PLASMA FIBRONECTIN CONCENTRATIONS IN BLEEDERS AND NON BLEEDERS PATIENTS WITH HEPATOSPLENIC SCHISTOSOMIASIS

THESIS

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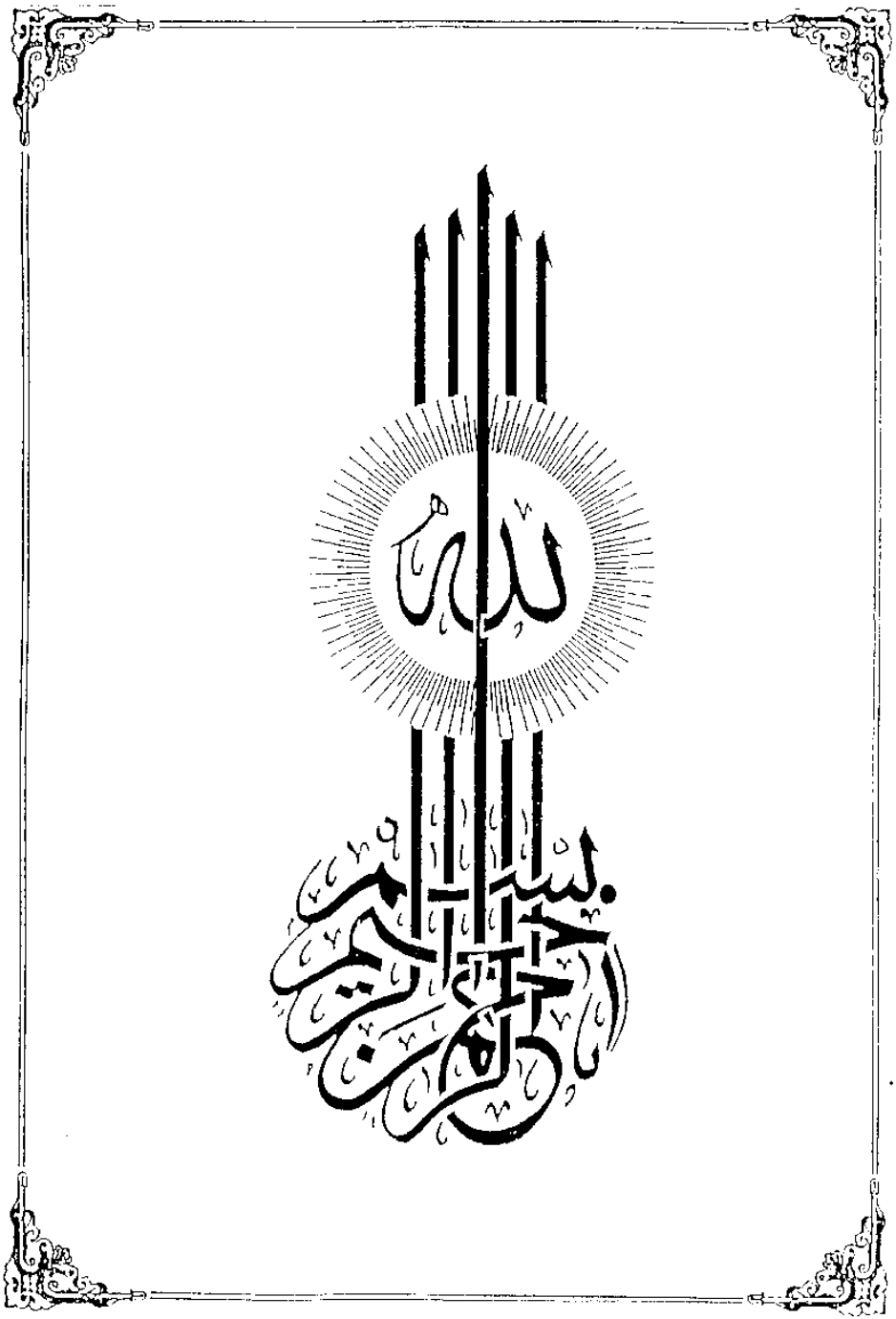

