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A STUDY OF COAGULATION DISTURBANCES IN INFECTIOUS DISEASE WITH PARTICULAR REFERENCE TO THE ROLE OF COAGULATION DISTURBANCES IN SALMONELLA TYPHI INFECTIONS

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A thesis submitted to the Faculty of Medicine, the University of the Witwatersrand, Johannesburg, for the Degree of Master of Medicine.

Johannesburg, 1986.

This thesis is my own work. No part of it has been presented at any other university. I obtained the information used in this thesis while training as a Registrar in the Department of Medicine, Johannesburg Hospital and the University of the Witwatersrand.

D.C. Spencer

ABSTRACT

Approximately 4,000 cases of typhoid fever are notified annually in South Africa. While localized gastrointestinal bleeding is common, a generalized haemorrhagic state remains unusual. Nevertheless recent reports have drawn attention to the high incidence of a sub-clinical, laboratory diagnosed, intravascular coagulopathy. This study evaluates the incidence of coagulation disturbances in Black patients with typhoid fever. In addition to the contribution of disseminated intravascular coagulation (D.I.C.), the role of disturbed liver function in impaired haemostasis is assessed.

The records of 32 patients seen consecutively over a two year period at the Hillbrow Hospital, Johannesburg, were evaluated. The final 13 were evaluated with full haematological and coagulation indices. All blood results reflect sampling during the first 24 hours after admission. The following blood tests were examined in this latter group; a full blood count including the platelet count and the peripheral blood smear, the prothrombin ratio, bleeding time and the activated partial thromboplastin time, the plasma fibrinogen and fibrin degradation products (FDP's), fibrin monomers, clotting factors VII, VIII and XII, levels of protein C, prekallikrein and plasminogen, and finally the concentrations of alpha-1 antitrypsin and C1-esterase inhibitor.

Two of the 32 patients had evidence of generalized bleeding at the time of admission. Both patients had a confirmed D.I.C. (6,2%). However, 5 of the 13 in whom coagulation tests were performed had a laboratory diagnosis of D.I.C. (38,5%). This includes the two patients with clinical D.I.C. In no patient was the D.I.C. life-threatening. The results must be evaluated with caution in view of the small sample size and the presence of referred patients in this study i.e. the patients in this study may have been generally more severely ill than those patients treated at more peripheral hospitals.

Elevated hepatic enzymes were noted in 87%. Significant reductions in protein C and albumin concentrations and an inverse correlation between values of albumin and lactate dehydrogenase (LDH) confirm the frequent disturbance of hepatic synthetic mechanisms. The activated partial thromboplastin time was generally prolonged in both patients with and without a D.I.C. Nevertheless specific hepatic-related factors VII and XII were not significantly reduced. In any patient with a coagulopathy therefore it appears likely that both D.I.C. and hepatitis may play a contributory role.

Thrombocytopenia occurred frequently but was never sufficiently severe to predispose to bleeding. Bleeding time and renal function were seldom abnormal. The lack of a positive correlation between the platelet count and absolute neutrophil count appears to exclude bone marrow failure as a

major contributor to the thrombocytopenia.

Although cleavage of factor XII has been postulated as the mechanism of prekallikrein activation (Colman et al, 1978), activation of this factor has not been confirmed in this study. Prekallikrein levels were generally normal in the group studied, however low values were recorded in two patients with D.I.C.

A significant reduction in the peripheral mononuclear cell counts, particularly the monocyte count, is reported in the study. This finding has not apparently been noted previously. This lends further support to the postulated role of "activated" tissue macrophages in the pathogenesis of typhoid.

The first two sections of this report review the physiology of haemostasis and the influence of a variety of infectious agents upon haemostatic control.

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PREFACE

THE ORGANIZATION OF THIS THESIS

This thesis is divided into three sections. The first section entitled "The Physiology of Haemostasis and Fibrinolysis", follows a brief introduction to the thesis as a whole. This introduction outlines the relevance of a study of coagulation and infectious disease to the Southern African community.

The second section deals with a variety of infectious diseases and associated alterations in the homeostasis of coagulation.

In the third section, particular attention is given to typhoid fever and the possible pathogenetic mechanisms influencing the control of coagulation. Discussion is related to local experience in Johannesburg, South Africa.

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GENERAL INTRODUCTION

Infectious disease still contributes significantly to worldwide morbidity and mortality. This is particularly true of the less developed regions. While disturbances of the coagulatory system are uncommon, such a complication frequently heralds a worsening of prognosis. This report will discuss the occurrence of coagulation disturbance and infection under the following headings:

The Physiology of Haemostasis and Fibrinolysis.

Infection and Disturbance of the Coagulation System.

Typhoid Fever and Coagulation Disturbances; Experience at the Hillbrow Hospital, Johannesburg, during the years 1983 and 1984.

Although typhoid fever is now a rarity in the developed world, it is still responsible for outbreaks of preventable disease in Africa and Asia. The number of typhoid notifications in South Africa is almost 4000 per annum with the provinces of Natal and the Transvaal notifying 80% of all cases (Somerville et al, 1981). More recently these numbers have increased possibly as a result of severe drought and socioeconomic and political instability. Not only is typhoid a disease capable of inflicting much individual suffering but it carries the potential of rapid transmission throughout a community. Hence it remains a disease of great importance to South Africa and to the wider community on the African continent.

SECTION 1

THE PHYSIOLOGY OF HAEMOSTASIS AND FIBRINOLYSIS

1. THE PHYSIOLOGY OF HAEMOSTASIS AND FIBRINOLYSIS

The integrity of normal haemostasis requires a complex integration of the blood vessel and the humoral and particulate components of the blood-fluid system. Following injury, the vascular smooth muscle contracts and the defect in the endothelium is rapidly plugged by circulating platelets. A more effective 'seal' is facilitated by the action of the serine protease, thrombin, which promotes the local formation of a stable fibrin clot. The enzyme plasmin, lyses clots; plasmin and the products of the degradation of fibrin viz. fibrin(ogen) degradation products which have anticoagulant activity, help to maintain the fluidity of the blood.

For purposes of discussion these components of haemostasis and fibrinolysis can be regarded individually, however the system behaves as an integrated whole. (Table 1.1).

Table 1.1 Participants in Haemostasis and Fibrinolysis.

1. The vasculature.
2. The particulate blood components.
    a. The platelet.
    b. The leucocyte
    c. The erythrocyte.
3. The humoral blood components.
    a. The blood clotting factors (intrinsic/extrinsic systems).
    b. Regulators of clotting factors.
    c. The fibrinolytic system.

1.1 THE VASCULATURE

Morphologically most vessels may be regarded as having three distinct layers. The inner intima with a single layer of endothelial cells which is in immediate contact with the circulating blood. The media containing smooth muscle cells that are able under appropriate stimuli to de-differentiate into endothelial cells and migrate to the intima.* And the supportive connective tissue, the adventitia, which surrounds the outer surface of the vessel. (Fig. 1.1)

The luminal surface of the endothelial cell consists of both a fixed and a movable layer. The former comprises sialic acid, glycolipids, oligosaccharide moieties of cell membrane glycoproteins, glycolipids and glycosaminoglycans such as heparin sulfate. Plasma proteins constitute the principal component of the movable layer. Vesicles within the cell engage in transportation and storage of materials. Workers have described the presence of 'Weibel-Palade bodies' in the endothelium of arteries: These tubular structures appear to be related to the Golgi system and are believed to play a role in fibrinolysis and blood pressure regulation (Mackie & Pittilo, 1985).

* BICK R.L., 1982. pg 303.

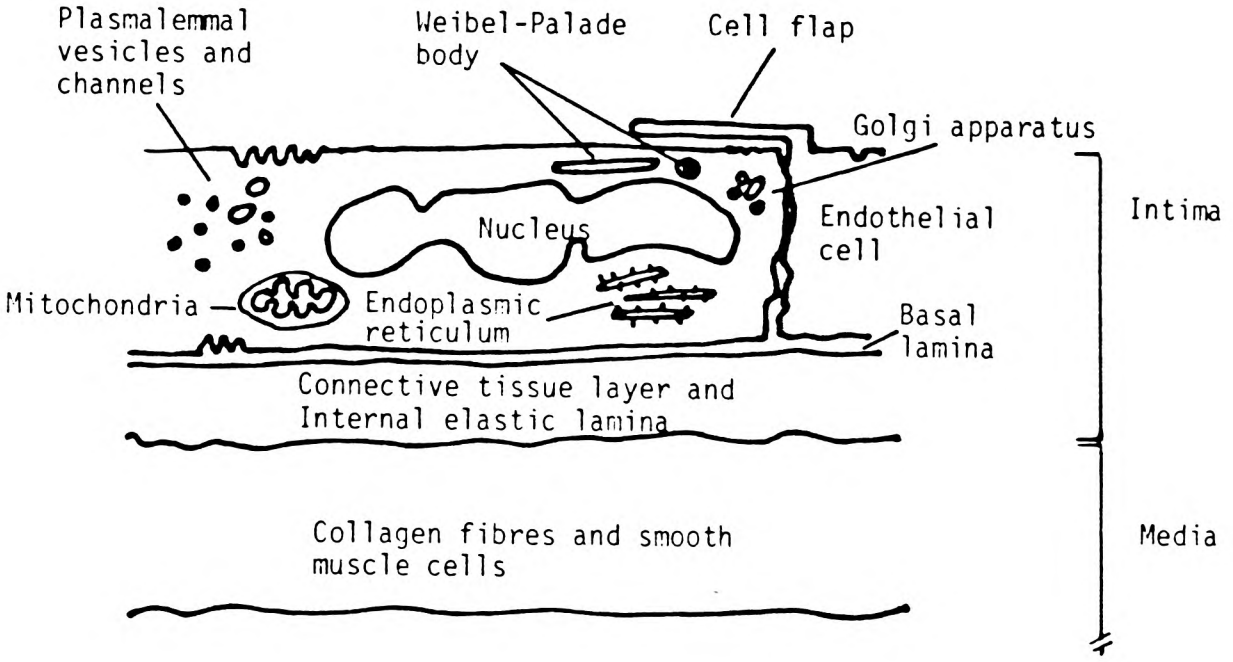


Figure 1.1 Endothelial cell morphology. This schematic diagram represents the intima and media as drawn from a transmission electron micrograph of rabbit thoracic aorta (Mackie & Pittilo, 1985, pp. 4).

The endothelial cell appears to influence coagulation through a variety of mechanisms:

1. Prostacyclin (PGI_2) production by endothelial and to a lesser extent, smooth muscle cells of the media, contributes to the inhibition of platelet adherance and to the dilatation of the vessel wall. Thrombin, bradykinin, histamine and angiotension 11 are promoters of PGl_2 production. Released PGl_2 binds to specific membrane receptors on platelets and stimulates the enzyme adenylate cyclase with resultant increase of cyclic adenosine monophosphate (cAMP) within the platelet. Consequently calcium ions are sequestered within the platelet leading to an inactivation of phospholipase A_2; a net result of this is the fall in production of the promoter of platelet aggregation and release, namely, thromboxane A_2 (TXA_2). Inactivation of phospholipase A_2 also results in decreased PGl_2 synthesis thereby acting as a regulator of PGl_2 activity.
2. The net negative charge on the surface of the endothelium accounts for an electrostatic repulsion of platelets: Such forces may be altered by hypoxia or direct infection of these cells and contribute towards an increased tendency to platelet deposition.

3. Antithrombin lll present within the cytoplasm of endothelial cells is probably the body's most significant inhibitor of thrombin.
4. Thrombomodulin, a product of the endothelial cell is required in the thrombin-initiated activation of protein C and thereby shares a role in the degradation of activated factors V and Vlllc by that protease.
5. Release of plasminogen activator from the endothelial cell facilitates the conversion of circulating plasminogen to plasmin and the promotion of local fibrinolysis. The local infusion of catecholamines and stimuli such as exercise, stress and venous occlusion increase blood concentrations of plasminogen activator. Thrombin appears to be inhibitory in the synthesis and release of this protein.
6. Von Willebrand factor VIII (VWF), which is believed to mediate platelet adhesion to subendothelium in regions of high shear rates of rapid blood flow. VWF also acts as a carrier protein for factor VIII coagulant protein (factor VIIIc).
7. Tissue factor. This protein is elaborated and released upon injury to the endothelial cell and occupies a central role in the extrinsic pathway of the coagulation cascade.

8. Collagen, the major structural component of the extracellular matrix, is synthesized by a number of cells, including the vascular endothelial cell. Together with other products of the endothelial cell namely VWF, fibronectin and thrombospondin (TSP), collagen promotes the adherance of platelets to the vessel wall.

1.2 THE PARTICULATE BLOOD COMPONENTS

1.2.1 The Platelets

These anucleate cell fragments serve a fundamental role in the maintenance of haemostasis. Quantitative deficiencies and qualitative defects result in serious bleeding tendencies. Four zones appear to form the morphological and functional regions of importance within the platelet (Fig. 1.2).

a) The Peripheral Zone. Composed of the limiting membrane of the platelet and the openings of the surface connecting system (SCS), together with the submembrane filaments., the peripheral zone is concerned chiefly with adhesion. Circulating thrombin and adenosine diphosphate (ADP) have binding sites on the surface membrane.

b) The Sol-gel Zone. This zone comprises a matrix of microtubules and microfilaments encompassing both the perimeter and the mid-region of the platelet. The polymerized tubulin of these tubules govern the

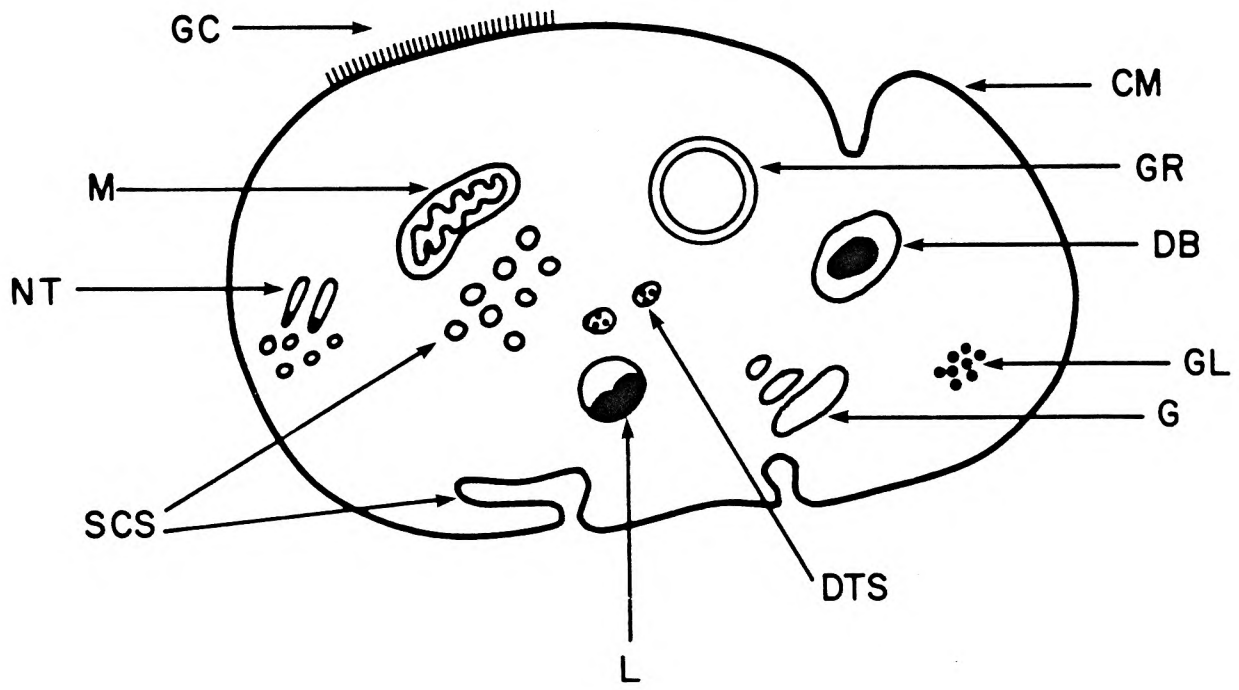


Figure 1.2 Platelet morphology. Schematic diagram taken from a transmission electron micrograph of a rabbit platelet (Mackie & Pittilo, 1985, pp. 11). Key: CM = cell membrane; DB = dense body; DTS = dense tubular system; G = Golgi apparatus; GC = glycocalyx; GL = glycogen; GR = alpha granule; L = lysosome; M = mitochondria; M = microtubule; SCS = surface connecting system.

maintenance of the discoid shape of the platelet and the contractions occurring during the 'release reaction'. Changes in shape, and contraction of platelet pseudopods is associated with the activity of actin and myosin in the filamentous section.

c) The Organelle Zone consists of a large variety of intracellular "bodies" including ribosomes, Golgi bodies, mitochondria and granules. The latter include lysosomes containing several enzymes, alpha granules capable of releasing stored substances such as beta-thromboglobulin, fibrinogen, platelet-factor 4 and platelet mitogenic factor., and dense bodies containing calcium, histamine, serotonin, pyrophosphate, ADP and ATP. These substances suggest the large variety of metabolic effects mediated via platelet activation.

d) The Membrane System. This region incorporates the surface connecting system (SCS) and an interdigitating dense tubular system (DTS). Internally this system is in contact with the organelle zone, and provides convenient access to the exterior for the products of these organelles. The presence of calcium/magnesium ATPase in the system suggests storage of calcium required in platelet contraction. A rich supply of cyclo-oxygenase and lipoxygenase enzymes implies a relationship between this system and platelet prostaglandin synthesis.

The 'contact interaction' between the platelet and the vessel wall occurs in several defined phases:

1 Platelet adhesion.

2. Granule release reaction.

3. Platelet aggregation.

4. Platelet fusion and clot retraction.

Stimuli to platelet adhesion include ADP, thrombin, TXA_2 and endotoxin (Hawiger et al, 1975). Platelet shape alters to that of a 'spiky sphere' and a so-called 'flip-flop' rearrangement of inner and outer membrane phospholipids occurs, facilitating binding of the platelet to factors V and Xa. The binding of clotting factors to the platelet surface results in the rapid generation of thrombin (Walsh, 1985; Schmaier, 1985). The complex biochemical events governing platelet function are reviewed by Mackie and Pittilo (1985) and will not be detailed in this report. Nonetheless it is important to recall the modulating role of cAMP which functions to generate intraplatelet kinase, an enzyme responsible for diminishing cytoplasmic levels of calcium ions. The more cAMP present in the platelet, the less the free calcium ion concentration, and hence the less the interaction between actin and myosin (Bick, 1982). Thrombin and collagen, and to a lesser extent ADP, are able to mobilise calcium ions and thereby promote the phenomenon of granule release. These same activators are capable of inducing platelet aggregation. This reaction allows the formation of a surface lipoprotein, "platelet factor 3",

upon which the coagulant enzymes and their substrates may interact. Platelets fix to the fibrin strands formed from the action of thrombin on fibrinogen. Platelet retraction contracts the volume of the clot, extruding entrapped fluid and thereby enhancing the stability and localization of the thrombus. Deposition and adherance of platelets to the vessel wall requires a complex interaction in which products of both are involved. Houdijk and Sixma (1985) have suggested a role for tissue-fibronectin together with VWF in this event.

1.2.2 The Leucocyte

The presence of white blood cells within thrombi has been recognized for more than a century, however the participation and function of these cells is still far from clear (Harlan, 1985).

Approximately one tenth of the total blood neutrophil pool appears to migrate from the circulation each hour by adhering to and then diapedesing through the vascular endothelium to enter extravascular sites. Both exercise and epinephrine mobilize the marginating leucocyte pool; release of endothelial cAMP may be a factor in the latter event (Boxer et al, 1980). Experimental infusion of ADP, serotonin and collagen extract, promote the adherance of white blood cells to one another and to the vascular endothelium (Hoover et al, 1978). Infective and inflammatory states also promote such adhesive interactions possibly as a result of a reduction in net electronegative

surface charges. Neutrophil degranulating stimuli and chemotactic forces have also been suggested (Needleman & Hoak, 1982).

Substances produced and released from leucocytes may alter the host environment: Highlycharged macromolecules, namely arginine-rich, lysosomal cationic proteins (LCP) and acidic muco-substance (AMS) resembling chondroitin sulfate, promote the paracoagulation of fibrin monomers. These macromolecules are found within the granular phase of the cell. Increased serum levels follow the experimental infusion of endotoxin (Fedorko & Morse, 1965).

Endotoxin has also been shown to enhance the activity of tissue factor released from neutrophils and monocytes. In the in vitro system, the addition of antibodies raised to placental-derived tissue factor to the supernatant of incubating leucocytes blocks the procoagulant effects of the latter. Immunoglobulin and soluble antigen-antibody complexes appear to be potent stimuli to the in vitro production of tissue factor by leucocytes (Zimmerman et al, 1977). The presence of a coagulopathy and clinical deterioration in the Dengue Shock Syndrome parallels the formation of antigen-antibody complexes and the increase in antibody titres (Halstead, 1982).

Some years ago Plow and Edington (1975) described leucocyte-related proteases with fibrinolytic activity. Ohlsson in 1971 reported elevated serum levels of neutrophil proteases in some patients with coagulopathies, particularly

in association with leukaemia. More recently, Bevilacqua and co-workers (1984), have described the monokine, interleukin-1, as inducing the synthesis and cell-surface expression of procoagulant activity in cultured endothelial cells. Direct endothelial damage has also been postulated as an occasional consequence of the subendothelial accumulation of macrophages; deleterious effects being mediated by a variety of monocyte-derived substances (Harlan, 1985).

1.2.3 The Erythrocyte

Present understanding of the role of the erythron does not allow it a significant status in the control of coagulation during health. Alterations in the dynamics of circulation such as occurs during dehydration or with certain hyperviscosity syndromes may theoretically promote haemostasis (Schafer, 1985). Disseminated intravascular coagulation (DIC) is a well described complication of incompatible blood transfusion: massive intravascular haemolysis may be accompanied by the release of quantities of ADP and tissue thromboplastin into the circulation. The thrombosis described in small vessels in fulminant malarial syndromes has in part been attributed to the release of red cell ADP (Wilson et al, 1982).

