SEROLOGIC STUDIES IN HEMORRHAGIC DIASTHESIS OF UNDEFINED ETIOLOGY

Thesis

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By

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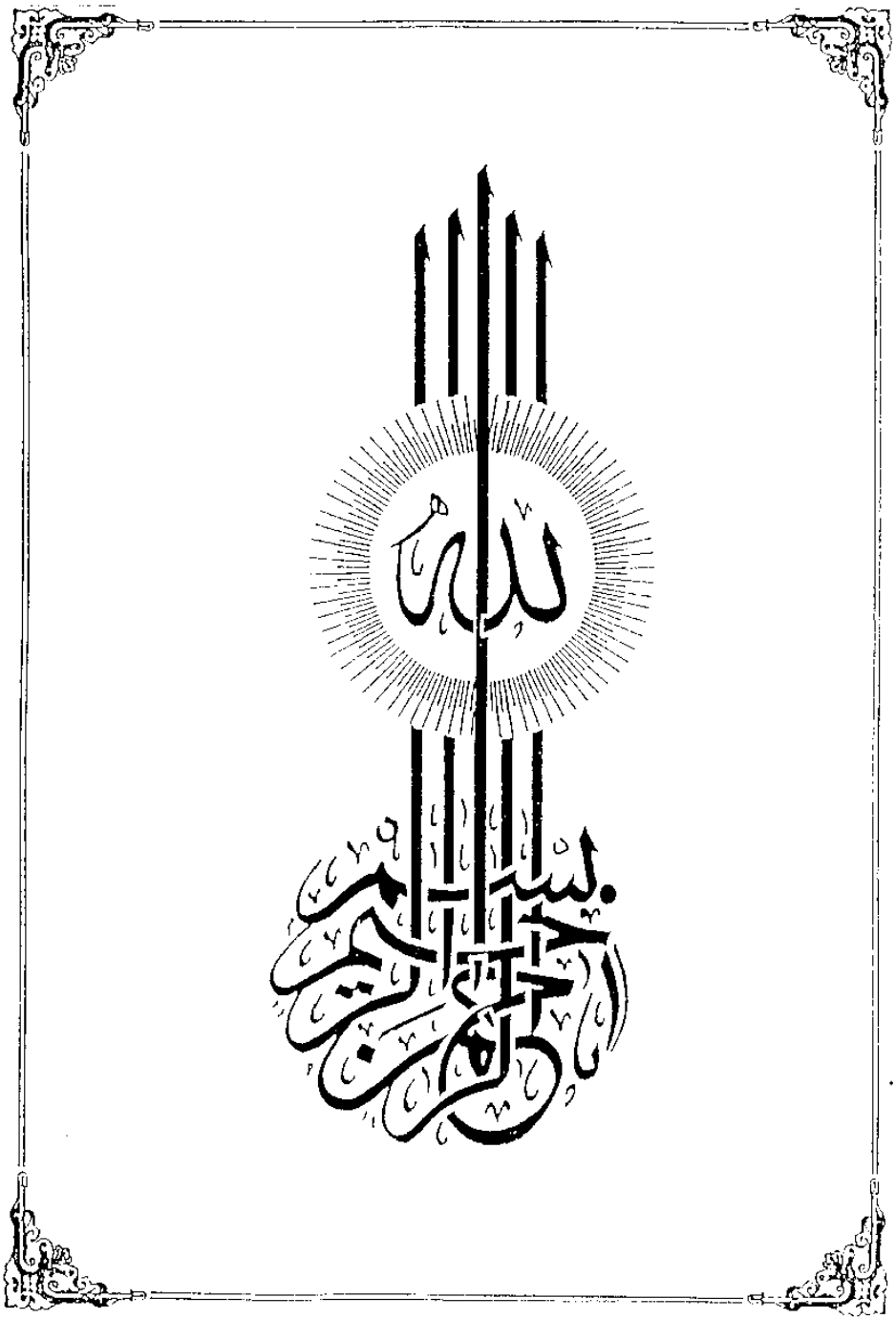


C O N T E N T S

AIM OF THE STUDY

Hemorrhagic diseases are common alarming diseases in infancy and childhood for which an etiologic diagnosis cannot often be immediately defined. Therefore , the present work aims at delineation of the role of some aspects that could be operative in the etiology of obscure cases of hemorrhagic diathesis namely immune mechanisms through the search for the presence of specific antibodies in the blood of those patients .