CONTENTS

INTRODUCTION AND AIM OF WORK

INTRODUCTION

Fibronectin (cold insoluble globulin) is a high molecular weight glycoprotein found both in an insoluble form in many tissues, such as interstilial connective tissue, in many basement membranes, around smooth muscle cells and fibroblasts and in the sarcolemma of striated muscle fibres **(Linder, et al., 1975, Stenman, et al., 1978)** and in soluble form in plasma and other body fluid **(Mosesson, et al., 1970; Kuusela, et al., 1978; Yamada, et al., 1978).**

A role of fibronectin in haemastasis is indicated by its interactions with fibrin and by its presence in platelets. Plasma fibronectin (cold insoluble globulin) can be cross-linked to itself and to fibrin by plasma transglutaminase "Factor XIII" **(Mosher, et al., 1975).** Thus, it is incorporated into fibrin clot **(Mosher, et al., 1980).**

Schistosomiasis is considered one of the main important cause of chronic liver disease resulting in portal hypertension and splenomegaly and the course of portal hypertension often is complicated by oesphageal varices. **(Hussein, et al., 1962).**

AIM OF WORK

The aim of the present study is to elucidate the concentration of plasma fibronectin in patients with and without bleeding varices in cases of hepatosplenic schistosomiasis as plasma fibronectin is a glycoprotein which has been associated with hepatic failure **(D'Ardenne, et al., 1984)** and decreases in patients with bleeding tendency due to its role in platelet aggregation and adhesion to fibrin thrombi **(Hynes, et al., 1978).**

Thus, we under took this prospective study to assess the prognostic value of plasma fibronectin in intestinal schistosomal hepatosplenomegaly patients with bleeding oesphageal varices.

REVIEW OF LITERATURE

DISCOVERY AND NOMENCLOTURE

In 1948, Morrisen et al. isolated a partially purified fraction of human plasma which they term "Cold insoluble globulin" (CIG). Other workers independly described the various proteins or factors as anti-gelatin factor **(Wolff, et al., 1967)**, microfibrillar protein **(Ross, et al., 1969)**, opsonic protein **(Saba, et al., 1970)**, galactoprotein a **(Gahmberg, et al., 1974)**, large external, transformation-sensitive "LETS" protein **(Hynes, et al., 1974)**, cell surface protein "CSP" **(Yamada, et al., 1974)**, fibroblast surface antigen "FSA" **(Ruoslahti, et al., 1974)** Zeta "Z" **(Blumberg, et al., 1975)**; cell spreading factor **(Grinnell, et al., 1976)**, cell attachment factor "CAF", "C. CAP" **(Pearlstein, et al., 1976)**. These factors are named according to sources or biological activities.

Recent evidences indicate that all these proteins are closely related, and they are probably only one or two specific proteins: Cell surface (Cellular) fibronectin, and plasma fibronectin. Cell surface fibronectin is a major constituent of the cell surface of many cultured cells, and was discovered when cell surface proteins or carbohydrates were labelled radio-isotopically or immunologically. This glycoprotein is also known as large, external, transformation sensitive "LETS" protein or cell surface protein "CSP" (Yamada et al., 1974).

Plasma fibronectin although known as cold insoluble globulin, purified plasma fibronectin is relatively soluble in the cold unless it is complexed with fibrin and fibrinogen **(Morrison, et al., 1948).**

Current information suggests that although the two forms of fibronectin are very similar, they are probably not identical. Whether they are products of different genes or of one gene and are modified post-transcriptionally, is not yet known. **(Yamada, et al., 1978).**

Distribution and Biologic Activities of Tissue Forms of Fibronectin:

Fibronectin is synthesized by a variety of different cells: fibroblast **(Ruoslahti, et al., 1973),** Astroglial cells **(Vaheri, et al., 1976),** certain epithelial cells including those derived from Carcinoma cell lines **(Chen, et al., 1977),** chondrocytes **(Dessau, et al., 1978),** myoblasts **(Furcht, et al., 1978),** endothelial cells **(Jaffe, et al., 1978)** Schwann cells **(Kurkinen, et al., 1979)** peritoneal macrophages have also been reported to synthesize and secrete fibronectin **(Johansson, et al., 1979),** although **Pearlstein, et al., (1978)** did not find the protein on the surface or within resident or activated peritoneal macrophage. Also isolated hepatocytes have been reported to synthesize and secrete fibronectin **(Voss, et al., 1979).** Fibronectin is a major component of the connective tissue matrix, immunofluoressent studies of normal tissues have demonstrated that it is present in many basement membranes, around smooth muscle fibres, in the sinusoidal walls of the Liver, in the stroma of lymphatic tissue, and in Loose connective tissues **(Stenman, et al., 1978).** Cellular expression of fibronectin is linked with cell differentiation and organogenesis. During embryo-

genesis fibronectin is first detectable on cells of blastula inner cell mass **(Wartiovaara, et al., 1978).** At later developmental phases, fibronectin is lost or becomes redistributed concomitant with differentiation of mesenchymal cells into muscle, cartilage and renal tubular epithelium **(Linder, et al., 1975).** In tissue culture, fibronectin usually appears as fibrillas matrices that are situated on cells, between cells, and between cells and the substratum **(Yamada, et al., 1978).** This pattern is consistent with the role it evidently plays in mediating the phenomenon of spreading and adhesion that takes places among cells and the substratum **(Pearlstein, et al., 1976).**

Studies of the effect of fibronectin addition on transformed cells have helped to clarify the role that this protein plays in regulating cellular functions. Adding fibronectin to cultures of transformed fibroblasts, which lack the extra cellular fibronectin matrix results in partial resumption of more normal behaviour and appearance **(Hynes, et al., 1978) & (Fircht, et al., 1978).**

The normalizing effects on the cell include restoration of a more flattened and elongated shape, improved adhesion to substratum, and reconstitution of prominent intracellular actin microfilament bundles **(Yamada, et al., 1976).** Fibronectin treated cells also display more rapid cellular locomotion **(Yamada, et al., 1978).**

STRUCTURE AND MOLECULAR INTERACTIONS

All fibronectins are composed of 200,000 - 250,000 molecular weight subunits. Plasma fibronectin is a disulphide-bonded dimer of two such subunits **(Mosesson, et al., 1975)**, cell surface fibronectin is found as disulphide-bonded dimers and multimers **(Hynes, et al., 1977).** The amino acid composition of fibronectin from different sources is very similar, but these are significant differences in Carbohydrate Composition **(Mosher, et al., 1980).**

Earlier observations suggested plasma and cell surface fibronectins to be immunohistochemically indistinguishable **(Yamada, et al., 1978)**, but a monoclonal antibody with specificity for cellular fibronectin has recently been described **(Atherton, et al., 1981).**

Recognition for fibronectin by this antibody was not dependent on Carbohydrate residues, and it was concluded that structural difference must exist between plasma and cellular forms. Some studies have indicated that plasma fibronectines have smaller apparent subunits than cell culture fibronectins **(Yamada, et al., 1979).** These authors also noted that, cell-surface fibronectin is 50 times more active than plasma fibronectin in restoring a more normal morphology to transformed cells. This property has been attributed to the multimeric form of the cell surface protein **(Mosher, et al., 1980).**

Fibronectin interacts with a variety of molecules including fibrin, collagen, glycosaminoglycans and DNA. In the presence of activated factor XIII (fibrin-stabilising factor), it is bound convalently

to fibrin and to itself **(Mosher, et al., 1975).** It binds more avidly to denatured Collagen (gelatin) than to native Collagens, but of the latter it has greatest affinity for Collagen type III **(Engvall, et al., 1978).** It binds to heparin, hyluronic acid and dextran sulphate, but not to dermaton sulphate or chondroitin sulphate **(Yamada,et al., 1980).** Affinity of gelatin or Collagen for fibronectin is increased in the presence of heparin, heparin sulphate or hyaluronic acid. It has been suggested that these interactions are involved in formation of extracellular matrices **(Jilek, et al., 1979).** Fibronectin can also bind to actin (an intracellular protein) **(Keski-oja, et al., 1980),** to staphylococcus **(Kuusela, et al., 1978)** and to cell surfaces.