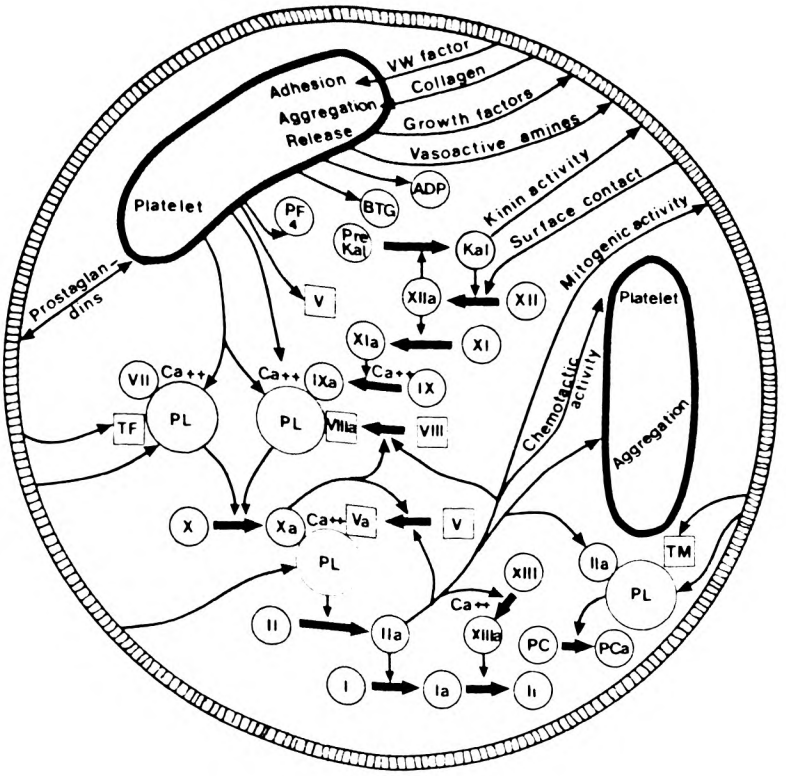


Figure 1.3 Thrombin generation. This complicated figure displays some of the major reactions by which the coagulation factors, vessel wall, and the blood platelets are intertwined in the expression of the coagulation response. The outer ring represents the blood vessel wall. The blood coagulation zymogens and enzymes are represented by roman numerals in circles. The blood clotting cofactors are identified by roman numerals in boxes. Circles identified as "PL" represent reactions that require a membrane (phospholipid) constituent. The membrane constituent may still be a portion of the cell, or in some cases may be released into circulation. The abbreviation "Pre Kal" represents prekallikrein, "TF" represents tissue factor, "TM" represents thrombomodulin, "PF 4" represents platelet factor 4, while βTG represents beta-thromboglobulin. Reprinted from Mann & Downing, 1984, pp.208.

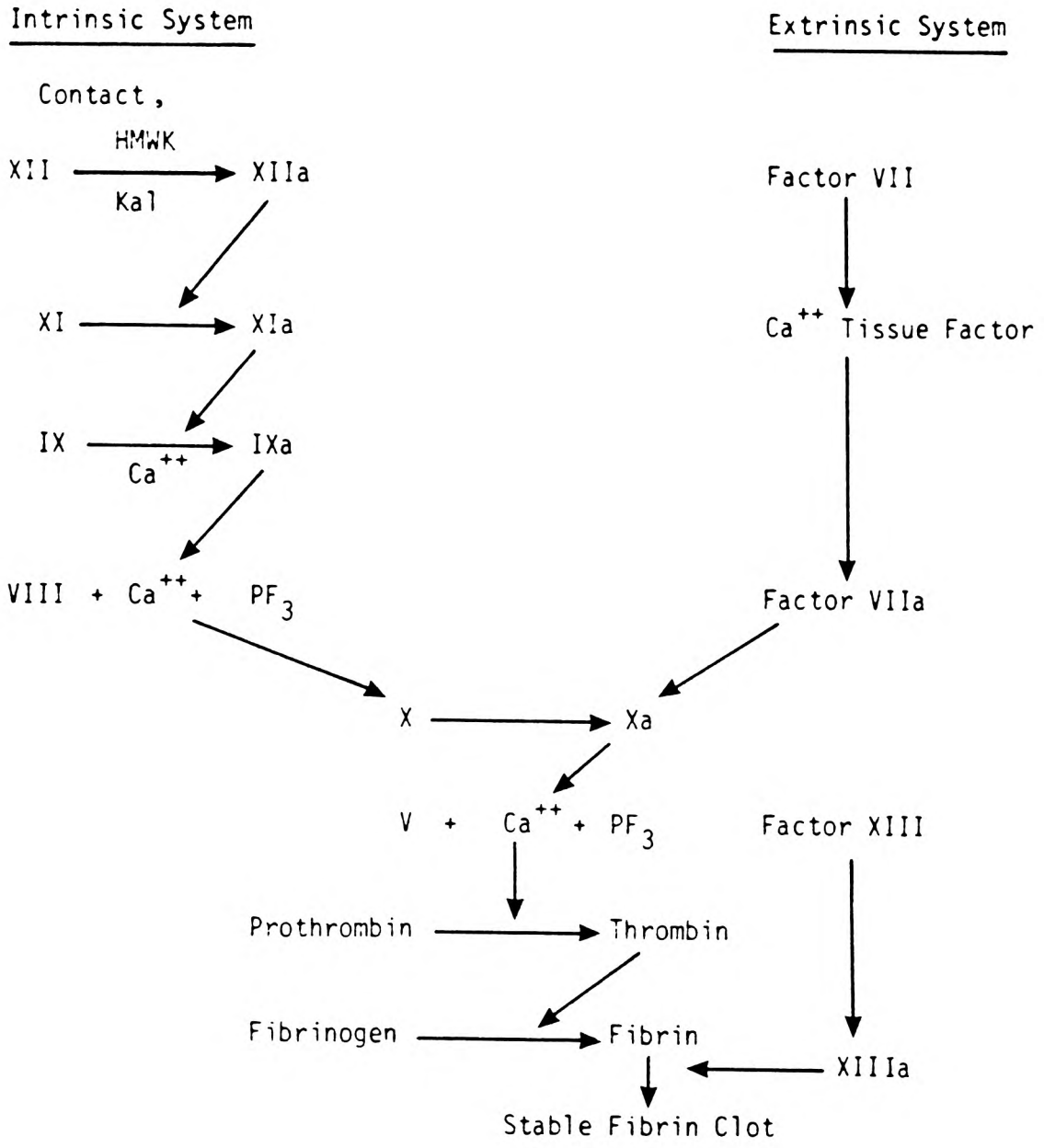


Figure 1.4 Compartmentalization of the blood coagulation system. The intrinsic system is evaluated with the activated partial thromboplastin time (APTT), and the extrinsic system with the prothrombin time (PT). Key: HMWK = high molecular weight kininogen; Kal = kallikrein; Ca^{++} = Calcium; PF_3 = platelet factor 3.

1.3 THE HUMORAL BLOOD COMPONENTS

1.3.1 The Blood Clotting Factors

The classic papers of Davie & Ratnoff (1964) and MacFarlane (1964) defined the intrinsic and extrinsic coagulation systems. Compartmentalization of the clotting mechanism is however now viewed as largely artificial. A more integrated system is represented in Figure 1.3 (pp.15). The classical pathway is represented in Figure 1.4 (pp. 16).

1.3.1.1 Activation of the coagulation sequence

1.3.1.1.1 The intrinsic system: Activation of factor XII (Hageman factor) appears to be the initiating step in this pathway. This may follow the exposure of blood to a negatively charged surface, either of a non-physiological nature such as talc or silicon dioxide or biological, such as skin, collagen and fatty acids. Sepsis has been linked to activation of the pathway on a number of occasions. The *in vivo* activation of factor XII is principally via kallikrein, and to a lesser extent, plasmin. Following exposure to a suitable stimulator, factor XII, prekallikrein and high molecular weight kininogen (HMWK) interact together to form a complex enzyme system in which activated factor XIIa is formed, prekallikren is activated to kallikrein and HMWK is cleaved with the release of the kinins (Mandle & Kaplan, 1977).

This initial activation of factor XII involves a conformational change in the molecule with exposure of its active enzymatic site. Prekallikrein and HMWK are able to

attach to the exposed surface in a one-to-one molar complex. Both prekallikrein and factor Xla circulate as a complex with HMWK. The role of the latter is to place factor Xll in a favourable position for interaction with factor Xl. Cleavage of factor Xlla gives rise to fragments of successively smaller molecular size possessing increased prekallikrein-activating ability and net negative charge. These fragments may initiate fibrinolysis via activation of plasminogen activator and generate changes associated with kinin release and enhanced vascular permeability.

Factor Xl is activated by adjacent molecules of factor Xlla. In a calcium-dependent reaction, Xla splits factor lX and induces enzymic activity by cleavage of a peptide from the heavy chain. Factor IXa forms a complex with factor VIII, calcium ions and phospholipid. The latter being derived largely from platelet membranes. Thrombin greatly enhances factor VIII activity. The factor IXa complex converts factor X to active factor Xa.

Factor Xa forms an enzyme complex called "Prothrombinase". This consists of factor Xa, factor Va activated by thrombin, calcium ions and a phospholipid provided by platelets namely platelet factor 3. Factor V appears to act as a surface binding agent. Phospholipid-bound prothrombin (factor II) is converted to the active enzyme thrombin, through the action of the factor Xa complex. Trace quantities of thrombin exert a facilitatory effect upon factor IXa and Xa complexes via a positive feedback

enhancement of factors Va and VIIIa.

The co-factors, Va and VIIIa, are believed to form receptors for their respective enzyme complexes. The complex as a whole may function to localise and amplify the activity of its enzyme. Factor Xa has been demonstrated to be less sensitive to inhibition by antithrombin III (ATIII) once it is bound with factor Va on a membrane surface (Brandt, 1984).

Protein C, synthesized by the liver, is responsible for the inactivation of factors Va and VIIIa, and the release of a plasminogen activator from the endothelial cell. Thrombin together with calcium ions, thrombomodulin and a phospholipid surface, form an enzyme complex, "protein C-ase" responsible for the conversion of protein C to its activated form (Esmon, 1983).

1.3.1.1.2 The extrinsic system: The enzyme complex comprising tissue factor, factor VIIa, calcium ions and a phospholipid surface is capable of activating factor X directly and indirectly, through an effect upon factor IX. Tissue factor is found in many sites including brain, lung and placenta, and is present in the granules of leucocytes and platelets. Injury to vascular endothelium, smooth muscle, fibroblasts, monocytes and macrophages results in the expression of tissue factor by these cells. Tissue factor is a mixture of phospholipid and apoprotein. The protein moiety participates in its interaction with factor VII. Factor VII exists in several forms; the single-chain

parent compound exhibits spontaneous enzymic activity. However, the doublechain alpha form is many times more active. Hepatic synthesis of functional factor VII is Vitamin K dependent. Acidic mucopolysaccharides released by injured cells appear to stimulate the activation of factor IX by the factor VII-tissue factor complex. While no physiologic inhibitor of alpha VIIa has as yet been reported, at low concentrations both the parent and double-chain form bind to tissue factor with equal avidity. Indeed the parent form may behave as a competitive inhibitor of its double chain product (Triplett, 1984).

Activation of the extrinsic clotting mechanism may occur during a variety of sepsis-related conditions. Extremely low serum levels of factor VII convey a grave prognosis in viral hepatitis. However, it has proven difficult to correlate this with either extrinsic pathway activity or primary liver failure (Mannucci & Forman, 1982).

1.3.2 The Regulators of the Clotting Factors

Maintenance of the fluidity of blood requires the inhibition of coagulation and a means of dissolution of thrombus already formed. Protease-inhibitors comprise approximately 20% of the globulin fraction of plasma, thus suggesting a major physiological role (Harpel, 1983) (Table 1.2).

Table 1.2 Naturally occurring protease-inhibitors.

Antithrombin III (ATIII)

Alpha-2 macroglobulin

Alpha-1 antitrypsin

C1-esterase inhibitor

Alpha-2 plasmin inhibitor

Alpha-1 antichromotrypsin

Interalpha-trypsin inhibitor

Heparin cofactor

Protein C inhibitor

Antithrombin III: This glycoprotein accounts for 75% of plasma antithrombotic activity. The formation by ATIII of a tight, covalent bond at its 385-386 position with thrombin, blocks the active enzymic site of the latter thereby reducing its proteolytic activity. ATIII binds similarly to factors IXa, Xa and XIa. It is also capable of binding to Hageman factor and kallikrein. Thrombin inactivation is surface mediated. The surface appears to be the endothelium. Binding of ATIII to the lysine residues of thrombin requires the presence of glycosaminoglycans, such as heparin sulfate, on the endothelial surface. Heparin significantly accelerates the complexing of AT III with

thrombin. The availability and expression of natural heparin activity therefore acts as a control to AT III function in vivo. Unlike the fluctuations in concentrations of various other protease inactivators such as alpha-1 antitrypsin in response to inflammatory stress, levels of AT III remain within a tight physiological range and control. Familial and acquired deficiency of ATIII is associated with recurrent thromboembolic disease. Antithrombin III levels are low in D.I.C. (Sharp, 1983). It is not altogether certain how much of this reflects consumption or decreased hepatic synthesis.

C1 - Esterase inhibitor and Alpha 2 macroglobulin: The former is a major inhibitor of the activated fragments of Factor XII and of kallikrein. Alpha-2 macroglobulin has similar, though less complete effects and in addition, is responsible for the slow-reacting antiplasmin component of plasma.

Protein C and Protein S: Since its isolation by Seegers and co-workers in 1960, the protease enzyme, Protein C, has been shown to inhibit thrombin formation through interference with prothrombin activation (Seegers, 1981). Thrombomodulin, an endothelial cell surface-bound protein, is believed to behave as a specific receptor, promoting the local binding of thrombin to the endothelial cell and thereby facilitating activation of Protein C (PCa). Although both factor Va and VIIIa are substrates for PCa activity, the consequent formation of the light-chain form

of Va is itself a block to further activation of Protein C. Protein S appears to enhance the binding of PCa to phospholipid and to accelerate factor Va inactivation. Recurrent thromboembolic disease has been linked to a familial deficiency of both these proteins (Griffin et al, 1981; Seligsohn et al, 1984; Comp & Esmon, 1984 a & b).

The in vitro admixing of Protein C and the IgG fraction from patients with the lupus anticoagulant prevents activation of Protein C: This may identify a molecular basis for the thrombotic sequelae associated with Systemic Lupus Erythematosis. Acquired deficiency of Protein C has been described in liver disease and with long-term oral anticoagulant usage. Griffin, Mosher and co-workers (1982) found decreased Protein C levels in patients hospitalised with DIC. Consumption of the protein was believed to be implicated in those patients without significant hepatic synthetic derangement.

1.3.3 The Fibrinolytic System

Activation of the intrinsic and extrinsic pathways of clotting, leads via a common pathway incorporating activated factor X, to the production of thrombin. Consequently fibrinogen is cleaved to form fibrin. Fibrinogen, is soluble in plasma: Although the core of the protein is non-polar, its outer side-chains are polar. The negatively charged peptides, fibrinopeptides A and B are released by thrombin action upon the parent protein. These peptides have been implicated in the pathogenesis of systemic and

pulmonary vasoconstriction. With the loss of these peptides, residual fibrin monomers are able to approach one another and form weak linkages. In the presence of calcium ions, factor XIII, variously called fibrinoligase and plasma transglutaminase, facilitates the covalent bonding between complementary lysine and glutamine receptors of adjacent monomers. The resulting fibrin polymer is insoluble (Doolittle, 1981). Thrombin activates factor XIII by causing dissociation of the two subunit pairs of the latter, thereby exposing the active site of the 'a' portion of the protein (Roath & Francis, 1985). In vivo, two fibrin strands or "mini-clot" may form: Fibrin I which retains the fibrinopeptide B (FPB) moeity and Fibrin II, lacking any fibrinopeptide. These fragments together with FPB are early markers of D.I.C. (Gaffney, 1983). The key enzyme involved in the dissolution of fibrin clot is plasmin. Eosinophils have been suggested as a source of plasminogen, the inactive precursor of plasmin. Naturally occurring plasminogen activators include Hageman factor and its fragments, kallikrein and factor XIa, and tissue plasminogen activator, a substance produced within the media of vessels and present in vascular endothelium, and released following damage to the vessel wall. Plasmin has equal affinity for fibrinogen and fibrin, and can hydrolyse factors XIIa, V, VII, IX and XI. It is able to activate the complement system and may lead to the generation of kinins.

The major plasmin inhibitor is the glycoprotein, alpha-

2 antiplasmin. Other naturally occurring inhibitors include alpha-2 macroglobulin, ATIII, alpha-1 antitrypsin and C1-inhibitor. Liver disease resulting in decreased synthesis of these inhibitors may play a role in fibrinolytic syndromes (Bick, 1982).

Digestion of fibrin/fibrinogen leads to the formation of smaller "fragments": An intermediate in the proteolysis of fibrinogen is the formation of fragment X. Further digestion gives rise to fragments Y and D. The former may also be digested further to form another D-fragment together with a small protein, fragment E (Fig. 1.5).

These products of plasmin activity are termed fibrinogen/fibrin degradation products (FDP's). Fragments X and Y exert an anti-thrombin effect and prolong the thrombin time *in vitro*. Through the formation of soluble complexes with fibrin, these fragments can impair normal fibrin polymerization. The thrombus is weakened by the interpositioning of fragment complexes between normal polymers of fibrin.

In addition, all four fragments (X, Y, D and E) are able to coat the membranes of platelets thereby impairing ADP-stimulated platelet aggregation. Such platelets become dysfunctional. The net effect of these FDP's is the promotion of anti-coagulation.

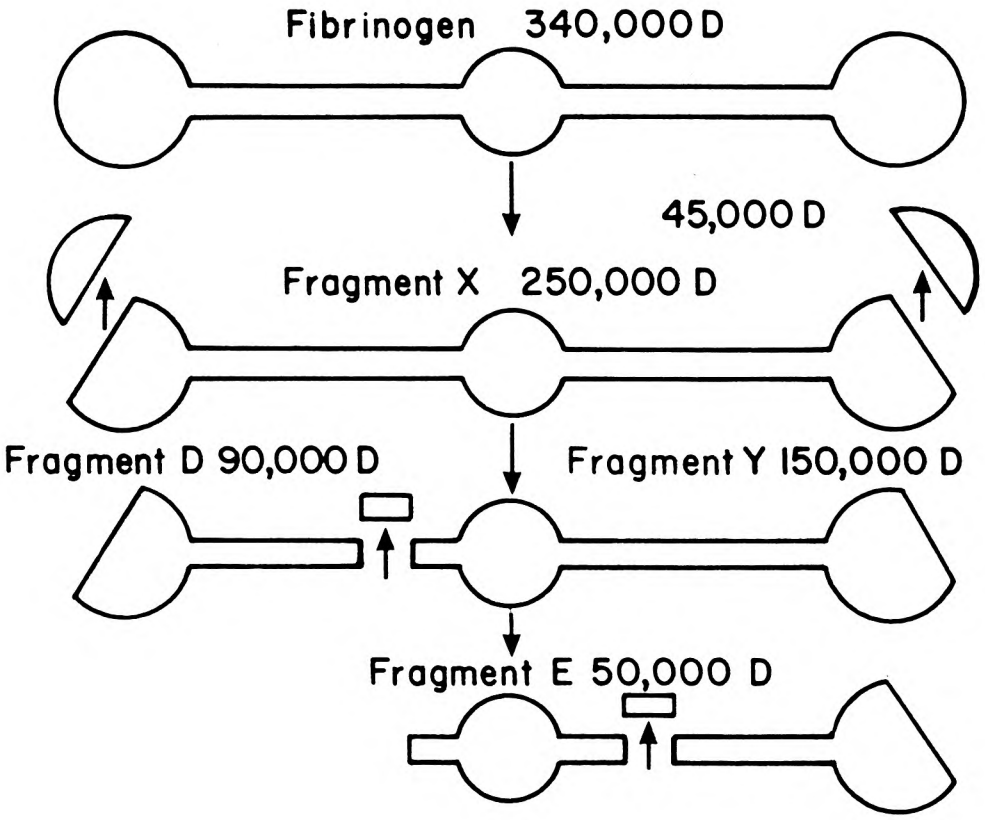


Figure 1.5 Digestion of fibrinogen. The trinodular model of the fibrinogen molecule is based upon electron micrographic appearances. Plasmin digestion of the molecule gives rise successively to the intermediate products X and Y and to the core fragments D and E. Molecular mass in daltons (D). Figure taken from Doolittle, 1981, pp. 95.

SECTION 2

INFECTION AND DISTURBANCE OF THE COAGULATION SYSTEM

2.1 DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

2.1.1 Introduction

Colman has defined this disorder as "a pathological syndrome resulting from the formation of thrombin, with subsequent activation and consumption of certain coagulant proteins and the production of fibrin thrombi" (Marder et al, 1982). The clinical events usually manifest as haemorrhagic phenomena, and follow the occlusion of the microcirculation with small thrombi of fibrin and platelets. Clotting factor and platelet consumption, plasmin activation, clot lysis and FDP formation continue the pathological process. Acute, chronic, localized and disseminated forms of the syndrome are described. The occlusion of small vessels viz. arterioles, venules and capillaries, with fibrin thrombi is the pathological hallmark of D.I.C.

Clinical features of the syndrome reflect the pathological process. Renal vessels are commonly involved and renal failure may result. Skin and mucous membrane petechiae reflect platelet consumption and dysfunction. Occlusion of the microcirculation may lead to skin and tissue infarction. Focal neurological defects are rare. An Adult Respiratory Distress Syndrome (ARDS) and interstitial alveolar haemorrhage may result in significant pulmonary compromise. Gastrointestinal haemorrhage and adrenal failure following haemorrhagic necrosis of the adrenal cortex have been described. While the conditions associated

with D.I.C. are legion (Table 2.1), it would appear that bacterial septicaemia is the most frequent (Yoshikawa et al, 1971). Wilson et al (1982) reviewed six major studies of D.I.C. involving more than 800 patients and found that infections could be implicated in up to 64% of cases.

A clinical suspicion of D.I.C. requires supportive laboratory data. Nevertheless, the diagnosis rests upon an overall assessment of all the available evidence. Individual laboratory tests have limited interpretative value if judged independently. Usually diagnostic, is the demonstration of a thrombocytopenia together with elevated levels of FDP's and the presence of fibrin monomers with reduced serum fibrinogen and ATIII levels. Prolongation of the activated partial thromboplastin time, the prothrombin and thrombin time is usual.

Tests of platelet function may be abnormal and examination of the peripheral smear may reveal deformation and fragmentation of erythrocytes. Absolute levels of fibrinogen tend to be low, however levels may be elevated in the presence of infection and neoplasia. Rapid fibrinogen turnover suggests thrombin mediated digestion: Unfortunately this test is not universally available. Paracoagulation tests with ethanol and protamine measure fibrin monomers; while simple to perform, these tests are relatively insensitive and difficult to accurately quantify. Better methods are generally too complex for routine use.

