In contrast, the "plasma clot" as formed for example by the recalcification of oxalated plasma , cannot be dissolved in urea nor monochloro-acotic acid. Therefore, it was assumed that the plasma clot which proved to be mechanically stronger than the fibrin clot is cross- linked not only by secondry but also by covalent bonds (Lorand and Jacobsen, 1958).

The fibrin stabilizing factor of plasma is a stoichiometric partner of fibrin in clot formation, acting as a resistant cement between the fibrin particles.

The ratio between the amount of FSF and fibrinogen appears to be critical , reminiscent of the antigen - antibody precipitation reaction. Between the zone of FSF excess and that of fibrinogen excess, there is the " zone of optimal combination" representing the real stoichiometric ratio between FSF and fibrin (Lorand and Jacobsen, 1958) .

Before the circulating factor XIII could exert its function in stabilizing the fibrin clot, it is changed into its active enzyme form"F XIII a"by action of thrombin (Buluk et al, 1961 ; Lorand and Konishi, 1964).

The rôle of FSF in formation of urea insoluble fibrin clot has been recently established (Schwartz et al, 1971). It catalyzes cross-linkages between fibrin monomers.

Neighbouring molecules are reciprocally linked by two ε - (γ - glutamyl) lysine cross bridges near their carboxy terminals (γ- γ cross - linking system). The two chains fit together quite naturally with the lysine-15 of one chain situated opposite glutamine-7 of the other and vice versa. Moreover, leucine- 11 sits comfortably next to leucine 11 of its neighbour (Schwartz et al,1971) .