REVIEW OF LITERATURE

HEMOSTASIS

When a small blood vessel is transected or damaged, the injury initiates a series of events that leads to the formation of a clot. The end result of this process of hemostasis is generally a sealing off of the blood vessel and prevention of further blood loss .

The initial event is constriction of the blood vessel and formation of a temporary hemostatic plug of platelets. This is followed by conversion of the plug into the definitive clot. The in-vivo action of the clotting mechanisms responsible for this conversion is balanced by limiting reactions that normally prevent clots from developing in uninjured vessels and maintain the blood in fluid state .

Local vasoconstriction :

The constriction of an injured arteriole or small artery may be so marked that the lumen is obliterated. The vasoconstriction is probably due to serotonin and other vasoconstrictors liberated from platelets which adhere to the walls of the

damaged vessels. It is claimed that, at least for a time after being divided transversely, arteries as large as the radial artery costrict and stop bleeding .

The temporary hemostatic plug :

When a blood vessel is damaged, the endothelium is disrupted and an underlying layer of collagen is exposed. Collagen attracts platelets, which adhere to it and liberate serotonin and adenosine diphosphate (ADP). The ADP in turn rapidly attracts other platelets and a loose plug of aggregated platelets is formed. ADP from disrupted red cells and tissue may also contribute to the initial aggregation (Ganong,1977) .

The clotting mechanism :

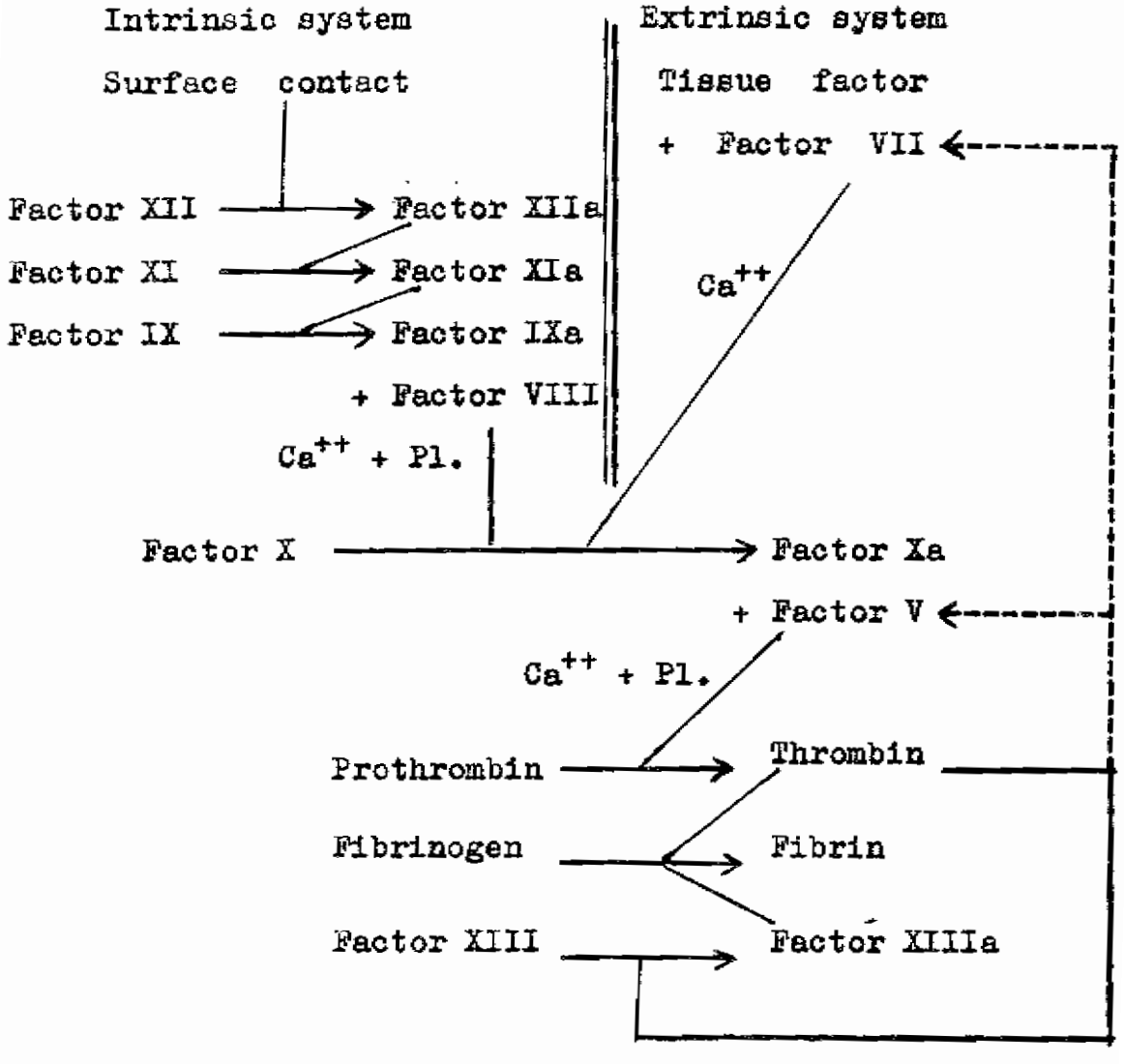
The loose aggregation of platelets in the temporary plug is bound together and converted into the definitive clot by fibrin. The fibrin formation is the end result of a large number of preceding biochemical events involving a series of soluble coagulation factors (table 1), the first step of which is the activation of factor XII (Hageman factor) by contact with a foreign surface such as damaged endothelium .