Table 2.1 Pathological States Associated With DIC *

1. Shock states
   a. Haemorrhagic
   b. Septic
   c. Traumatic
   d. Burns
   e. Anaphylactic
2. Obstetrical states
a. Abruptio placenta
   b. Amniotic fluid embolism
   c. Intrauterine fetal death with delayed delivery
   d. Severe eclampsia
   e. Septic abortion
   f. Hydatidiform mole
3. Neoplasms
   a. Prostate
   b. Pancreas
   c. Lung
   d. Stomach
   e. Ovary
   f. Gallbladder
   g. Colon
   h. Rhabdomyosarcoma
4. Infections
   a. Gram-positive bacteria
   b. Gram-negative bacteria
   c. Viruses
   d. Rickettsiae
   e. Purpura fulminans
   f. Miscellaneous
      1) Malaria
      2) Tuberculosis
      3) Kala-azar
      4) Aspergillosis
5. Haematological disorders
   a. Leukaemia
      1) Acute promyelocytic
      2) Acute myelogenous
      3) Acute lymphoblastic
   b. Incompatible blood transfusion
   c. Paroxysmal nocturnal haemoglobinuria
   d. Sickle cell crisis
   e. Acquired haemolytic anaemia
   f. Chronic idiopathic thrombocytopenic purpura
   g. Thrombotic thrombocytopenic purpura
   h. Haemolytic-uraemic syndrome
6. Cardiopulmonary disorders
   a. Pulmonary embolism
   b. Cyanotic congenital heart disease
   c. Hyaline membrane disease
7. Renal disorders
   a. Transplant rejection
   b. Glomerulonephritis
8. Trauma and surgery
   a. Crush syndrome
   b. Thoracic surgery
   c. Extracorporeal circulation
9. Miscellaneous
   a. Malayan pit viper
   b. Fat embolism
   c. Kasabach-Merritt syndrome
   e. Heat stroke
   f. Allergic drug reactions
   g. Acute haemorrhagic pancreatitis
   h. Cold exposure

* Yoshikawa, Tanaka & Guze, 1971. pg 238.

Measurement of FDP's and assays of specific clotting factors are frequently helpful in evaluating the clinical course and therefore often require to be repeated. Finally, the diagnosis of D.I.C. may be confirmed on pathological examination, for example with skin biopsy or occasionally, at post-mortem (Colman et al, 1979; Hamilton et al, 1978).

2.1.2 Infection and the Activation of Coagulation

Activation of the intrinsic pathway has been linked to sepsis on a number of occasions (Robboy et al, 1973; Hirsch et al, 1974; Mason et al, 1970). Colman (1982) summarized the sequence of events occurring with gram-negative sepsis as:-

1. Activation of Hageman factor with a subsequent fall of plasma levels.
2. The conversion of factor XIIa and fragments into activators of prekallikrein.
3. Prekallikrein activation is followed by a fall in plasma levels.
4. Depletion of plasma kininogen leads to the release of free kinins into the circulation.

The role of endotoxin-mediated activation of coagulation will be discussed in some detail below. It will be recalled that endotoxin is capable of activating both the intrinsic and extrinsic clotting mechanisms. Indeed Nossel (1982) cautioned that an endotoxin-related fall in the serum level of factor XII in D.I.C. may be a result rather than a cause of thrombin generation. Muller-Berghaus and co-

workers (1971) demonstrated that experimentally-induced inhibition of factor XII activation in rabbits injected with endotoxin did not prevent subsequent coagulatory disturbances. Nonetheless, profoundly low levels of factor XII were noted in animals demonstrating very severe D.I.C. These workers further demonstrated that prior use of Coumarin in this subset prevented the fall in factor XII - suggesting that the decreased levels were consequent upon thrombin formation.

Many years earlier, Thomas and Good (1952) had observed leucopenia to provide protection against fibrin-deposition and accelerated fibrinogen clearance in rabbits. The reinfusion of leucocytes in this model re-established susceptibility. Some years later it was noted that leucopenia at the time of endotoxin infusion seemed also to prevent the fall in factors V, VII and VIII., in addition to blocking fibrin formation (Horn & Collins, 1968).

Extrinsic pathway participation in coagulatory disturbances, mediated possibly via the release of leucocyte and/or monocyte-derived tissue factor, has been demonstrated in a number of studies (Edwards et al, 1979; Rivers et al, 1975; Garner & Evansen, 1974). Tissue factor induced D.I.C. is an established complication of amniotic-fluid embolism, incompatible blood transfusions and several malignancies.

Direct activation of the common pathway of coagulation probably occurs rarely. However, the staphylococcal coagulase enzyme is capable of direct activation of

prothrombin. Also the snake venoms of Echis carinatus, the carpet viper, and that of the Russell viper, activate prothrombin and factor X respectively.

2.1.3 The Role of Endotoxin

Experimental confirmation of the role of endotoxin in promoting intravascular thrombosis has been well established. In the local Shwartzman reaction endotoxin is injected into the skin of a rabbit. Intravenous endotoxin is administered 18 to 24 hrs later. Histological examination of the initial site confirms an inflammatory reaction and thrombosis of capillaries and vessels. A generalised Shwartzman reaction (gSr) follows two intravenous injections of endotoxin, 18 to 24 hours apart. Fibrin thrombi are present in many vessels but particularly the glomerular capillaries and splenic sinusoids. Appearances on pathological examination are indistinguishable from D.I.C. (Hjort & Rapaport, 1965). As early as 1935, Apitz was able to elicit a gSr in animals utilizing filtrates of meningococci and typhoid bacilli. Both platelets and leucocytes appear to be required in order for these reactions to occur (Hjort et al, 1964). Pretreatment of these animals with heparin prevents the gSr in the majority - establishing a role for thrombin in the pathogenesis of the D.I.C.

Endotoxin has been demonstrated to cause,

a) Direct damage to cell membranes (McGrath & Stewart, 1969).

b) Release of proteolytic enzymes and tissue factor-like material from incubated leucocytes (Zimmerman et al, 1977; Lerner et al, 1977).

c) Activation of the complement cascade (Levin et al, 1972). In the experimental model, prior depletion of C3 with cobra venom does not alter the occurrence of D.I.C. This suggests that complement utilization may be a feature of endotoxin-induced coagulopathy, but is not necessarily a triggering mechanism. Reduced levels of total haemolytic C3 are described in DIC.

d) Tissue injury: Endotoxin exerts its vascular effects principally upon small vessels. The resultant intense vasospasm promotes stagnation of blood leading to tissue hypoxia. Activation of factor XIIa and the formation of bradykinin lead to peripheral pooling of blood and alterations of capillary permeability. Cellular hypoxia results in uncoupling of essential oxidative phosphorylation and the development of lactic acidosis (Petersdorf & Dale, 1980).

Endotoxaemia is a frequent concomitant of gram-negative bacteraemia and of the clinical syndrome of 'septic shock'.

In a recent review of patients with gram-negative bacteraemia, 11% were noted to fulfill laboratory criteria of a D.I.C. (Kreger et al, 1980). The elaboration of tissue factor with procoagulant activity and direct invasion and injury of vessel walls by gram-negative organisms suggest the divers aetiological factors implicated in the coagulatory disturbance (Fumarola & Miragliotta, 1983; Cohen et al, 1978; Preston et al, 1973).

2.2 SPECIFIC INFECTIVE AGENTS AND CLINICAL SYNDROMES RELATING TO COAGULATION DISTURBANCES (Table 2.2)

2.2.1 Gram-Negative Bacterial Infections.

A great deal has already been mentioned regarding the role of endotoxin in these infections. Specific attention will only be given to meningococcal disease. More detail relating to Salmonella infections and specifically, typhoid fever will be discussed in the final section of this report.

2.2.1.1 Meningococcal septicaemia

D.I.C. plays a central role in the syndrome of fulminant purpura and bilateral adrenal haemorrhage described by Voelcker (1894), Waterhouse (1911) and Friderichsen (1918) - reported in Yoshikawa et al, 1971. Meningococci have been demonstrated within damaged endothelial cells and within the leucocytes participating in thrombus formation (Evans et al, 1969; Sotto et al, 1976). Both endotoxin and tissue factor have been incriminated; the bulk of available evidence favours the former as a primary

Table 2.2 Haemorrhagic Fevers

- Virus Infections
  - Enterovirus: Coxsackie B hepatitis, enterovirus 72 = hepatitis A.
  - Arthropod-borne virus (arbovirus) infections
    - Alphavirus
      - Chikungunya
      - Sindbis
    - Flavivirus
      - Mosquito-borne - yellow fever, dengue, West Nile
      - Tick-borne - Congo-Crimean fever
    - Bunyavirus
      - Rift Valley fever
    - Rodent-associated virus infections
      - Lassa fever
        - Mopeia
      - Hantaan
    - Virus infections possibly associated with vervet monkeys
      - Marburg virus disease
      - Ebola virus disease
    - Herpesvirus group
      - Herpesvirus hominis (ordinary print)
      - Varicella
      - Cytomegalovirus
      - EB virus
    - Acute infectious fever
      - Morbilli
      - Rubella
- Chlamydial infections
  - Psittacosis
- Rickettsial infections
  - Epidemic louse-borne typhus fever
  - Tick-bite fever, South African-tick typhus
  - Q fever
- Bacterial septicaemia
  - Meningococcal
  - Staphylococcal
  - Streptococcal
  - Gram-negative bacteria
  - Plague
  - Anthrax
- Protozoal infections
  - Malaria
  - Trypanosomiasis

instigator of the syndrome (McGehee et al, 1967; Dennis et al, 1968). In a Scandinavian study, low levels of ATIII correlated with the onset of D.I.C. and with the severity of the septicaemia. These authors noted also the presence of complement (C3) split products: However levels of C3, alpha-1 antitrypsin and alpha-2 macroglobulin bore no prognostic significance (Laursen et al, 1981).

2.2.2 Gram - Positive Bacterial Infections

Discussion will be confined to the Staphylococci and Streptococci. Endotoxaemia is a less frequent complication of gram-positive infections.

2.2.2.1 The Staphylococci

A large number of species of S.aureus possess an "anti-phagocytic" capsule which enhances spread and invasion. Nevertheless the exposed cell wall of 'naked' species may also be harmful; the cell wall matrix comprising teichoic-acid and peptidoglycan may mimic the effects of endotoxin and in particular, may activate the alternate complement pathway. Direct access of these organisms to the intravascular compartment such as through a contaminated intravenous catheter, may theoretically precipitate cardiovascular shock and D.I.C.

Protein A, a component of the cell wall of many strains of S.aureus has a particular affinity for the Fc fragment of circulating immunoglobulins. Through such an interaction, the staphylococcus is able to fix complement and initiate haemodynamic changes in the host (Sheagren, 1984 a,b;

Sjoquist & Stalenheim, 1969). Hawiger and co-workers (1979) have also demonstrated *in vitro* platelet injury and clumping induced by protein A-IgG interaction. Both alpha toxin and coagulase enzyme liberated from these organisms, appear to possess thrombogenic activity (Tuazon et al, 1981). Indeed while a fulminant D.I.C. remains unusual, Murray and Sheagren (1977), in a literature review of Staphylococcal septicaemia, suggest that coagulation disturbances are not altogether uncommon. Although cardiovascular collapse and thrombocytopenia are commonly associated with the "toxic shock syndrome", D.I.C. remains rare (Tofte & Williams, 1982). The elaboration of enterotoxin F, alternatively called pyrogenic exotoxin C, has been implicated in this syndrome; serum levels are elevated. The toxin is able to diffuse across mucous membranes, possibly from contaminated vaginal tampons, and either directly damage tissue or enhance an otherwise 'sublethal' effect of gram-negative endotoxin, upon host tissue (Kaplan & Tannenbaum, 1982; Schlievert, 1983). Elevated levels of plasma teichoic-acid antibody occasionally resolve diagnostic uncertainty (Sheagren, 1984c).

2.2.2.2 The Streptococci

This discussion will include the coagulatory disturbances associated with asplenia and purpura fulminans.

According to laboratory-induced haemolytic patterns on blood agar plates, the Streptococci are categorized into various groups; alpha-haemolytic e.g. S. pneumoniae and

S. viridans, beta-haemolytic e.g. Lancefield's streptococci including S. pyogenes, and gamma or non-haemolytic classes. A further family, the peptococci, include organisms of varying haemolytic potential. The pathogenetic potency of these gram-positive cocci depends upon their protective coating, namely the hyaluronic acid capsule, various cell wall constituents such as the 'M' protein which provides resistance against phagocytosis, and the ability of the bacteria to elaborate toxins. Streptolysins O and S are toxic to erythrocytes and in addition, damage neutrophil and platelet surface membranes and may facilitate the rupture of subcellular organelles (Bisno, 1980).

2.2.2.2.1 Anatomical or functional asplenia: These individuals are particularly susceptible to serious infection, often with encapsulated organisms such as the Streptococci. Both D.I.C. and a Waterhouse-Friderichsen type syndrome have been well documented (Bisno & Freeman, 1970; Coonrod & Leach, 1976; Jewett, 1973). In a recent review of over 7000 asplenic subjects, Wilson and Johnson (1980) noted an overall incidence of serious bacterial infections of between 4 and 12%. Where severe infection had been recorded, the mortality rate was between 50 and 70%. Organisms responsible for severe infections in aspenic subjects included, S. pneumoniae 50%, N. meningitidis 12-15%, H. influenzae 8-12%, "other" Streptococci" including Group A species 7%, and a collection of miscellaneous organisms such as Staphylococci, E. coli, Pseudomonas etc. 16%.

The severity of infection in this group of patients appears to relate to:

1. An extremely high level of bacteraemia.
2. Deficiency of the protein, tuftsin: a phagocytosis promoting tetrapeptide (Gelfand & Grabbe, 1983).
3. Impaired clearance of encapsulated organisms. Clearance of particles and organisms from the blood stream occurs in both the liver and spleen. IgG-coated particles are removed largely by the spleen, possibly through a mechanism requiring slow "percolation" of blood within the sinusoidal system. This allows more prolonged exposure of organisms to specific phagocytic cells. On the other hand, the liver clears complement-coated particles (C3b) more efficiently. Nevertheless its greater vascular supply ensures the liver as the major site of particle and organism clearance in the non-immunized host. However in the asplenic state, the host requires prior IgG-sensitization to facilitate improved clearance of IgG-coated particles via the liver (Brown et al, 1981).

Alterations of coagulation may follow direct platelet-pneumococcal polysaccharide antigen-antibody interaction or may occur indirectly following complement activation (Guckian, 1975; Kelton et al, 1979). Reduced levels of C3 and C_4 have been reported in fulminant Streptococcal infections (Rytel et al, 1974). It has been suggested that

the production of the complement fragment C5c, may stimulate the aggregation of neutrophils and release of proteolytic enzymes, liberation of TXA_2, platelet-activating factor and toxic free-radicals. Endothelial damage resulting from bacterial invasion or consequent upon platelet and/or leucocyte deposition and degranulation, are further postulated mechanisms promoting a coagulopathy.

2.2.2.2.2 Purpura fulminans: This rare syndrome is characterized by an initial thrombotic tendency followed by D.I.C. The recent paper of Marciniak et al (1985) draws attention to the subdivision of this syndrome into two groups viz.

1. Neonatal purpura fulminans.
2. Acquired purpura fulminans.

The former would appear to be related to a homozygous expression of protein C deficiency, such patients presenting shortly after birth. The acquired form probably reflects an immune reaction to various forms of infectious agents, the most common of which have been the Streptococci and viruses. The heterozygous state for familial protein C deficiency has not as yet been related to an increased thrombotic tendency and is unlikely to play a significant role in the acquired syndrome.

In their review of the acquired form, Spicer and Rau (1976) noted the association and general frequency of the following organisms: 'Scarlet Fever Syndrome' viz. mainly Group A Streptococci 27%, unidentified 'upper respiratory'

infections 23%, Varicella 20%, 'other' Streptococci 11%, and a miscellaneous group of conditions such as Rubella, Roseola, small pox vaccination and repeated exposure to Diphtheria antiserum in 19%.

Fever, hypotension, cutaneous haemorrhage involving lower extremities and abdomen and less frequently the upper half of the body, characterize the clinical expression of disease. A polymorphonuclear leucocytosis is usual and the peripheral smear suggests a microangiopathic haemolytic anaemia. Megakaryocytes are increased in the hypercellular marrow. Nonetheless a thrombocytopenia suggesting sequestration or peripheral destruction is usual. Laboratory tests confirm a D.I.C. Autopsy examination reveals widespread thrombosis of small arterioles (Antley & McMillan, 1967). Early anticoagulation with heparin has reportedly been beneficial in an otherwise fatal condition (Little, 1959). Few adults manifest the acquired form; childhood and adolescence is the period of greatest susceptibility. The pathogenetic mechanism of the secondary form remains obscure. Its occurrence after the onset of infection and its occasional relationship to vaccine or antiserum exposure suggest an activation of immunological pathways.

2.2.3 The Viruses and Coagulation Disturbances

Viruses may profoundly alter normal haemostatic mechanisms (McKay & Margaretten, 1967). Nevertheless, a clinically significant intravascular coagulopathy appears to

be an infrequent event (Fisher-Hoch, 1983; Halstead 1981; Viral Haemorrhagic Fevers, 1981). Damage to the endothelial cell appears to be a common step in the initiation of vascular changes. This injury may follow direct invasion or may follow the activation of the immunological system. Thrombocytopenia and damage to the liver with reduced synthesis of clotting factors and protease inhibitors, may account for additional aspects of the haemostatic derangement.

Rather than examining each group of viruses individually, the subject will be considered under the broad and somewhat arbitrary headings of:

a) The viral hepatitic syndromes.

b) The viral exanthems.

c) The viral haemorrhagic fevers.

Clearly there is a significant overlap of common pathogenic mechanisms between these three groups. While the first two occur commonly throughout the world, the latter grouping is no less important: Although largely confined to the less developed regions, the viral haemorrhagic fevers excite much interest and anxiety amongst both health professionals and the general public.

2.2.3.1 The viral hepatitic syndromes

The Picorna group of RNA viruses, and particularly the enteroviridae, including the hepatitis A virus and the coxsackie A and B viruses have occasionally caused a D.I.C. Less universal but regionally important, are the Arboviruses

of which the Yellow Fever Virus is an example. The frequently quoted study by Dennis and co-workers (1969) of experimentally-induced Yellow fever infection in Rhesus monkeys demonstrated the onset of coagulatory and haemorrhagic disturbances prior to the onset of severe hepatic changes. Nevertheless, laboratory and post-mortem support for D.I.C. in these animals was scanty.

The DNA viruses particularly hepatitis B virus and the herpes virus group including the Epstein-Barr virus, have been associated with clinical disease in which both hepatic and vascular components may be significant (Sullivan & Harris, 1984).

Intravascular coagulation in the setting of liver disease may reflect;

1. Activation of coagulation factors secondary to the release of hepatic debris and tissue thromboplastin into the circulation.
2. The inability of the damaged liver to adequately filter and neutralize activated coagulation factors.
3. Diminished hepatic production of ATIII.
4. Enhanced access of endotoxin derived from gastro-intestinal bacteria, to the general circulation via the opening of portosystemic vascular shunts or through impaired macrophage-mediated liver clearance (Mannucci & Forman, 1982).

Herold and Straub (1973) have however suggested that coagulation disturbances may rather reflect a generalised

proteolytic state: Intracellular proteases are released from the damaged liver and their natural inhibitors are inadequately synthesized by that organ. Elevated levels of factor VIII measured commonly in acute liver disease do not support the presence of a D.I.C. A fulminant hepatitis is often associated with a modest thrombocytopenia viz. 100-150 x 10^9/l platelets, and with reduced levels of liver-related coagulation factors. Low factor VII levels are of prognostic significance and probably reflect injury to synthetic mechanisms in the liver rather than activation of the extrinsic clotting cascade. The report of D.I.C. and acute hepatitis by Singh, Singh and co-workers (1983) indicates the increased frequency of D.I.C. in pregnant women with hepatitis and in the subgroup of patients with coma.

2.2.3.2 The viral exanthems

While a thrombocytopenia is not unusual in the course of, or following, infection with measles, rubella, varicella and variola viruses, D.I.C. appears to be extremely rare (Horstmann, 1978). Focal infection of the endothelial cell has been reported with varicella and variola infections.

A fulminant though uncommon syndrome described within the paediatric population is that termed the "virus-associated hemophagocytic syndrome (VAHS)". This condition has been linked to infection with the cytomegalic, herpes simplex, Epstein-Barr and adenoviridae. Clinical manifestations include fever, hepatosplenomegaly,

derangement of liver function and thrombocytopenia, in addition to D.I.C. Examination reveals histiocytic hyperplasia with erythrophagocytosis. At the present time the condition is uniformly fatal. It's pathogenesis remains obscure and therapy is supportive (Risdall et al, 1979; Wilson et al, 1981).

2.2.3.3 The viral haemorrhagic fevers

Coagulatory disturbances may result from the interplay of a variety of contributory mechanisms:

1. Vascular damage. Direct invasion of vascular endothelial cells has been described in Dengue and Junin virus disease and in Korean haemorrhagic fevers (KHF) (Andrews et al, 1978; Fisher-Hoch, 1983). A form of chronic proliferative arteritis has been noted in Rhesus monkeys infected with Lassa fever virus (Callis et al, 1982).
2. Coagulatory disorders. Intravascular coagulation has been confirmed, albeit occasionally, in yellow fever, KHF and in Dengue haemorrhagic fever (DHF). A D.I.C. has also been described in Marburg and Ebola virus infection (Gear et al, 1975; Fisher-Hoch et al, 1983). Endothelial prostacyclin production was demonstrated to be defective in the latter, and contributed to the associated platelet dysfunction.
3. Platelet abnormalities. Thrombocytopenia is almost invariable but usually modest. Platelet dysfunction has been confirmed in studies incriminating the Dengue,

Ebola and Junin viruses. Virus has been recovered from platelets in DHF. Viral antisera has been shown to fluoresce in the presence of megakaryocytes in Junin virus infection suggesting an immunologically-mediated role for the thombocytopenia (Molinas et al, 1981).

4. Activation of the immune system. Lymphocytosis, the presence of atypical lymphocytes and occasionally, leucopenia, have been reported in these fevers. Immune complex generation and deposition has been described in DHF and the group of diseases within the general classification of 'haemorrhagic fever with renal syndrome' (HFRS) namely, nephropathia epidemica as described in Scandinavia and the U.S.S.R., and KHF due to the Hantaan virus reported in Korea, Japan and China (Cohen, 1982). Immune complexes may activate the intrinsic clotting system and may interact with complement.

2.2.3.4 Discussion on some specific viral haemorrhagic fevers

2.2.3.4.1 The arthropod-borne virus infections (Arboviruses): Togavirus family

a. Alphaviruses or Group A viruses

e.g. Chikungunya virus, Sindbis virus.

b. Flaviviruses or Group B viruses

e.g. Mosquito-borne infections: Yellow Fever, Dengue, West Nile Viruses.

e.g. Tick-borne infections: Omsk Haemorrhagic Fever, Kyasanur Forest Disease.

Bunyavirus family

Rift Valley Fever virus, Congo-Crimean Fever virus.

These RNA viruses are transmitted by insects between susceptible vertebrate hosts. Chikungunya and Sindbis infections rarely produce major haematological or haemostatic complications. A mild haemorrhagic illness has been described in Asian children infected with the former virus (Deller & Russel, 1968).