A model of fibronectin molecule has been proposed (Fig. 1) based on binding properties of polypeptide fragments produced by proteolytic cleavage. The glutamine residue required for cross linkage to fibrin, Collagen and the surface of staphylococci is present at the N-terminal end. The gelatin-binding site is close to, but separated from the N-terminal end, and the glycosaminoglycans-binding site is at the C-terminal end. the cell attachment site is between gelatin and heparin-binding sites **(Mosher, et al., 1980).**

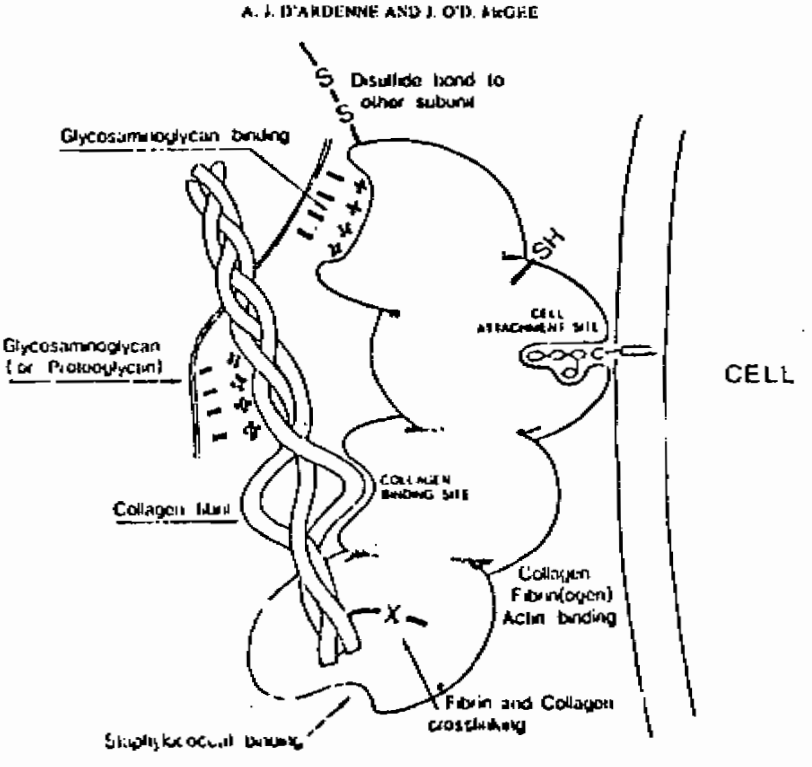

Fig. (1): Model of a fibronectin subunit. (Reproduced from Ruoslahti, Engvall and Haynian [6] with kind permission of the authors).

FIBRONECTIN AND MACROPHAGE FUNCTION

Macrophages are phagocytic cells situated in many organs and tissues. Collectively they comprise the mononuclear phagocytic system, a fundamental component of host defense mechanisms. The system has been known in the past as the reticuloendothelial system (RES). These cells serve an important function in clearance of fibrin-fibrinogen complexes **(Lee, et al., 1962)** and other foreign and particulate matter **(Saba, et al., 1970).**

In many instances, uptake by cells of this system is determined by the presence of opsonic signals on the surface of the targets of phagocytosis. The best studies opsonic agents are Ig G and C_3b of the complement system **(Bianco, et al., 1977).**

A protein in serum termed α_2-surface-binding glycoprotein (α_2-SB-glycoprotein), is also believed to interact with such targets prior to macrophage recognition and clearance **(Saba, et al., 1978).**

Saba and Coworkers have purified this protein and shown it to be immuno-chemically identical and structurally very similar to cold insoluble globulin.

Saba, et al., (1978) reported that patients developing sepsis following trauma, burn, or surgery manifest a significant reduction in level of α_2-SB glycoprotein as measured either by immuno-assay **(Laurell, et al., 1972)** or by an in vitro liver bio-assay technique **(Saba, et al., 1966).** Reduction in the level of this protein appears