Macfarlane (1964) first suggested that the subsequent chain of events could best be explained by a cascade of proenzyme-enzyme transformation , each enzyme activating the next until the final substrate, fibrinogen , is reached (Fig. 1) .

Coagulation factors would normally be circulating in inactive state . Macfarlane pointed out that a mechanism acts as a " biochemical amplifier " whereby a small initial stimulus would result in a massive final reaction since each enzyme might activate ten times its own number of proenzymes. Rapid fibrin formation seems to be essential for sufficient hemostasis .

Table 1 : The coagulation factors

Factor	Synonym
I	Fibrinogen
II	Prothrombin
III	Thromboplastin , Tissue extract
IV	Calcium
V	Labile factor , ac-globulin , Proaccelerin
VII	Stable factor , Proconvertin , Serum prothrombin conversion accelerator (SPCA)
VIII	Antihemophilic globulin (AHG), Antihemophilic factor , Antihemophilic factor A
IX	Christmas factor , Plasma thromboplastin component (PTC) , Antihemophilic factor B
X	Staurt - Prower factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor
XIII	Fibrin stabilizing factor



Transformation

Action

Catalytic action

Ca^{++} = Calcium

Pl. = Phospholipid

Fig. 1 : A cascade mechanism for blood clotting

A similar theory was proposed by Davie and Ratnoff (1964). There have been minor modification since (Macfarlane,1968 ; Davie et al.,1969) with evolvement of evidence suggesting that factor VIII and factor V do not function as proenzymes but as cofactors for the action of the enzyme generated in the preceding stage, viz. IXa and Xa respectively .

Platelets participate, by PF_3 or phospholipid availability, at the stage of interaction between activated factor X and factor V. Calcium ions are necessary for most of the steps .

The coagulation factors involved in the series of reactions beginning with activation of factor XII and ending in fibrin formation constitute the intrinsic coagulation system, all the components of which are present in, or intrinsic to, the circulating blood . There is also an extrinsic coagulation system involving tissue thromboplastin and a further factor (VII) not included in the intrinsic system .