The tick vector of the Congo-Crimean Fever virus belongs to the genus, Hyalomma. Both the tick and the viral disease have been noted in a large area stretching across Africa into the Middle East, the Soviet Union and Pakistan. A severe haemorrhagic syndrome may characterize the disease in a susceptible human host. Antibody titres suggest a high incidence of subclinical exposure to the virus - implying that severe clinical disease is relatively infrequent. Nevertheless case fatality rates range between 15% and 70% in sporadic and hospital-related outbreaks respectively. D.I.C. is reported in 5-25% of persons showing signs of the disease. Histological examination has confirmed vascular damage (Suleiman, 1980; Viral Haemorrhagic Fever, Dept of Health, U.S.A., 1984).

The Dengue virus is endemic to S.E. Asia. Outbreaks have however been recorded in Africa and Europe. Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) occur predominantly in children in endemic regions. Adults

manifest a benign form of disease although epistaxis and skin purpura or petechiae are described. The primary infection is initiated by one of the four "types" of the virus. This occurs at an early age and results in the development of IgM antibodies. During childhood, exposure to a second dengue virus, of a different type, results in the raising of IgG antibodies. These antibodies fix complement and form immune complexes which are believed to be crucial to the development of DHF and/or DSS (Churdboonchart et al, 1984). Halstead (1982) has proposed a phenomenon of "Immune Enhancement" in which the dengue virus forms a complex with non-neutralizing antibody. This complex is engulfed by macrophages which themselves become infected. Consequently such activated monocytes are able to release proteases, tissue thromboplastin and monokines including platelet-aggregating factor and vascular permeability factor (Pang, 1983). Halstead (above) has noted the excretion of urinary histamine to parallel the severity of the disease.

Although the DIC and immune complex deposition accounts in part for the thrombocytopenia and platelet dysfunction of DHF, megakaryocyte-maturation arrest has also been demonstrated early in the course of the disease. Characteristically factors V, VII, IX and X are depressed. Hageman factor and prekallikren may also be low (Edelman et al, 1975). While hepatic synthesis of clotting factors may be defective., rising levels of FDP's, thrombocytopenia and

hypofibrinogenaemia correlate with a worsening of the clinical state and suggest the onset of D.I.C. The reason for the severity of the infection in childhood rather than adulthood is still uncertain. (Pathogentic mechanisms in D.H.F., WHO Bulletin, 1973).

2.2.3.4.2 The primate-related virus infections: Human outbreaks of infection with the Rhabdovirus, Ebola, have been reported from the Sudan and Zaire. A laboratory-acquired infection related to exposure to African Rhesus monkeys was reported from the United Kingdom in 1977. In the experimental model, activation of the intrinsic clotting pathway, increased FDP's and a microangiopathic haemolytic anaemia were thought to follow upon endothelial injury and platelet aggregation. Renal and hepatic changes were insufficient to account for the haemostatic abnormalities (Fisher-Hoch et al, 1983).

Vervet monkeys were incriminated in the outbreak of Marburg fever described in West Germany and Yugoslavia in 1967. The country of origin of the primates was Uganda. An outbreak reported in Johnnesburg in 1975 demonstrated clinical and laboratory features of D.I.C. (Gear et al, 1975). Derangement of liver function may have contributed to the haemorrhagic diathesis.

2.2.3.4.3 The rodent-related virus infections:

a. Arenaviruses

e.g. Lassa virus, Junin virus, Machupo virus (cause of Bolivian Haemorrhagic fever, BHF).

b. Bunyavirus (Fisher-Hoch, 1983)

e.g. Hantaan virus.

Evidence supporting a D.I.C. in these infections is unusual. However vascular damage and alterations in coagulation with thrombocytopenia and prolonged APTT are not infrequent. Lassa virus in the rhesus monkey causes major hepatic and pulmonary lesions. Necrosis of vascular endothelium has been demonstrated (Callis et al, 1982).

Infection with the Hantaan virus has been associated with activation of immunological pathways (Penttinen et al, 1981). Junin virus has been recovered from vascular endothelial cells and macrophages: Activation of the complement system and utilization of clotting factors appear to be concomitant features of Argentine Hemorrhagic Fever (AHF). Halstead (1981) has also suggested a role for 'immune-enhancement' in AHF in view of the virus's predilection for the reticuloendothelial system.

2.2.4 The Rickettsiae and Coagulatory Disturbances

This family of organisms is primarily responsible for vasculitic disease. Describing pathological specimens from Polish civilians and soldiers infected with and dying from Epidemic typhus during the First World War, Wolbach in 1919 noted widespread intravascular micro-thrombi (reported in Walker & Mattern, 1980). Large vessels were spared. Vascular occlusion apparently occurred approximately a week after the appearance of the rash. It was thought that in untreated cases, the vasculitis may predispose to a

thrombotic state.

Rickettsial organisms have been recovered from vascular smooth muscle and endothelium (Silverman & Bond, 1984). Decrease in fibrinogen and factors II and VII have been noted. Both the prothrombin time and APTT may be prolonged. While platelet production is usually normal, thrombocytopenia may occur in 40-60% of patients with Rocky Mountain Spotted Fever. Thrombocytopenia has been shown to correlate positively with increased mortality (Hattwick et al, 1976). Nevertheless major changes in the coagulation profiles of these patients remains unusual.

Tick-bite fever in southern Africa is caused by Rickettsia conori var. pijperi, transmitted by various ixodid or 'hard' ticks. A severe form of the disease may manifest as a haemorrhagic rash with petechiae and very rarely digital gangrene; a clinical syndrome more common to elderly patients (Gear et al, 1983; Gear, 1978).

2.2.5 The Protozoa and D.I.C.

2.2.5.1 Malaria

A clinically obvious D.I.C. is rare and occurs late in the course of the illness (Butler et al, 1973; Dennis et al, 1967). Over the past decade, a major resurgence of malaria has been reported; an increase of related haematological abnormalities can be anticipated. Wyler (1983) noting that worldwide, some 300 million people are afflicted annually, states that malaria remains one of the major infective hazards of this century.

Aikawa and co-workers (1980) have noted the deposition of fibrin in pulmonary lymphatics, and the occlusion of the microcirculation of hamsters with mononuclear cells. Similar changes may be found in severely ill patients. Thrombotic events may follow upon altered electronegative repulsive forces between parasitized red cells and the vascular endothelium. Mohanty and colleagues (1985) reported increased procoagulant activity of erythrocytes from infected subjects. The fragmentation of these cells may liberate ADP and thereby promote platelet aggregation (Wilson et al, 1982). It is also possible that endotoxin-like substances may be released from the parasite and activate coagulation and complement pathways (Hall, 1976). Deposition of immune complexes have been described in both P. falciparum and P. malariae infections (Malaria and Immunology, 1978). Vascular damage may follow hypoxia consequent upon the sludging of erythrocytes in the microcirculation. Intravascular coagulopathy occurs most frequently within the context of a worsening anaemia and the onset of pulmonary oedema. While thrombocytopenia is frequent, it is seldom sufficiently severe when isolated, to produce a bleeding tendency (Perrin et al, 1982).

2.2.5.2 Other protozoal infections

African trypanosomiasis has on occasion been associated with a D.I.C. Barret-Connor and colleagues (1973) describe D.I.C. together with a Coombs positive haemolytic anaemia, marked thrombocytopenia and hypocomplementaemia. The

frequent description of cold haemagglutinins in trypanosomiasis supports an immune-mediated pathogenesis. Indeed, a wide spectrum of antibody production including anti-IgG, anti-single stranded DNA and anti-erythrocyte, is characteristic. The parasite is believed to be capable of releasing haemolytic toxins - supported experimentally, unproven in man (Wéry et al, 1982). A striking increase of cholesterol, beta-lipoprotein and free fatty acids is commonly noted in the plasma of patients. Whether this hyperlipidaemia contributes to the haemolytic process is still uncertain. Bone marrow failure occurs as a preterminal event.

Although a D.I.C. is rare in Leishmaniasis, a variety of haematological alterations are characteristic. Hypersplenism and the high level of immune complexes in Kala-azar contribute to the anaemia, leucopenia and thrombocytopenia. A Coombs positive anaemia occurs frequently (Meiser & Belehu, 1982).

Hepatic derangements are common to all the protozoal infections, however this is usually insufficient to promote a bleeding diathesis.

2.2.6 Miscellaneous infections and coagulation disturbances

Virtually every infecting organism has at one time or another been implicated in the pathogenesis of an intravascular coagulopathy. Occasional reports have noted an association of D.I.C. with miliary tuberculosis (Kraus & Walker, 1979; Goldfine et al, 1969), and with disseminated

fungal infections (Prochazka et al, 1971). The latter incriminated direct invasion of vascular endothelium by fungal hyphae as a possible trigger of the thrombotic event (Doughten & Pearson, 1968).

Human spirochaetal disease, including Leptospirosis and Relapsing Fever, has rarely been associated with a coagulopathy. However, the leptospira may injure capillaries and produce a vasculitis. Hypoprothrombinaemia may follow hepatic endothelial cell damage subsequent to interaction with and deposition of immune complexes (Johnson, 1977). Haemolysis and thrombocytopenia may be partially immunologically mediated. Nonetheless, production of haemolysins with phospholipase activity is probably a more important mechanism (Trowbridge et al, 1981). A D.I.C. in leptospirosis has been suggested by the concomitance of thrombocytopenia and renal failure (Raoult et al, 1983; Edwards et al, 1982). Gallway and co-workers (1977) noted that elevated endotoxin levels in infection with Borrelia organisms correlated with decreased factor XII, pre-kallikrein and complement. Clumping of the organisms and capillary endothelial cell injury appears to parallel the appearance of skin and mucous membrane haemorrhages.

2.3 NON-D.I.C. RELATED COAGULATION DISTURBANCES AND INFECTIOUS DISEASE

2.3.1 Thrombocytopenia and Infection

Although low platelet counts are not uncommonly

described with many infectious conditions, an isolated thrombocytopenia is rarely responsible for significant haemostatic failure.

2.3.1.1 Thrombocytopenia and bacterial infections

A platelet count of less than 50 x 10^9/l suggests a D.I.C. in bacterial septicaemias. However, counts between 50 and 150 x 10^9/l usually suggest alternative mechanisms (Neame et al, 1980). Nevertheless, increased destruction rather than production failure appears to be the more important. Platelets bind endotoxin via a specific membrane receptor which facilitates the platelet release reaction (MacIntyre et al, 1977). The severity of thrombocytopenia in gram-positive and gram-negative septicaemias bears a direct relationship to the plasma levels of platelet-associated immunoglobulins (PA-IgG); suggesting an immunologically mediated aetiology (Wilson et al, 1982). The recurrence of idiopathic thrombocytopenic purpura (ITP) following an infection or vaccination, may represent the activity of anti-platelet antibodies produced during the infective episode (Veenhoven et al, 1979).

2.3.1.2 Thrombocytopenia and viral illness

A hypocellular bone marrow with reduced megakaryocytes has been noted in dengue and congenital rubella infections as well as following vaccination with attenuated measles and vaccinia viruses. Anti-platelet antibodies are described in infectious mononucleosis. Immunological pathways of platelet destruction have also been implicated in mumps and

rubella infection (Andrews & Bart, 1975; Boyd & Reid, 1973; Graham et al, 1974; Myllylä et al, 1969).

The thrombocytopenia of mumps has been associated with adsorption onto the platelet surface of soluble viral protein and in some instances, the formation of antigen-antibody complexes. Between 50 and 80% of acute ITP in childhood has been said to be associated with a recent viral illness (Wilson et al, 1982).

2.3.1.3 Thrombocytopenia and protozoal infection

Longitudinal studies of P. falciparum infections have revealed an extremely frequent occurrence of thrombocytopenia. Counts below $100 \times 10^9/l$ were noted in almost 100% of subjects in two studies quoted by Perrin and Mackay (1982). Elevated PA-IgG correlate with the parasitaemia and return to non-recordable levels once the platelet count normalises. Occasional evidence of marrow dyserthropoiesis and the demonstration of parasites within platelets suggest additional causes of the thrombocytopenia. Parasitized platelets display an increased sensitivity to ADP and epinephrine (Abdalla et al, 1980; Essien & Ebhota, 1981).

2.3.2 Hepatic Disease and Infection

The plethora of infectious agents causing hepatic pathology has already been alluded to, as have the variety of mechanisms predisposing to haemostatic disturbance. Haemorrhagic manifestations of chronic liver disease usually present as local gastrointestinal bleeds. Generalized

haemorrhage may reflect inadequate prothrombin and clotting factor synthesis.

2.3.3 Concluding Remarks on Infection and Coagulation Disturbances

The interaction between the microbe and the haemostatic system may be modified by environmental factors and the pre-existence of various diseases. It is not unusual to find infections complicating renal, cardiac or pulmonary failure. Disorders such as diabetes mellitus and ethanol abuse, cancer and its treatment, commonly predispose the host to both infection and haemostatic alterations. A D.I.C. in the context of infection is fortunately rare and usually self-evident: The treatment of D.I.C. is still unclear though therapy of the underlying infection and general supportive measures remain as primary goals.

SECTION 3

TYPHOID FEVER AND COAGULATION DISTURBANCES

3.1 A STUDY OF PATIENTS ATTENDING THE HILLBROW HOSPITAL, JOHANNESBURG, DURING 1983 AND 1984.

3.1.1 Aims of Study

1. To assess the frequency of coagulation disturbances in typhoid fever.
2. To ascertain the role, if any, of deranged hepatic and metabolic function in the coagulation disturbance.
3. Evaluate pathogenetic mechanisms contributory to the disturbances of typhoid fever.

3.1.2 Methods of Study

During the two-year period from January, 1983 to December, 1984, thirty-two patients with typhoid fever were admitted to the Hillbrow Hospital, Johannesburg. All patients were Black. Eighteen were female and fourteen were male. Their ages ranged from twelve to seventy years. Median age was 28,3 years.

Most patients were domicile within the Johannesburg urban region. A small number were referred from distant hospitals in the Northern and Eastern Transvaal. No patient had commenced anti-bacterial therapy or received any form of blood product before their admission to the Hillbrow Hospital.

The diagnosis of typhoid fever depended upon a compatible clinical history and examination, and the culture of Salmonella typhi from the patient. Blood cultures were positive in twenty-seven of the patients, while the remaining five had positive stool cultures. Isolation of

the organism was performed by staff of the typhoid laboratory, South African Institute for Medical Research (SAIMR), Johannesburg.

Data reflected in this study records blood tests performed during the first twenty-four hours following hospital admission. As data was gathered retrospectively from the records of individual patients, full haematological, serological and biochemical information is not available in every case. However a full coagulation profile was performed in a prospective manner on the final thirteen patients. The coagulation screen was performed by Mrs N. Pienaar of the Haematology/Coagulation laboratory, SAIMR, Johannesburg Hospital. Prekallikrein, plasminogen and protein C estimations had to be performed on plasma stored and frozen at -70°C. All results were compared with samples from normal healthy adult controls.

The laboratory indices included:-

Haematological system

The haemoglobin, total leucocyte count, platelet count, platelet-crit, distribution width and mean platelet volume and the mean red cell corpuscular volume were measured on a Coulter S Plus automated analyser in the haematology laboratory of the Johannesburg Hospital. The peripheral blood smear and differential cell count were performed manually by staff of the same laboratory.

The coagulation screen

The prothrombin ratio utilized Quick's one-stage method and was performed using human-brain thromboplastin prepared by the laboratory's reagent unit and standardized against British Comparative Thromboplastin Reagent.

The Activated Partial Thromboplastin Time (APTT) was performed with Ortho APTT reagent and Ø.Ø25 molar calcium chloride.

Fibrinogen levels were estimated with the Ellis and Stransky method (Ellis & Stransky, 1961).

All clotting factor assays utilized the one-stage method. Factor-deficient plasma was obtained from General Diagnostics.

Prekallikrein activity utilized reagent from Boehringer Mannheim Diagnostics.

Plasminogen estimation was by means of a chromogenic assay incorporating reagents from the Behring Institute.

Protein C was measured with the Elisa method, using reagent from Diagnostica Stago.

Fibrin/Fibrinogen degradation product (FDP's) measurement utilized material from Wellcome Diagnostics viz. The Thrombo-Wellco Test.

Fibrin monomers were determined using serial dilutions of protamine sulphate viz. The Protamine Sulphate Test.

C1-esterase inhibitor was measured with the electrophoretic method requiring Tris buffer and C1-esterase inhibitor anti-sera available from the Behring Institute.

Alpha-1 antitrypsin was measured by the turbidometric method requiring alpha-1 antitrypsin antiserum from Daka.

The Biochemical System

Serum sodium and potassium, blood urea and creatinine values were recorded from the automated analyser, Technicon SMA 6:60.

Total protein, serum albumin, bilirubin, alkaline phosphatase, alanine transaminase (ALT), Aspartate transaminase (AST), gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) were performed on the automated analyser, Technicon SMA 12:60. All biochemical tests were performed in the Biochemistry Laboratory, SAIMR, Hillbrow Hospital.

3.1.3 Results

3.1.3.1. Morbidity and Mortality

In a series as small as this, and in which patients were referred from distant centres, statistics relating to morbidity and mortality are likely to be unreliable. The only death occurred in the eldest patient, a seventy year old male transferred from a Northern Transvaal hospital. This patient had an extensive and inoperable carcinoma of the floor of the mouth. On admission he was febrile, dehydrated and in renal failure. Despite appropriate antibiotic therapy and intravenous rehydration, he died nine days after admission.

All patients were febrile. Twenty-seven of the total thirty-two had symptomatology referable to the

gastrointestinal tract; eight presented with bloody diarrhoea. Two patients had clinical features suggestive of a generalised bleeding disorder. Four had pneumonia. Two had a myocarditis and one, an endocarditis.

The duration of hospitalization was compared with LDH levels, no correlation was found (Figure 3.1): The duration of hospital stay is probably a poor index of morbidity in view of the large variety of economic and social factors influencing this parameter.

3.1.3.2 Coagulation disturbance

Results in this study are compared with the normal range in healthy white adult controls. Unfortunately such normal values are not available for Black South Africans. A student 't-test' was applied where results were normally distributed and analysed on an IBM computer. The Chi-Square Test was applied to the analysis of LDH levels.

Laboratory criteria supportive of a diagnosis of D.I.C. included a combination of three of the following four indices in individual cases:

a) A thrombocytopenia of 140 x 10^9/l or less.

b) Prolongation of the prothrombin-time expressed as a ratio, and/or prolongation of the activated partial thromboplastin (APTT) time also expressed as a ratio.

c) Plasma fibrinogen levels below 2 gm/l.

d) The presence of fibrinogen degradation products (FDP's) in excess of 40 /ug/ml.

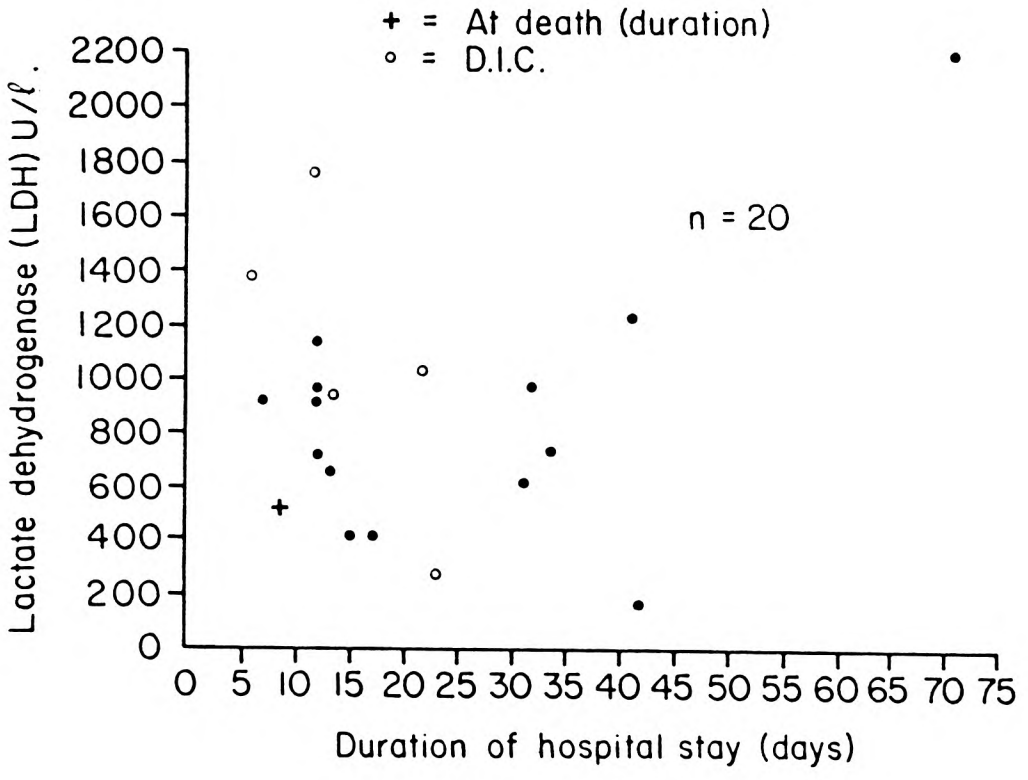


Figure 3.1: Duration of hospital stay compared to levels of lactate dehydrogenase. These parameters appear to bear no relationship to one another.

Of the series of 13 patients in whom a full coagulaion screen could be performed, four fulfilled the above criteria, with a fifth patient demonstrating evidence of an "overcompensated" D.I.C. in which fibrin monomers and FDP's were present but the serum fibrinogen was above 2 gm/l. A brief summary of the case histories of these five patients follows:

Case 1 Mr H.Z., a 23 year old labourer domicile in Johannesburg. A week earlier he had returned from a vacation in the Transkei. Three days prior to admission headache, fever and diarrhoea were noted. Fever and abdominal tenderness were found on admission and a diagnosis of typhoid fever confirmed on blood culture 48 hours later. Intravenous ampicillin was commenced but changed to chloramphenicol five days later owing to persistent fever and the development of a toxic myocarditis. Admission bloods revealed a subclinical DIC: Thrombocytopenia of 78 x 10^9/l, prothrombin ratio of 1,43 and APTT (ratio) of 2,58, and FDP's of 120 - 240 /ug/ml. The fibrinogen was elevated at 4,58 g/l and may have represented "over compensation" or an "acute phase" response. A moderate elevation of hepatic transaminases was also present at the time of his admission. Repeat liver function tests and hepatitis serology results were not recorded in the patient's file. He was transferred to a convalescent hospital eight days after admission.

Case 2 Mrs J.T., a forty year old domestic worker who had returned to Johannesburg two weeks earlier after visiting family in Swaziland. Fever, rigors and diarrhoea commenced five days before admission. Initial examination revealed fever and hepatomegaly. S. typhi was cultured from both blood and stool several days later. Although intravenous ampicillin was started, a bronchopneumonia and persistent fever in the second hospital week prompted a change to chloramphenicol. The growth some days later of E. coli from the blood, resulted in the addition of an aminoglycoside: At the time of this septicaemia, the patient was reported to have clinical and ECG features of a toxic myocarditis.

Admission bloods suggested a subclinical D.I.C. and a hepatitis; Thrombocytopenia of 96 x 10^9/l, elevated prothrombin ratio of 1,28 and APTT ratio of 1,36, FDP's between 240-480 ng/ml and the serum fibrinogen in the low, normal range of 2,13 g/l. Hepatic transaminase levels became markedly elevated by the end of her first hospital week. At the time of discharge 40 days after admission, the patient was well and both the myocarditis and hepatitis appeared to have resolved. The D.I.C. resolved spontaneously by the middle of the second week.

Case 3 Miss J.W., a 22 year old domestic worker domicile in Johannesburg. No recent travel history. Fever, headache and abdominal discomfort had been noted for the preceding two weeks. Two days prior to the admission her family observed a rash and occasional confusion. The admission

examination revealed a pyrexia, purpuric rash, and episodes of delirium. Bleeding occurred spontaneously from venepuncture sites: A clinical DIC was suspected and the patient admitted to the intensive care unit (ICU). Intravenous ampicillin was administered together with rehydration fluids and plasma. Initial blood tests confirmed the presence of a D.I.C. and hepatitis. Platelet count of 83 x 10^9/l, elevated prothrombin ratio of 1,39 and APTT ratio of 1,89, elevated FDP's of 120-240 /ug/ml and reduced fibrinogen of 0,99 g/l. Hepatitis A and B markers were negative. Blood cultures were repeatedly positive for S. typhi. Cerebrospinal fluid was normal. The patient appeared to be well twelve days after admission. Hepatic enzymes had returned to normal and tne D.I.C. had resolved at the time of her discharge.

Case 4 Mrs S.M., a 20 year old housewife domicile in Johannesburg. No travel history. Admitted to the gynaecological department of the Hillbrow hospital following three days of abdominal pain, fever and a vaginal discharge. Two days later the patient was noted to be febrile, confused and to have abdominal distension with the passage of melaena stools. A marked leucopenia was noted. Intravenous ampicillin was commenced and a diagnosis of typhoid fever confirmed on blood culture. Admission bloods revealed a "compensated" D.I.C., platelet count of 133 x 10^9/l, normal prothrombin ratio of 1,06 and mildly elevated APTT ratio of 1,42, with elevated FDP's of 120 - 240 /ug/ml, in addition

to positive fibrin monomers. The fibrinogen was markedly elevated at 5,84 g/l. Deteriorating renal function with the development of the nephrotic syndrome and a left lobar pneumonia and persistent toxicity and fever, necessitated a transfer to the I.C.U. Hepatic transaminases were mildly elevated. During her second week in I.C.U., blood cultures positive for S. faecalis required the addition of intravenous aminoglycosides. By the time of her discharge 25 days after admission, the D.I.C. and hepatitis had resolved and the nephrotic syndrome appeared to be improving.

Case 5 Miss P.N., a 19 year old scholar who recently had visited family in the Northern Transvaal. Headache, fever and diarrhoea began three days before admission. Examination confirmed a fever, bronchopneumonia and hepatosplenomegaly. Bleeding was evident at venepuncture sites and a D.I.C. confirmed; thrombocytopenia of 49 x 10^9/l, normal prothrombin ratio of 1,Ø8 with an elevated APTT ratio of 1,83, elevated FDP's of 48Ø-96Ø /ug/ml with fibrinogen of 1,18 g/l. Intravenous ampicillin was started on admission and S. typhi cultured on blood several days later. The patient was admitted to the I.C.U. where three days later chloramphenicol was substituted in view of a persistence of the 'toxic' state. A mild hepatitis and nephritis were noted; both had resolved by the time of her discharge 22 days after admission. Markers for viral hepatitis remained negative. The D.I.C. appeared to improve

spontaneously with general supportive and antibiotic therapy.

The measurement of the platelet count (Figure 3.2) and the platelet-crit (Figure 3.3) yielded a statistically significant thrombocytopenia in the group as a whole, $p < Ø,Ø5$. Figure 3.4, represents the Fibrin Degradation Products in a group inclusive of the thirteen patients studied prospectively. Fibrinogen levels for the latter group, are recorded in Figure 3.5; results were not statistically significant viz. $p > Ø,1$. The two patients with elevated levels of fibrinogen may have represented 'overcompensation' by the liver or an 'acute phase reaction' of the protein.

Results of the prothrombin ratio (Figure 3.6) approached statistical significance; $p = Ø,Ø76$. However the activated partial thromboplastin time (Figure 3.7) was not significantly elevated although individual values were occasionally high ($p > Ø,1$). Those patients with a D.I.C. tended to belong to the latter group. The general tendency of these results to lie within the normal distribution may reflect a 'compensated state' in which accelerated production of clotting factors by the liver may mask increased sequestration. Patients with a D.I.C. and normal results may have reflected the early phases of evolution of a coagulopathy: all blood samples record admission findings.

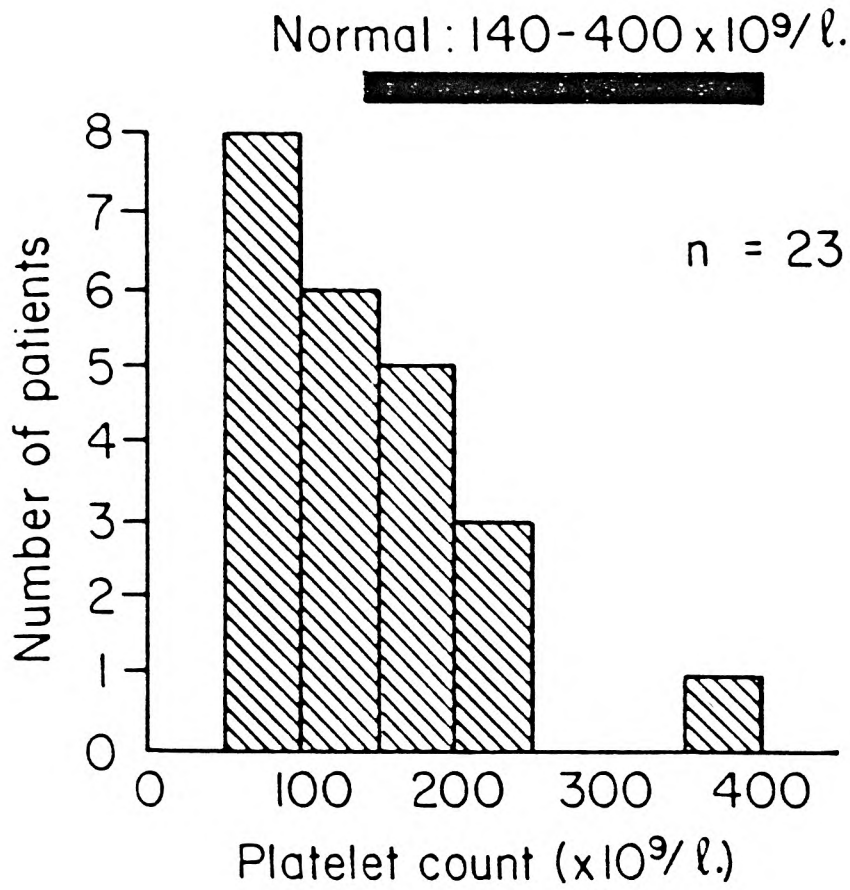


Figure 3.2 Admission platelet count in patients with Typhoid Fever.

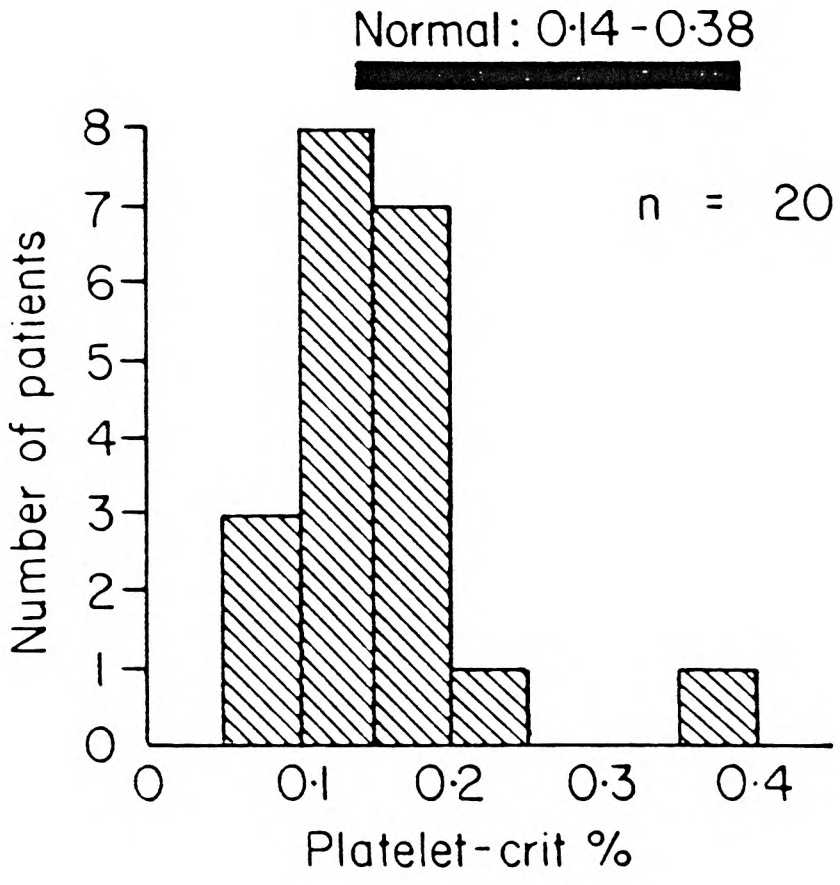


Figure 3.3 Admission platelet-crit values in patients with Typhoid Fever.

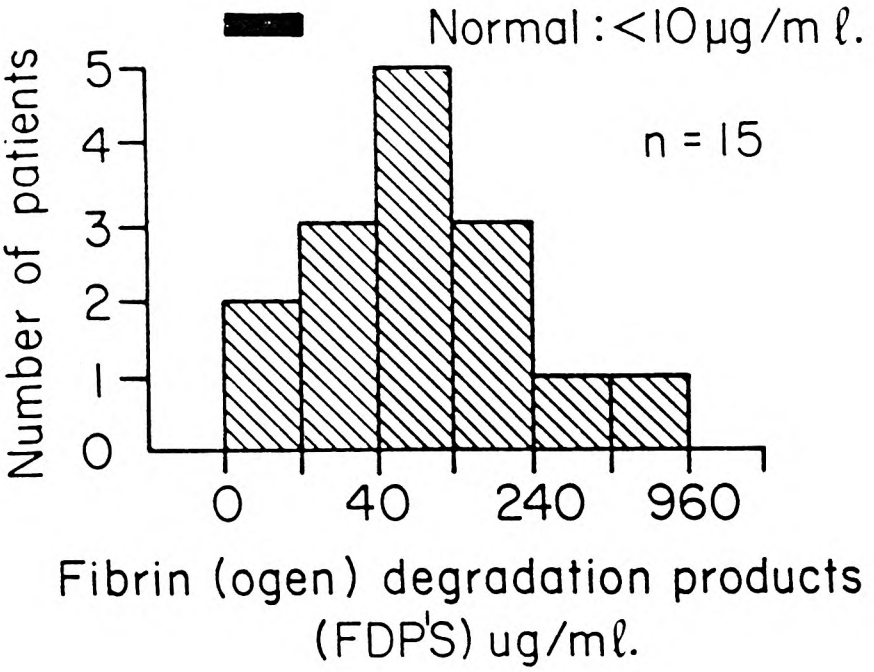


Figure 3.4 Fibrin degradation products on admission samples of blood from Typhoid Patients.

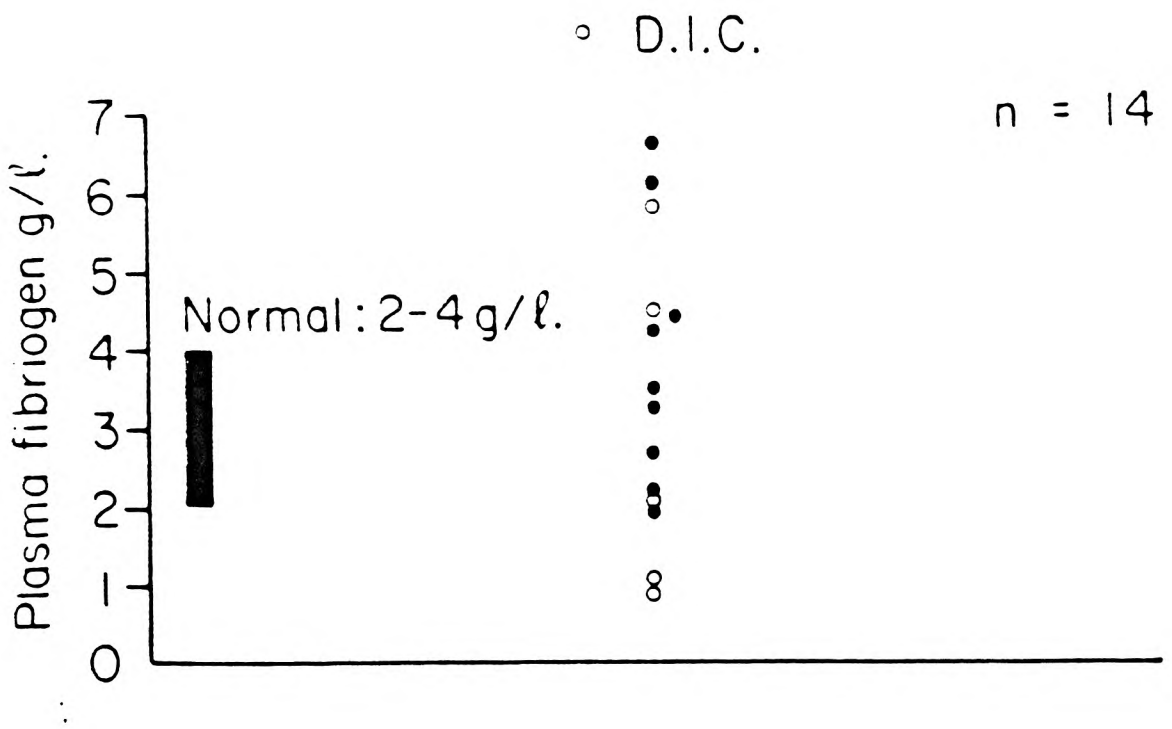
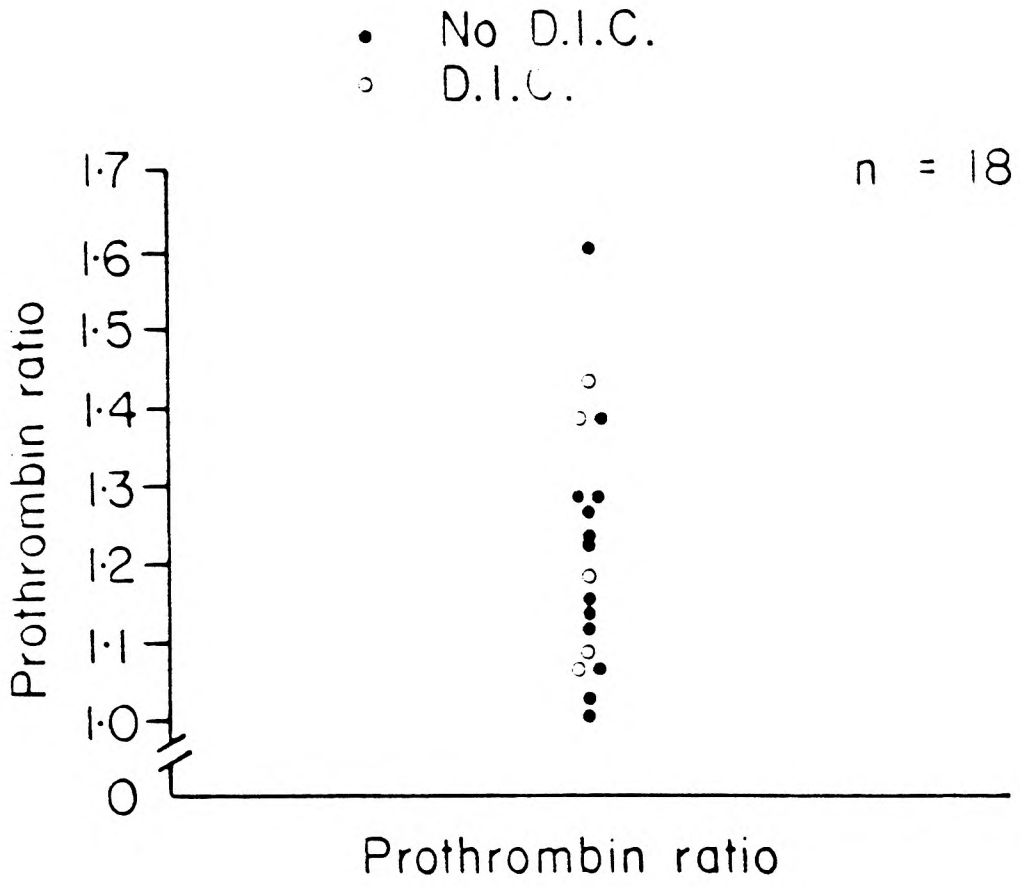


Figure 3.5 Plasma fibrinogen levels on admission in Typhoid patients.



Mean	=	1·216
SD	=	0·16
SD(control)	=	0·083
Z	=	1·77
p	=	0·076

Figure 3.6 Prothrombin ratio in Typhoid fever. Blood samples taken on admision. Patients with D.I.C. represented by open circles.

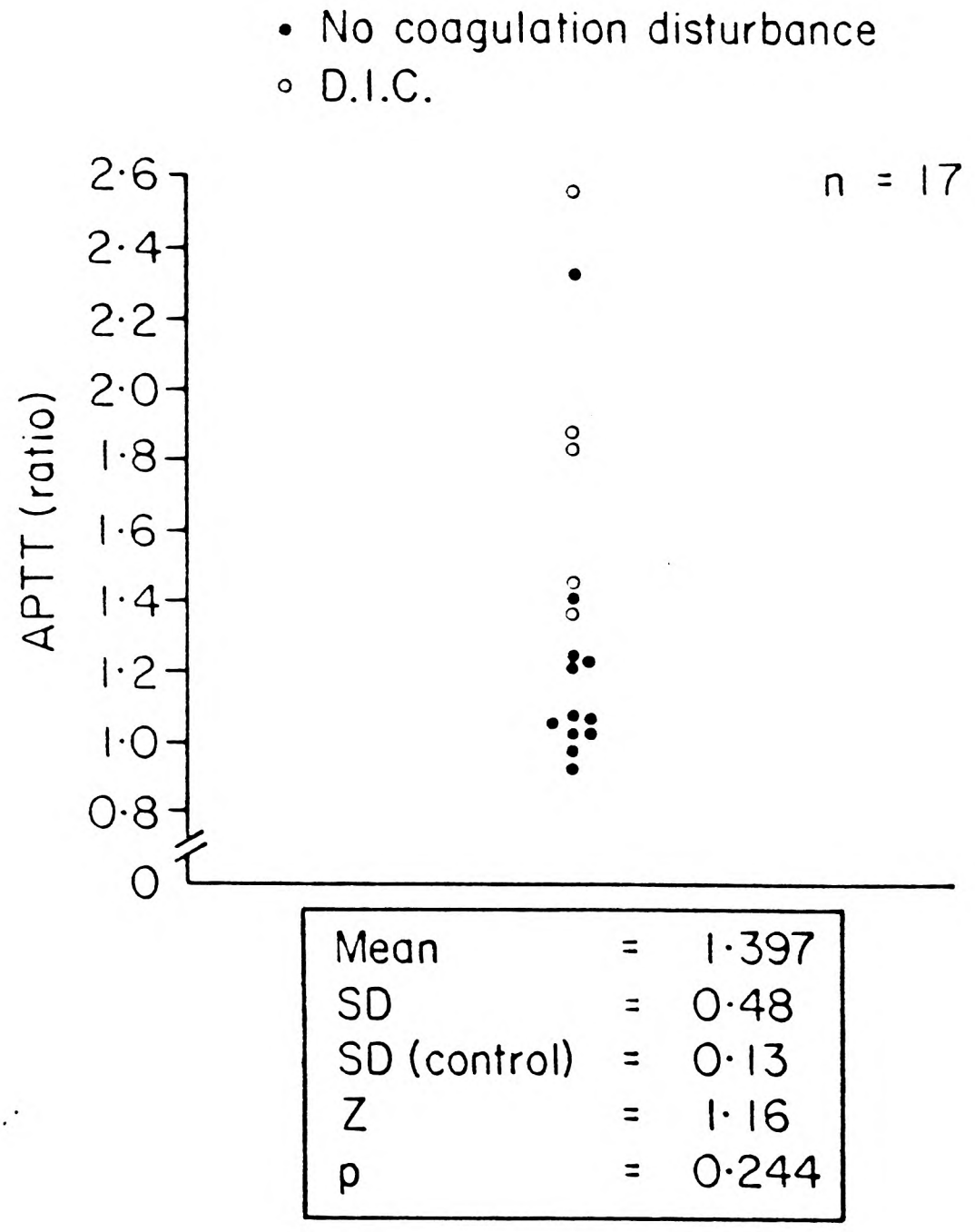


Figure 3.7 Activated partial thromboplastin time (APTT) in Typhoid Fever. Patients with D.I.C. represented by open circles.

The measurement of coagulation factors VII, VIII and XII is represented in Figures 3.8 and 3.9. Statistical analysis shows no significant alteration viz. $p > Ø,1$. A single low value of factor VII was recorded in a patient with reduced protein C and a coagulation profile suggestive, but not diagnostic of a D.I.C. Unfortunately hepatic enzymes and serum albumin levels were not recorded in this patient.

Plasma prekallikrein activity and levels of plasminogen were not significantly altered viz. $p > Ø,1$. (See Figures 3.1Ø and 3.11). However, of the 5 patients with laboratory evidence of D.I.C., the two with clinically evident bleeding, recorded particularly low levels of prekallikrein. Neither had depressed levels of factor XII.

Figures 3.12 and 3.13 describe results for alpha-1 antitrypsin and C1-esterase inhibitor respectively. Both are statistically significantly elevated i.e. $p < Ø,Ø5$. These levels may reflect an 'acute-phase' response. Lack of evidence supporting factor XII and prekallikrein utilization suggests that C1-inhibitor is unlikely to be elevated in response to intrinsic clotting pathway activation.

3.1.3.3 Hepatitis and deranged liver synthetic function

Seventeen of a total of twenty-three patients in whom hepatic transaminase enzymes were recorded, had elevated ALT (74%) and twenty had elevated AST levels (87%). These results are demonstrated in Figures 3.14 and 3.15. Closed dots in these figures reflect patients with a D.I.C.