Both systems have a final common pathway from factor X to fibrin formation (Stormorken and Owren, 1971). In the presence of platelets (phospholipid), Ca^{++} and factor V, activated factor X catalyzes the conversion of prothrombin to thrombin .

Thrombin cleaves fibrinopeptides A and B from the alpha and beta chains of fibrinogen. The residual peptide chains aggregate by means of loose hydrogen bonds to form fibrin monomer. Under the influence of factor XIII, itself activated by thrombin, further covalent cross links are formed which convert it into a more permanent form of fibrin .

The fibrinolytic system provides a mechanism for removal of physiologically deposited fibein(Fig.2). Plasminogen from the circulating plasma is laid down with fibrin during the formation of thrombi. Activator present in the walls of blood vessels is capable of transforming this plasminogen into the free proteolytic enzyme plasmin the natural substrate of which is fibrin. Circulating inhibitors of fibrinolysis, antiplasmins , are present in the circulating plasma but are excluded from the interior of thrombi. The products of digestion of fibrin by plasmin are a series of fibrin degradation products (Willoughby,1977) .

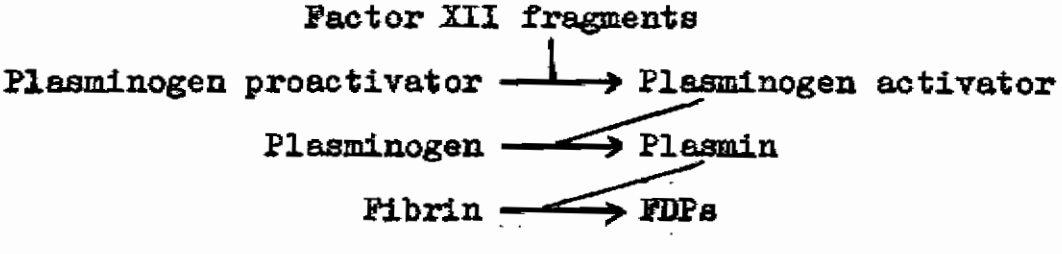


Fig. 2 : Fibrinolytic System

IMMUNOLOGIC HEMORRHAGIC DIASTHESIS

Introduction

The recognition that certain blood disorders were brought about by an autoantibody mechanism stems from observations made early in this century. In 1904 Donath and Landsteiner showed that the serum of patients suffering from paroxysmal cold hemoglobinuria contained an antibody , a hemolysin , which was adsorbed to red cells at a low temperature and which led to lysis by complement if the cell-serum suspension was subsequently warmed to body temperature .

A few years later Widal and his colleagues reported in France the first observations which suggested that aquired hemolytic icterus might have its origin in the development of autohemagglutinins, and a little later Chauffard and Fiessinger (1907) emphasized the role of hemolysins in the causation of hemolytic anemia associated with hemoglobinuria. The importance of these early observations was not,however, widely appreciated until the late 1930s and it was not until well after introduction of the antiglobulin test (Coombs test) in 1946 that the role of autoantibodies

in the causation of aquired hemolytic anemia was generally accepted and the etiologic term "autoimmune" was commonly used .

In addition, certain drug-induced blood dyscrasias are now known to be brought about by immunological mechanisms. After Ackroyd's (1949) pioneer observations on thrombocytopenic purpura following the administration of the hypnotic sedormid , a similar mechanism has been found to operate in purpuras due to certain drugs .

The idea that idiopathic thrombocytopenic purpura (ITP) might have a similar pathogenesis and be caused by antiplatelet autoantibodies is of more recent origin and is even now not much more than 20 years old .

It was Harrington and his colleagues (1951) who first showed that the blood or plasma of 60 per cent of patients suffering from ITP contained a factor capable of producing thrombocytopenia in a normal recipient and that this factor had many of the characteristics of an antibody. Later, Shulman et al (1964) extended these observations by showing that :

1- The factor was present in the 7 S γ- globulin fraction .