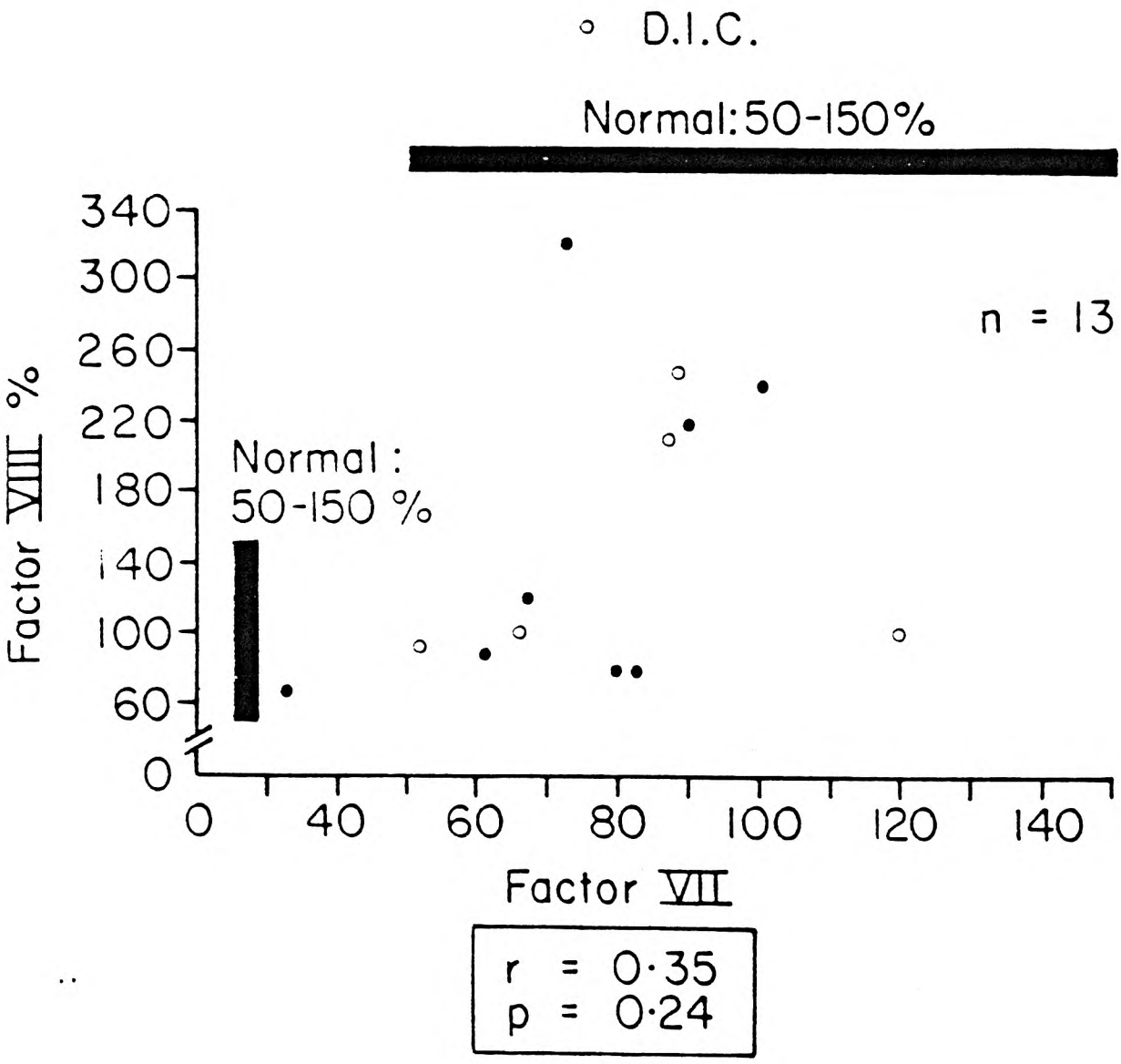


Figure 3.8 Clotting factor levels in typhoid fever: Factors VII and VIII.

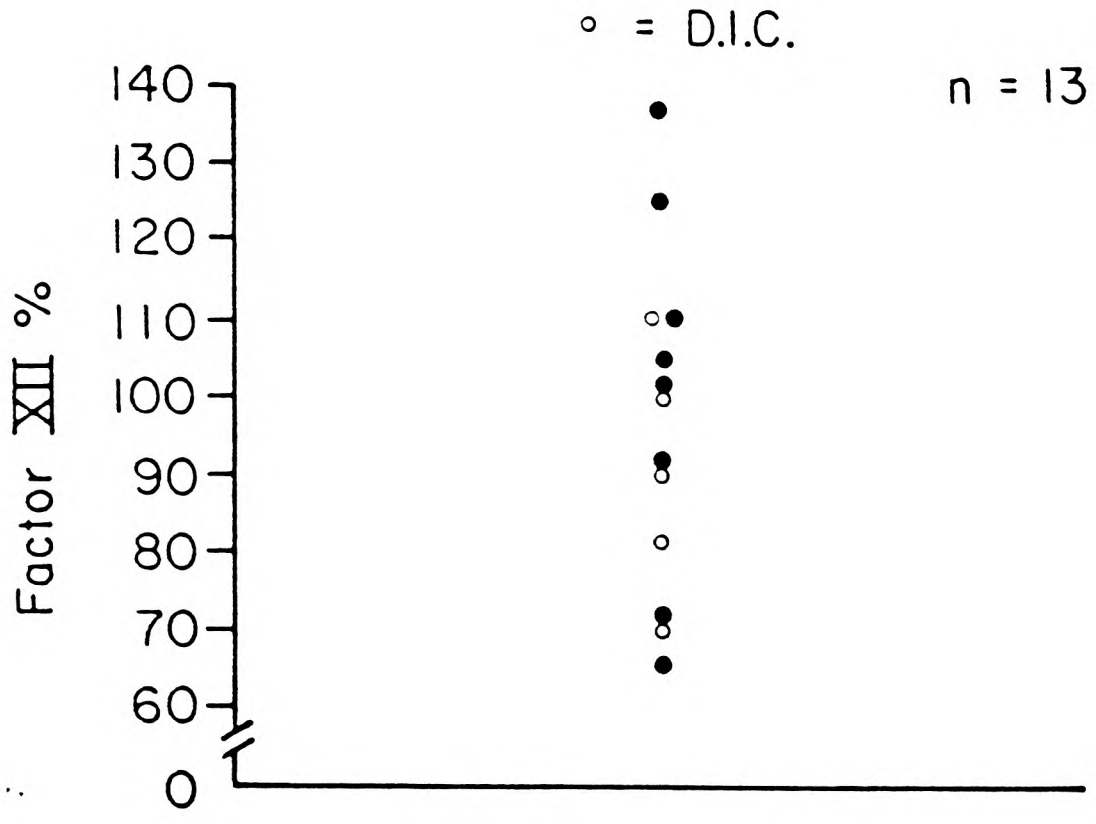


Figure 3.9 Clotting factor levels in Typhoid Fever: Factor XII.

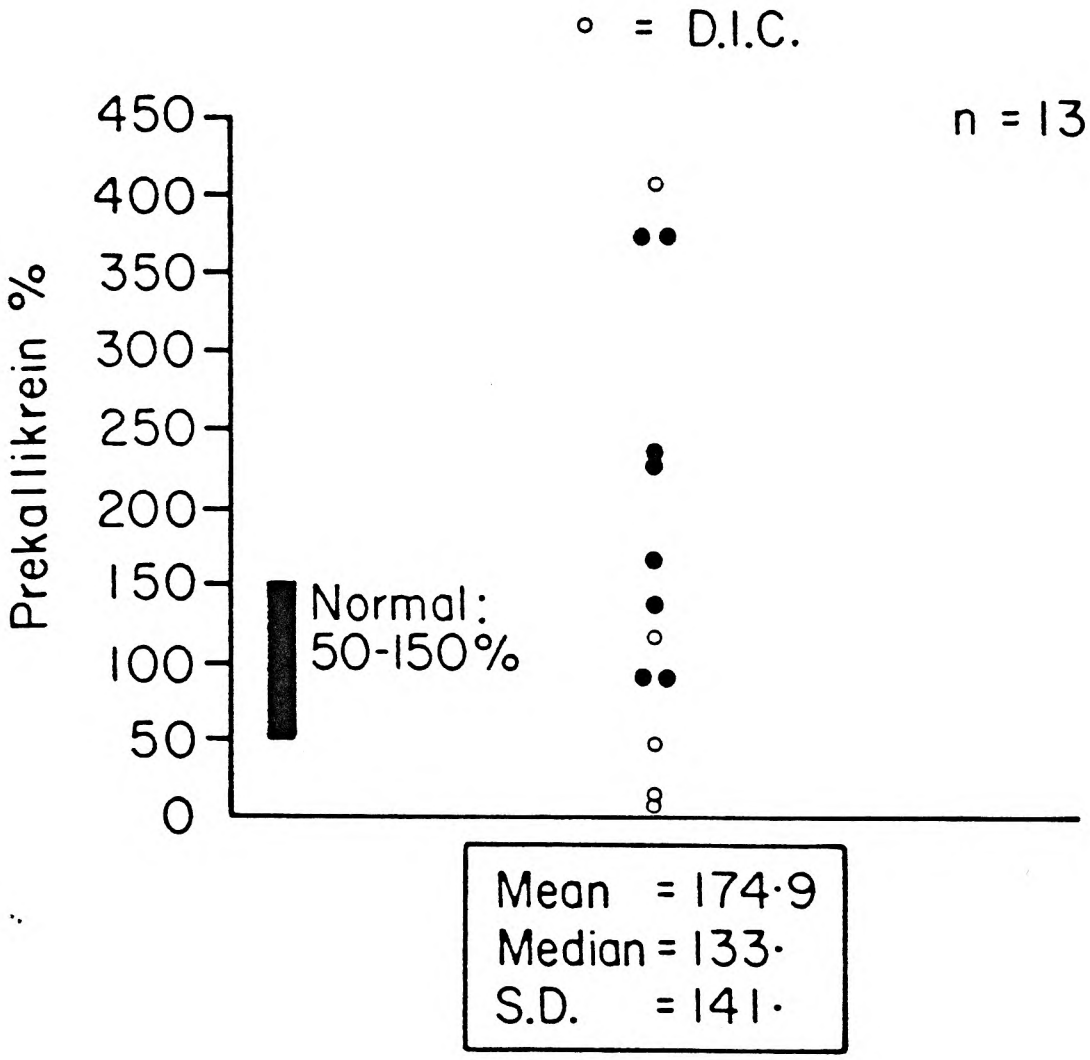


Figure 3.10 Prekallikrein levels on admission blood samples in Typhoid Fever.

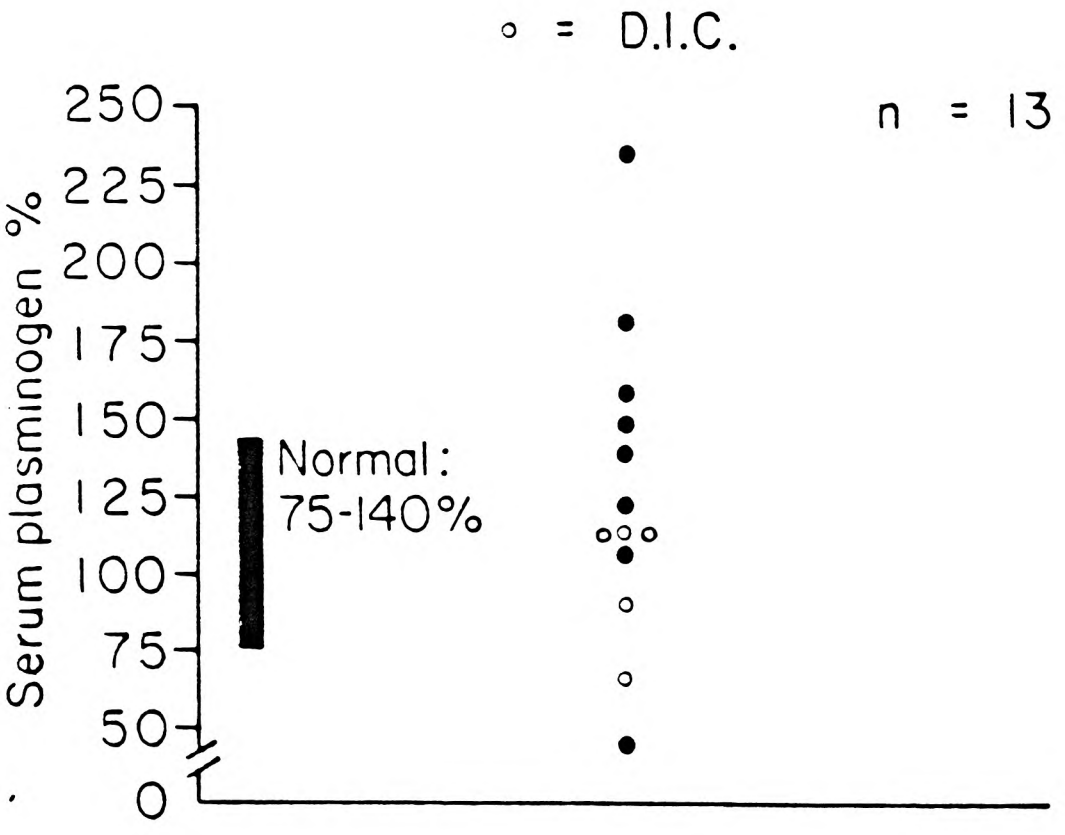


Figure 3.11 Plasminogen levels on admission blood samples in Typhoid Fever.

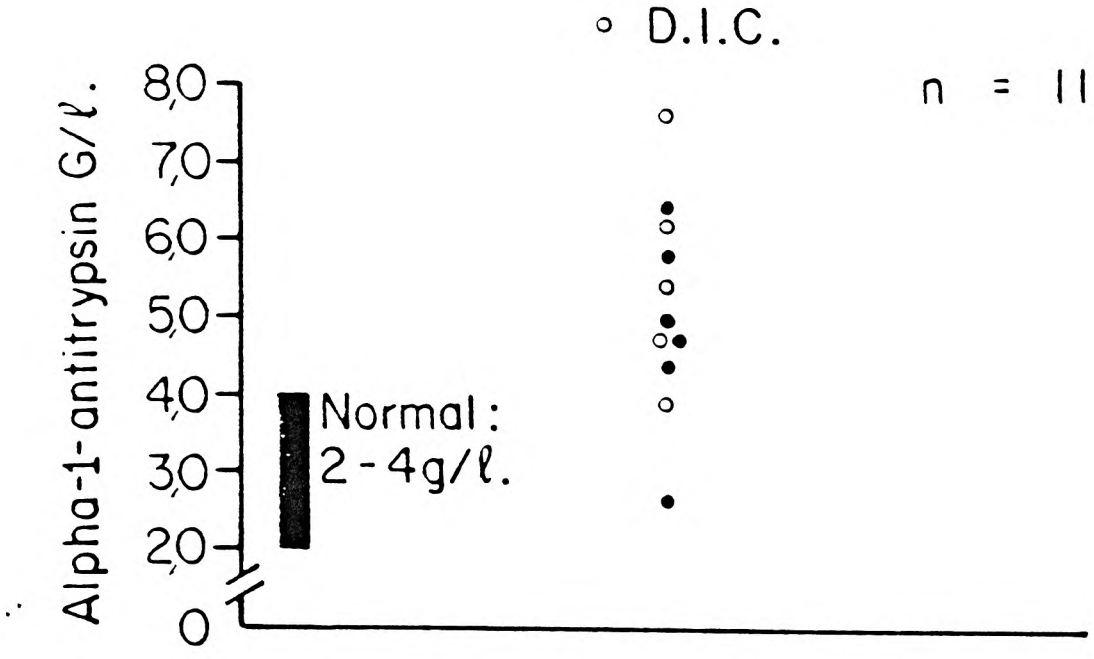


Figure 3.12 Alpha-1 antitrypsin in Typhoid Fever.

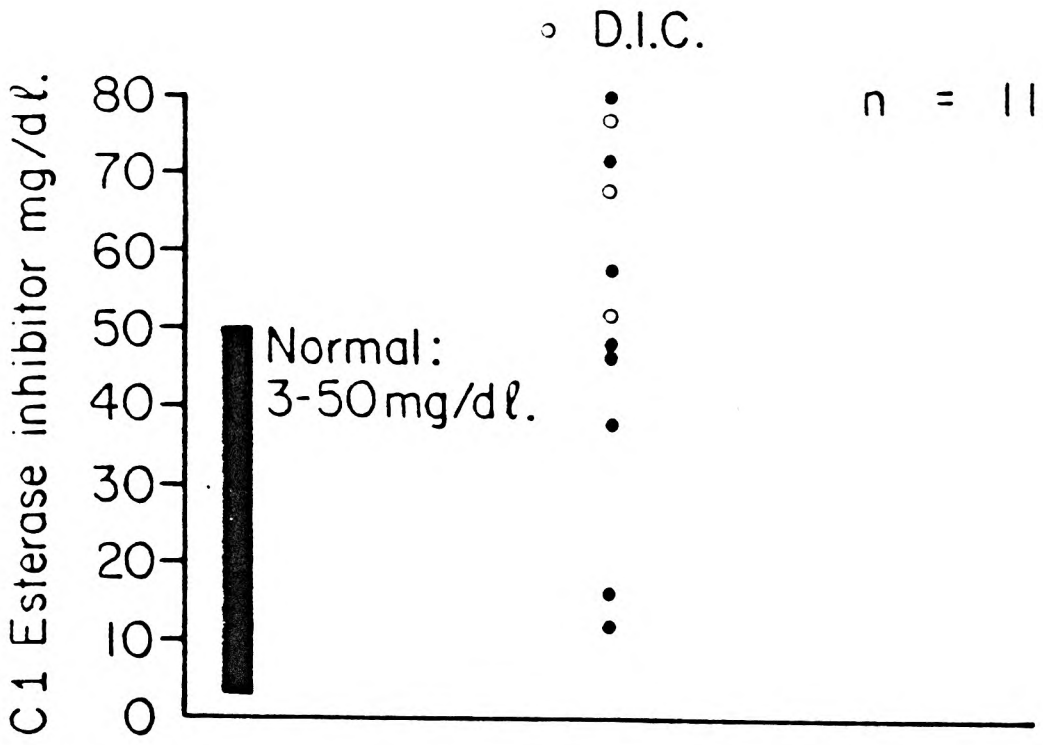


Figure 3.13 C1-Esterase inhibitor in Typhoid Fever.

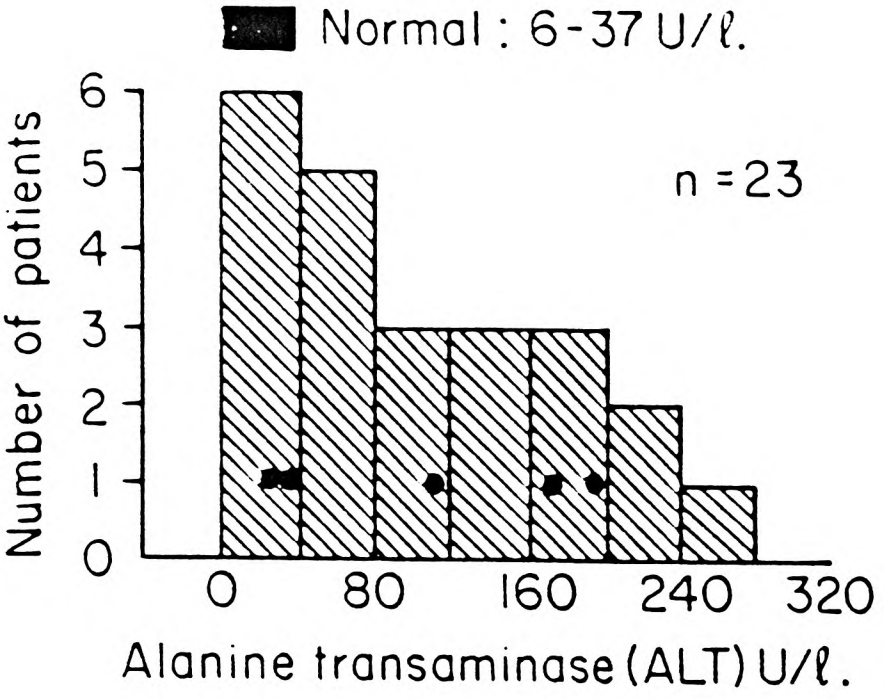


Figure 3.14 Hepatic enzymes in Typhoid Fever: (i) Alanine transaminase (ALT). Results from admission blood samples.

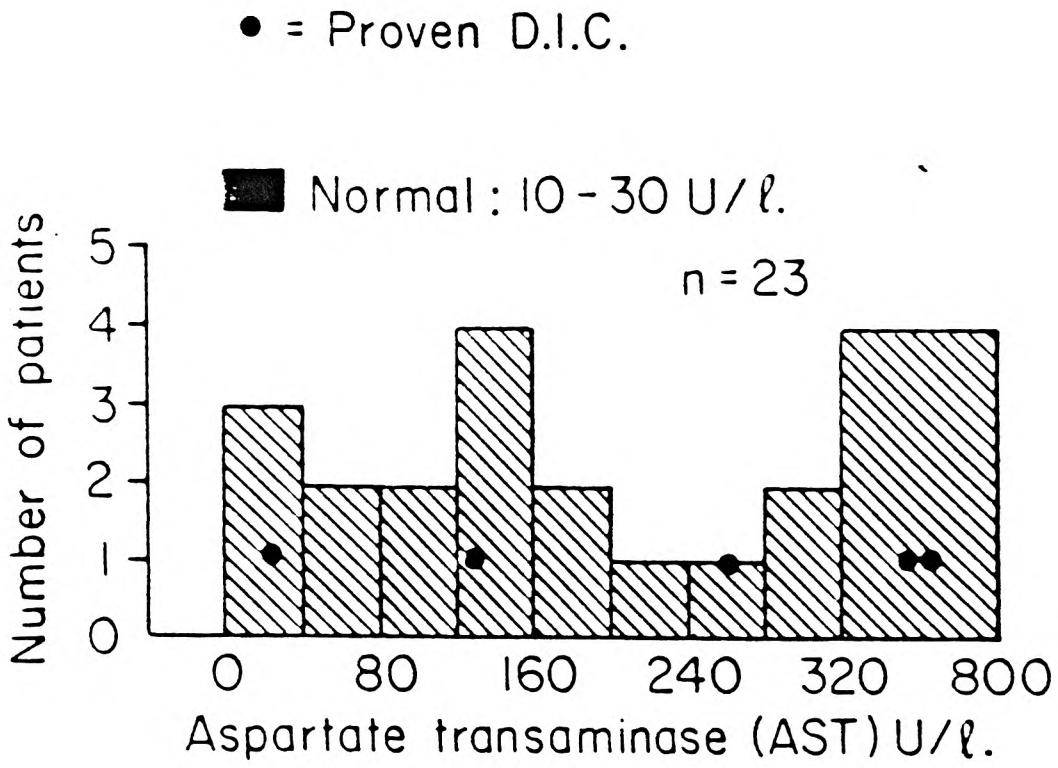


Figure 3.15 Hepatic enzymes in Typhoid Fever: (ii) Aspartate transaminase (AST). Results from admission blood samples.

GGT was recorded in seventeen patients and elevated in twelve (75%) - see Figure 3.16. Elevation of hepatic enzymes occurred with the same frequency in patients with and without laboratory evidence of a D.I.C. Nineteen of the twenty patients in whom LDH. levels were measured at the time of admission, had elevated values, $p < Ø,Ø5$ (Figure 3.17).

Although levels of factor VII, XII and fibrinogen were not significantly reduced, the levels of albumin and protein C were, $p < Ø,Ø5$ in each instant (Figures 3.18 and 3.19). Surprisingly, those patients with D.I.C. nevertheless appeared to have normal values of protein C. Griffen et al (1982) noted reduced levels of protein C in D.I.C. It is possible that longitudinal evaluation of protein C in these patients may have revealed a subsequent fall as these results reflect admission blood sampling. Unfortunately levels of Factor Va and protein C inhibitor were not measured though it seems doubtful that reduced levels of these inhibitors could be incriminated. The comparison of albumin and LDH reveals an inverse correlation $p < Ø,Ø5$; significant liver synthetic dysfunction is the most plausible explanation particularly in the context of reduced protein C levels and the elevation of hepatic enzymes (Figure 3.17). Results of the present study do not suggest that more severe hepatic dysfunction occurred in the small group with D.I.C.

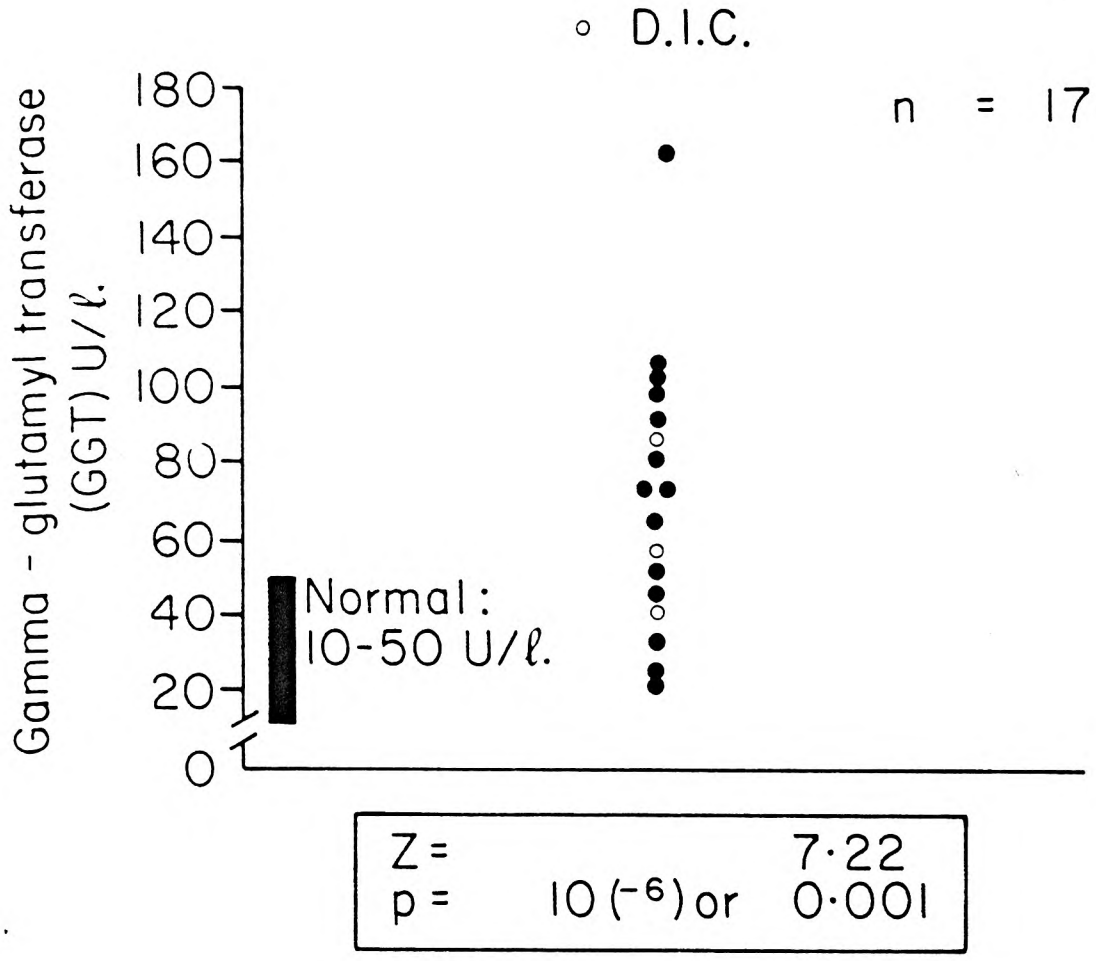


Figure 3.16 Hepatic enzymes in Typhoid Fever (iii) Gamma-glutamyl transferase (GGT). Results of admission blood samples.

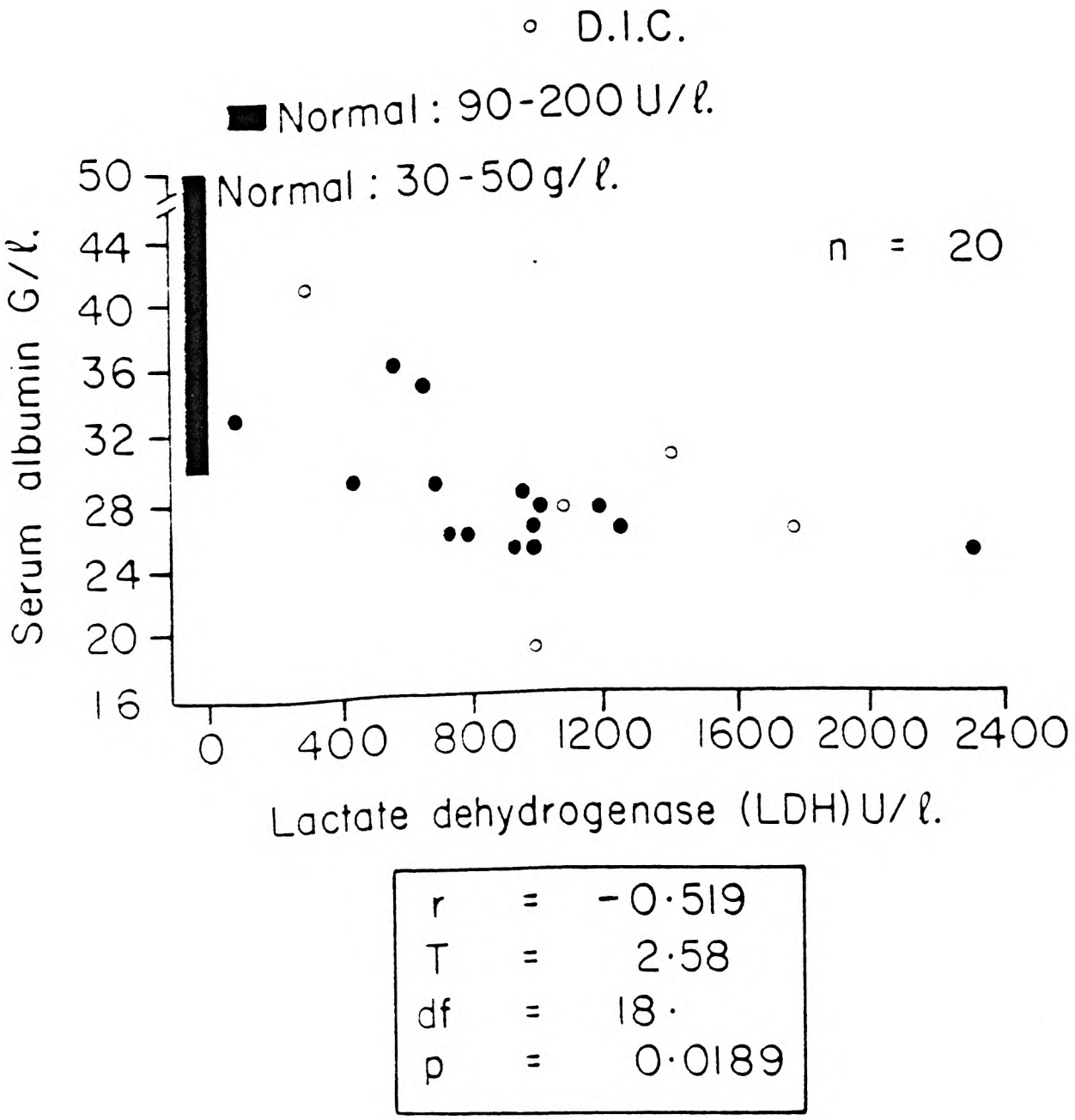


Figure 3.17 Comparison of serum albumin and lactate dehydrogenase levels in patients with Typhoid Fever. Open dots represent individual patients with a laboratory diagnosis of D.I.C.

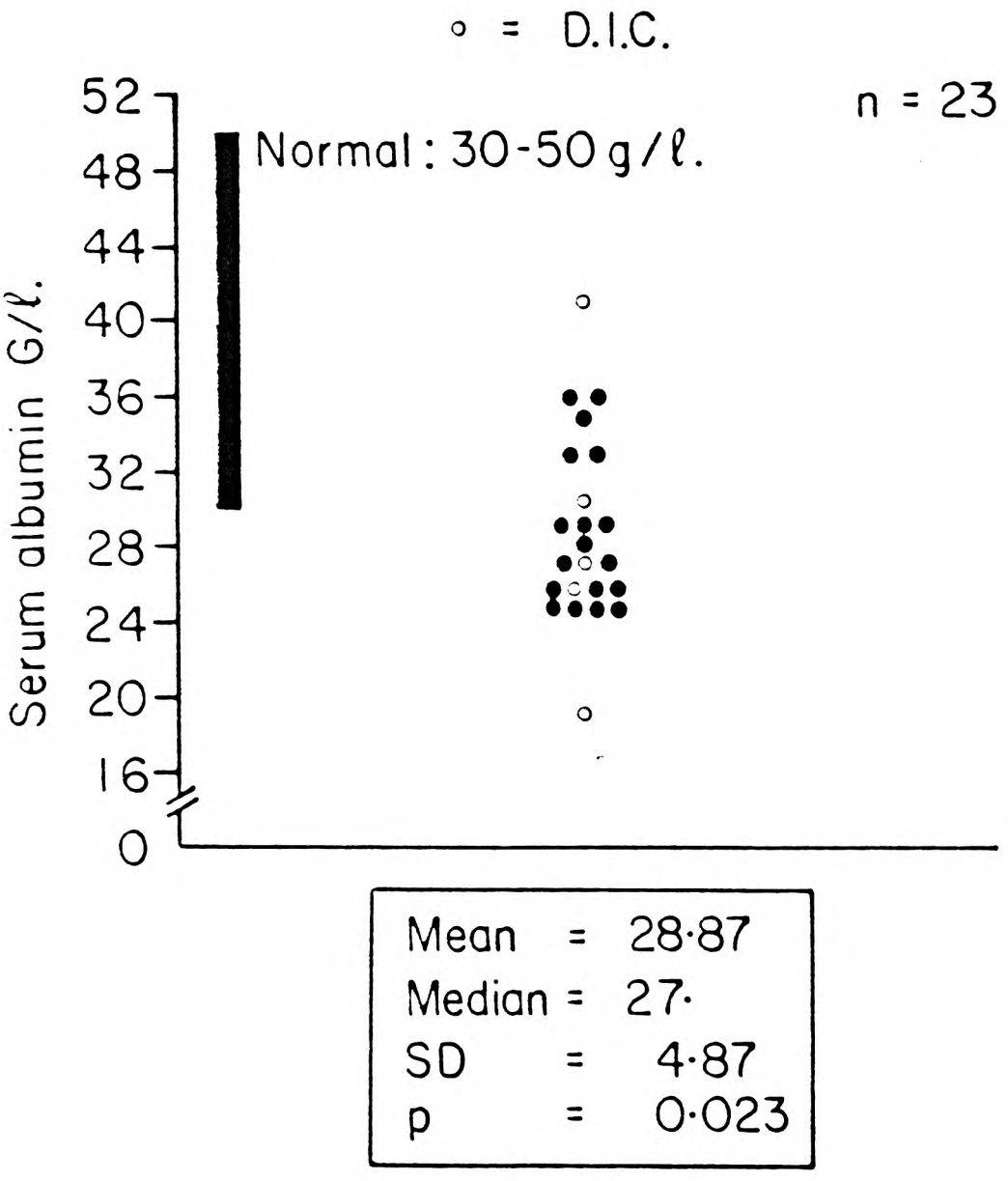


Figure 3.18 Serum albumin levels on admission in patients with Typhoid Fever.

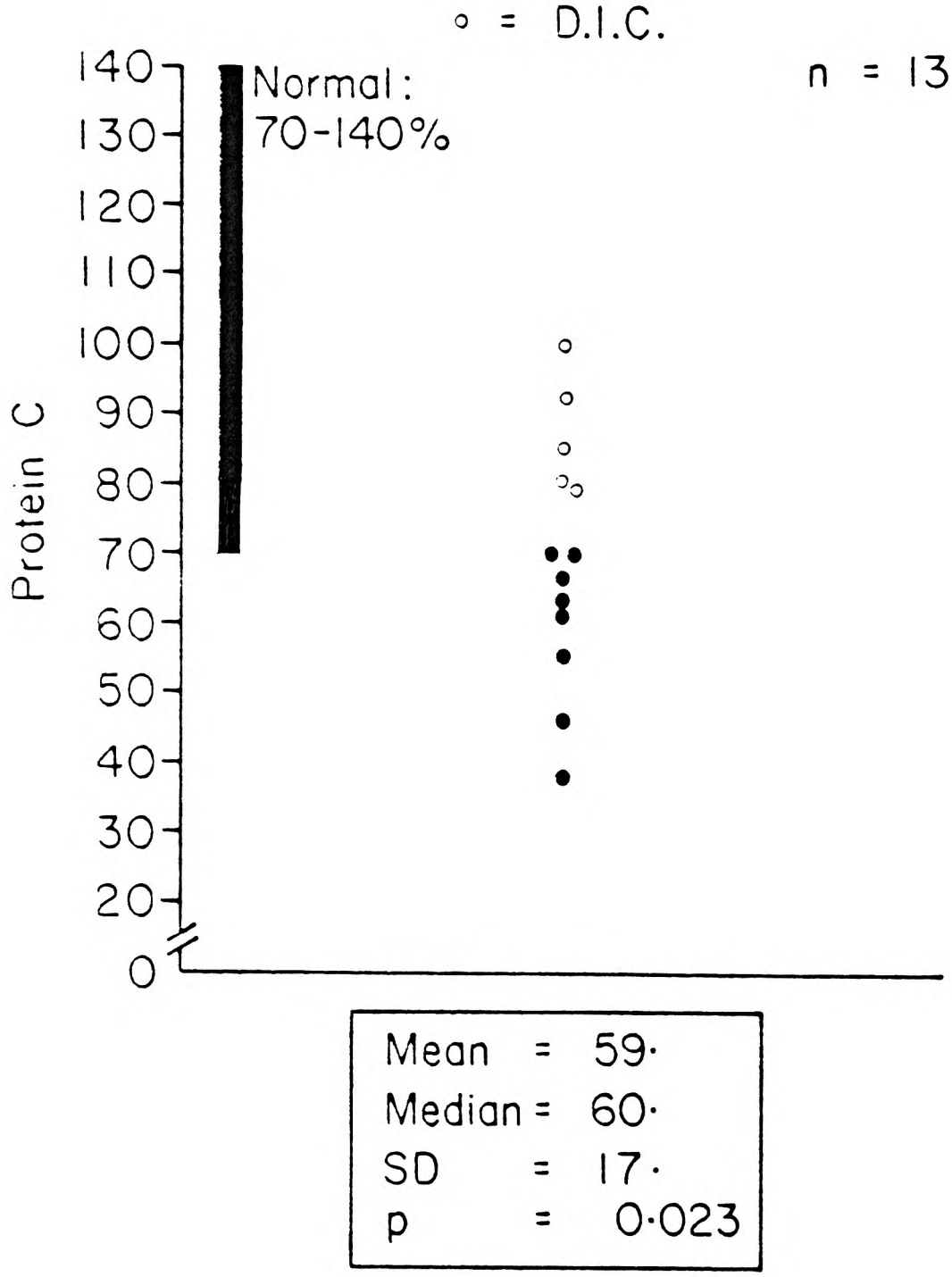


Figure 3.19 Protein C levels in patients with Typhoid Fever.

In addition, poor nutritional status and/or the sequestration of albumin through the capillaries and into the interstitium, may have contributed towards the hypoalbuminaemia.

In no patient was a significant elevation of total bilirubin or alkaline phosphatase recorded. And in no patient was jaundice noted during the course of the illness, nor was there clinical evidence of hepatic failure.

3.1.3.4 Metabolic abnormalities

Figures 3.2Ø and 3.21 record the levels of the serum electrolytes sodium and potassium. The finding of a hyponatraemia is statistically significant, $p < Ø,Ø5$. This possibly reflects the frequency of diarrhoea; twenty of the thirty-two patients (63%) volunteered a history of diarrhoea. Of the twelve patients reportedly confused or having neuro-psychiatric manifestations, eleven were hyponatraemic, of whom three had serum sodium levels below 12Ø mmol/l. While a number of individuals had markedly reduced potassium levels, the overall results were not significant, $p > Ø,1$. Diarrhoea and nutritional deficiency may have accounted for the hypokalaemia in certain individuals.

Renal dysfunction is unlikely to have contributed to a bleeding tendency in any of the patients in this study (Figure 3.22). A highly significant correlation exists between blood urea and creatinine, $p < Ø,Ø5$. This confirms a primary renal element in some patients with typhoid fever.

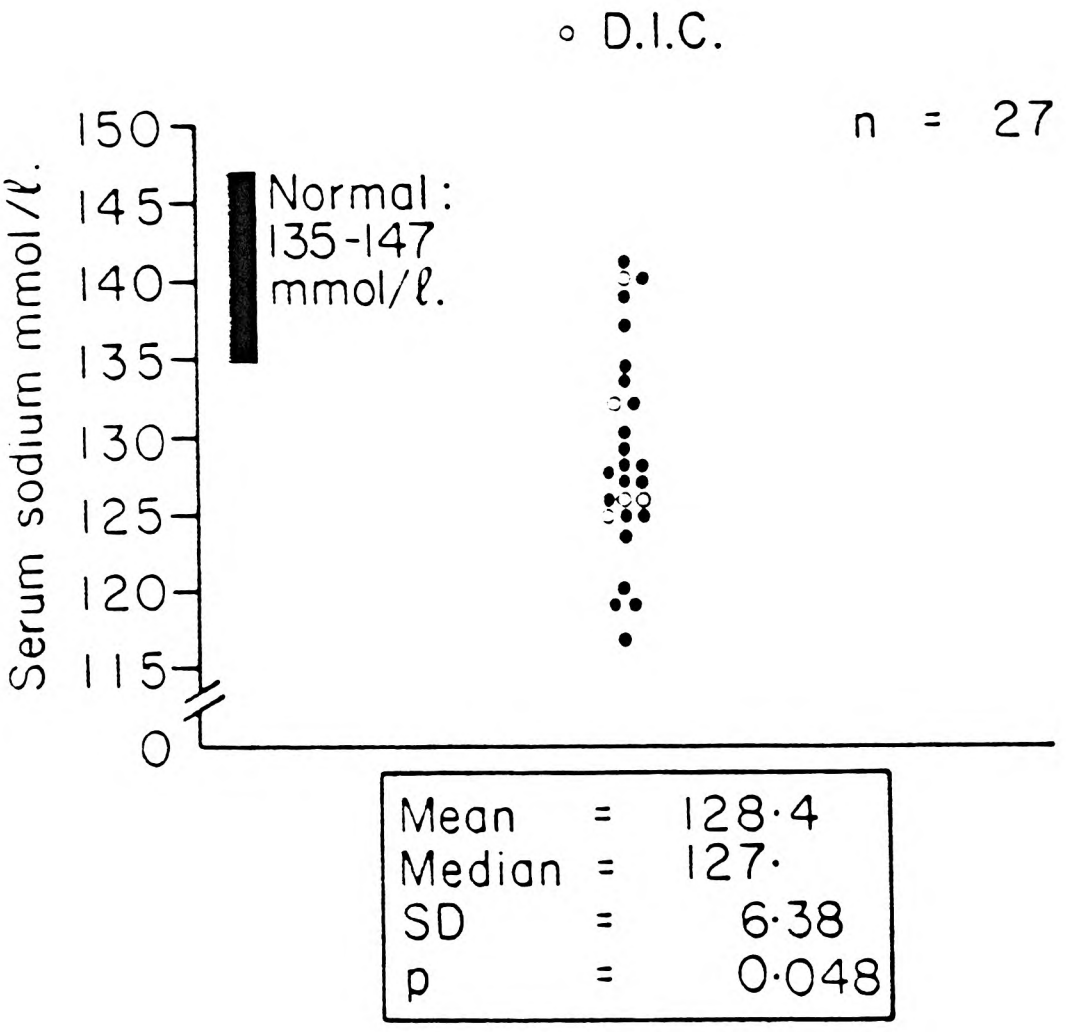


Figure 3.20 Serum sodium levels on acute admission in Typhoid Fever.

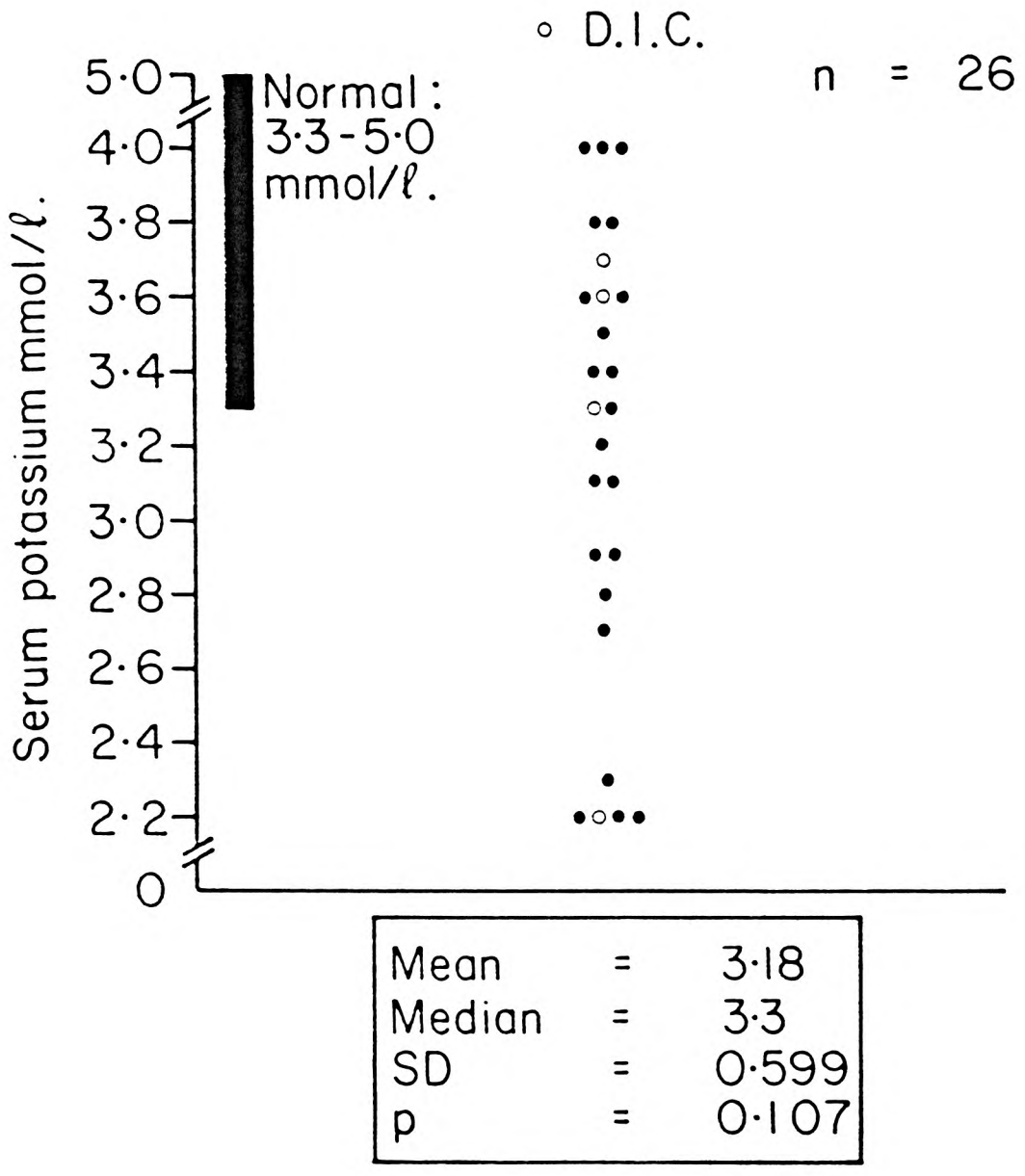


Figure 3.21 Serum potassium levels on acute admission in Typhoid Fever.

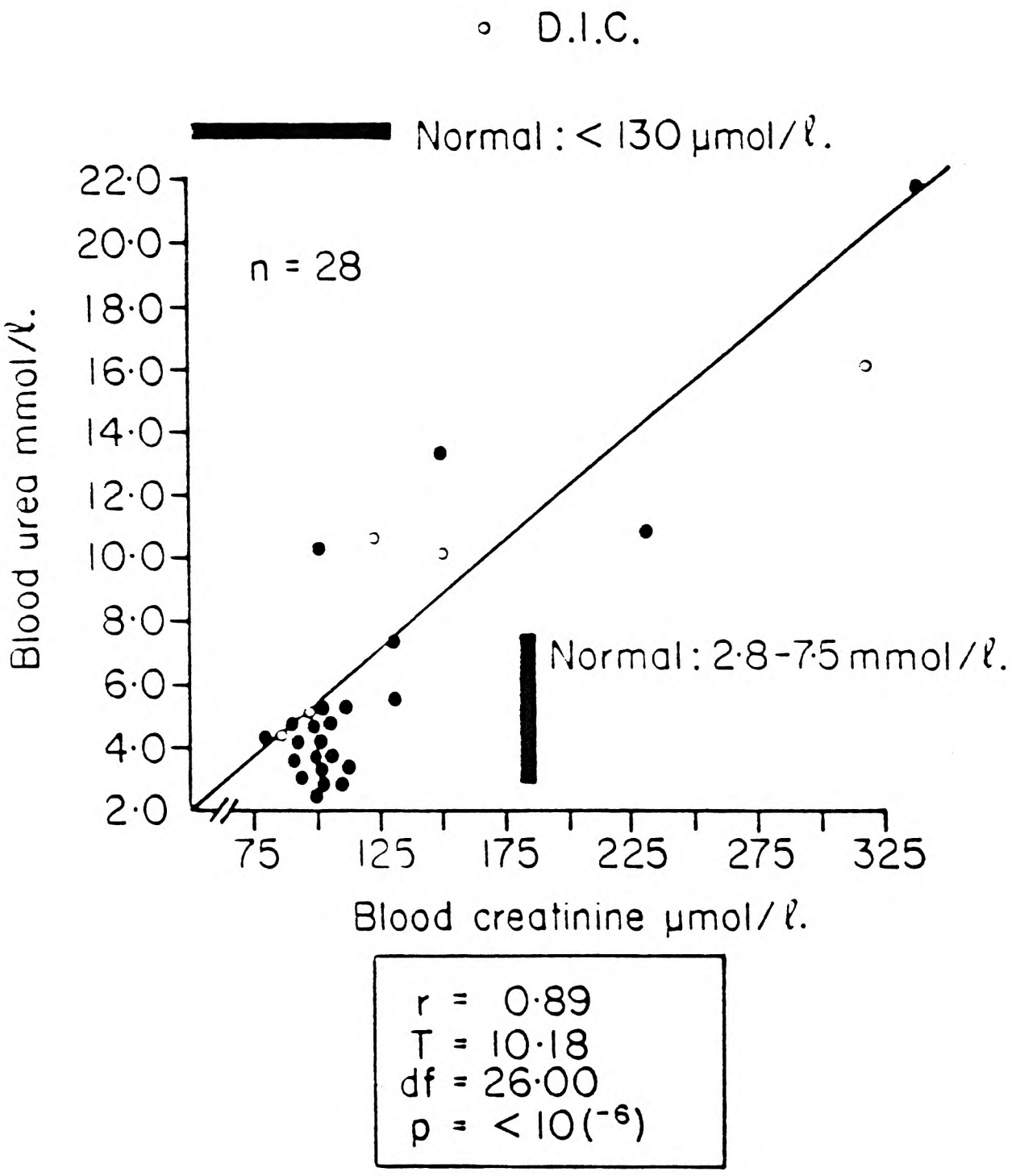


Figure 3.22 Comparison of blood urea and creatinine levels in Typhoid Fever.

3.1.3.5. Haematological abnormalities

Of the twenty-three patients in whom platelet counts were recorded, fourteen (61%) had a modest thrombocytopenia, viz. below 140 x 10^9/l (Figure 3.2). A comparison of the platelet count and the absolute neutrophil count gave no statistical correlation, $p > 0,1$ (Figure 3.23). Bone marrow failure as a major contributor to the thrombocytopenia seems therefore unlikely. Indeed, in a single thrombocytopenic patient included in this study and in whom bone marrow aspiration and trephine was performed, the marrow was hypercellular but otherwise normal.

Figures 3.24 and 3.25 confirm the general tendency of a leucopenia. However, there was no significant change in either absolute neutrophil or lymphocyte counts. Absolute monocyte counts were on the other hand significantly depressed, $p < 0,05$. Although the total numbers are small, those patients with D.I.C. appear to cluster within the lymphopenic and monocytopenic groups. Attention does not appear to have been focused on this finding in previous literature on typhoid.

An anaemia was almost universal in both male and female groups, $p < 0,05$ (Figures 3.26). Note that the normal values quoted in these figures refer to healthy white subjects at sea level. Mean corpuscular volume of the erythrocyte was in the low-normal to below normal range (Figure 3.27).

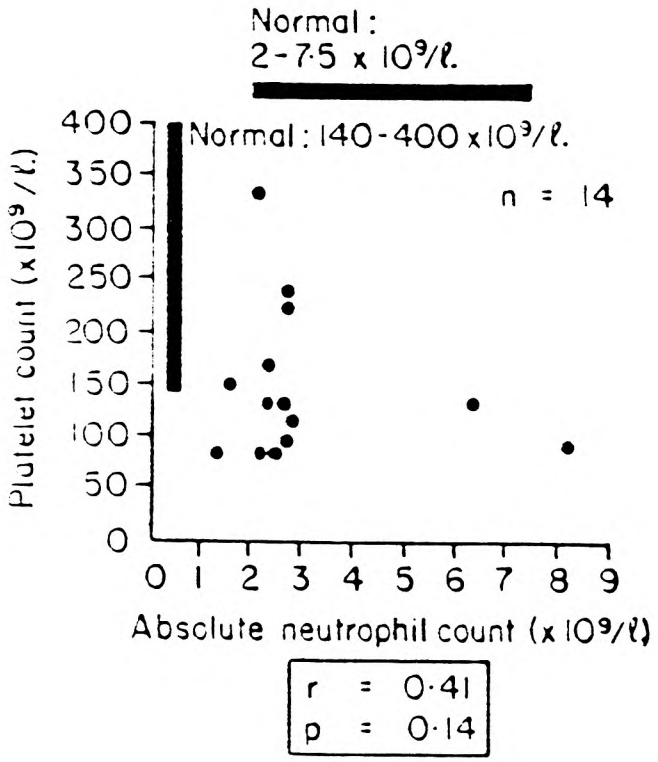


Figure 3.23 Platelet count and absolute neutrophil count in Typhoid Fever.

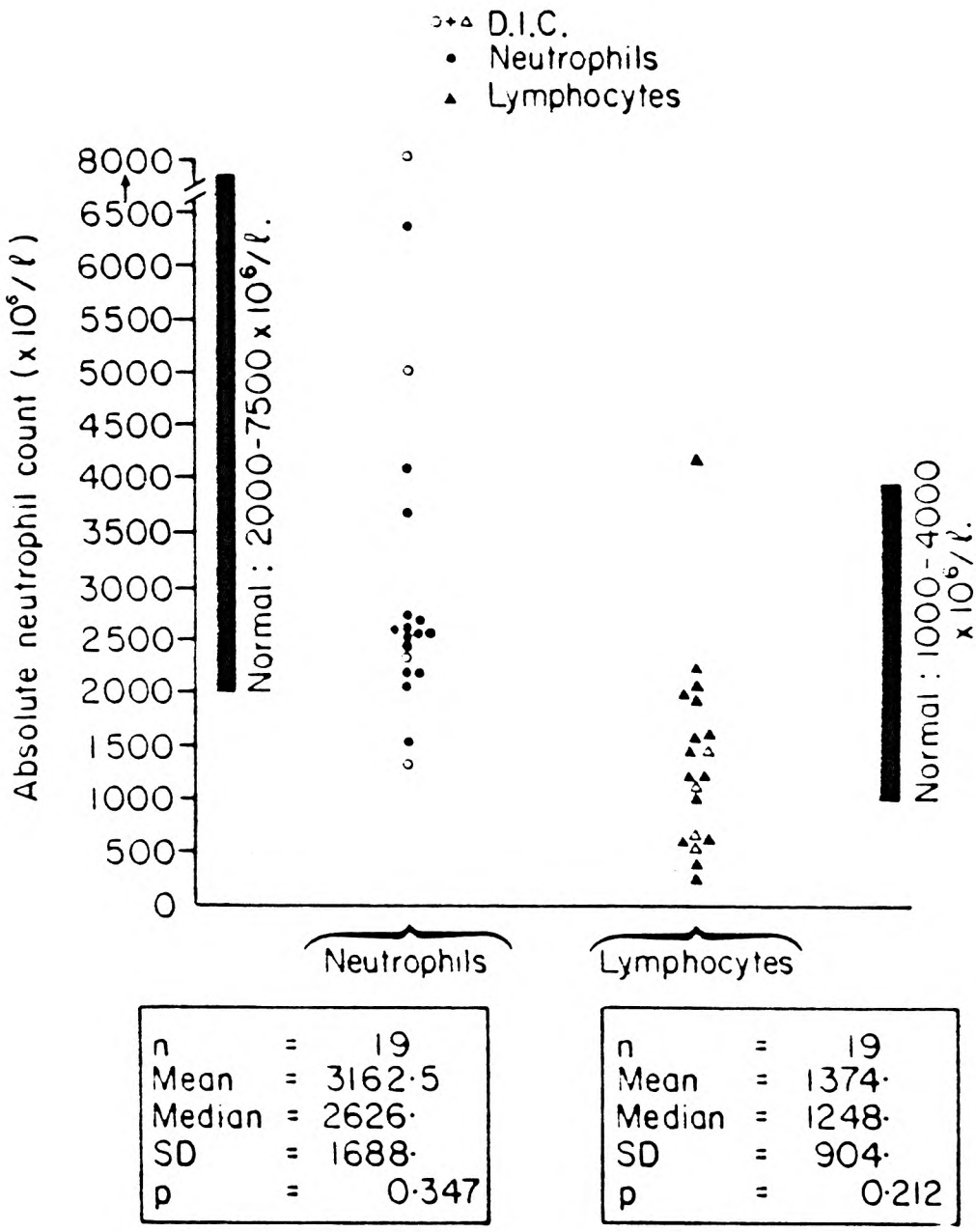


Figure 3.24 Leucocyte count on admission in patients with Typhoid Fever: Absolute neutrophil and lymphocyte counts.

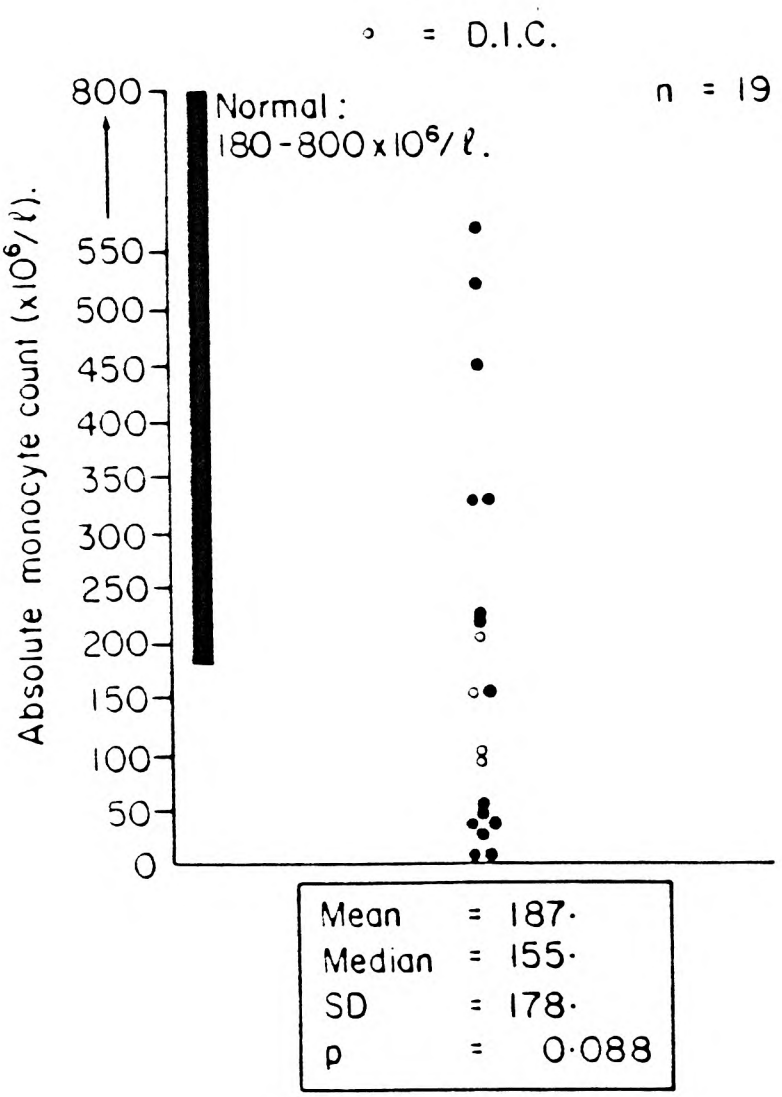


Figure 3.25 Absolute monocyte count in Typhoid Fever.

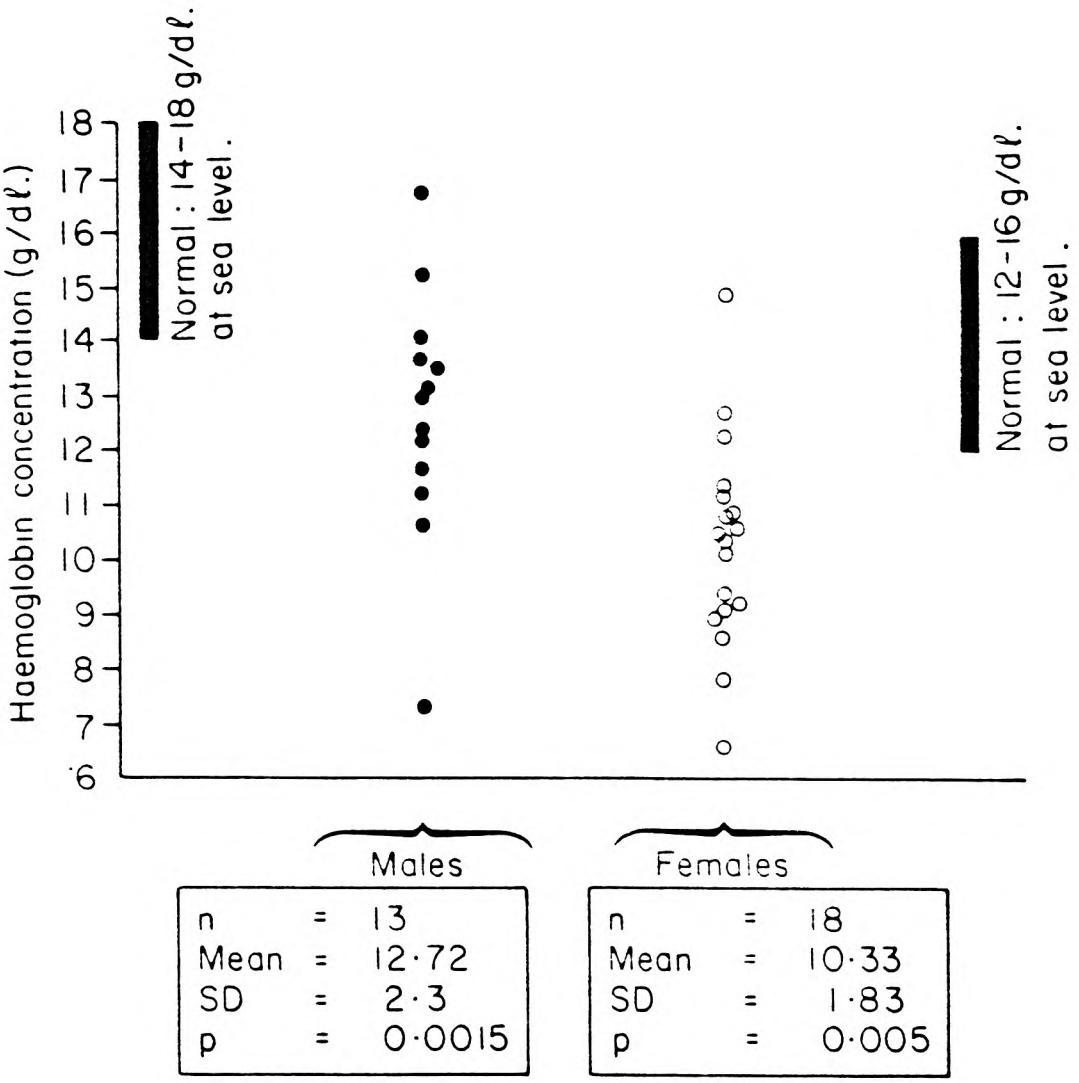


Figure 3.26 Haemoglobin concentration in males and females with Typhoid Fever.

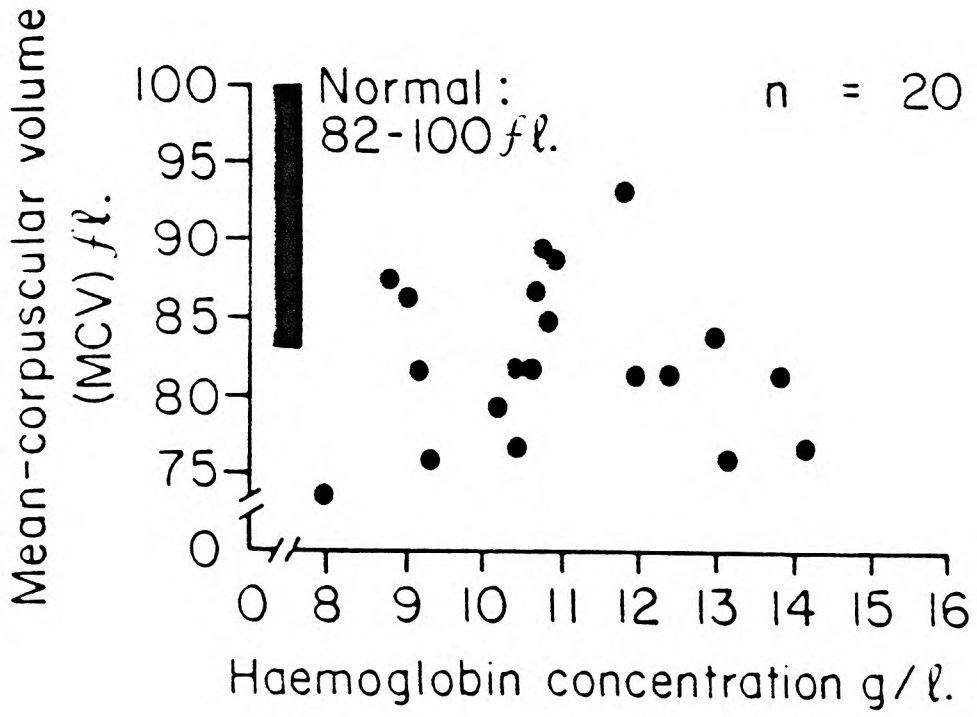


Figure 3.27 Comparison of the erythrocyte mean-corpuscular volume and haemoglobin concentration in Typhoid patients.

However the wide scatter of results suggests that a variety of factors possibly including iron deficiency, nutritional compromise and a degree of marrow failure would require to be evaluated. A haemolytic component to the anaemia has not been excluded, nevertheless bilirubin levels when recorded were normal. Elevated LDH levels can be explained without implicating haemolysis.

3.1.4 Discussion

Bleeding in typhoid fever takes two forms, either local haemorrhage from the gastrointestinal tract, or infrequently, generalized bleeding which may result from disturbed control of coagulation.

The former is usually trivial and presents as a self-limiting bloody diarrhoea often during the second week of illness. Ulceration of the mucosa overlying inflamed Peyer's patches with consequent erosion or penetration of vessels has been demonstrated. Occasionally the mucosa and submucosa, may slough. Subsequent to the antibiotic era, severe local bleeding requiring blood transfusions has been reported in fewer than 5% of cases (Huckstep, 1960; Foote & Hook, 1979). Persistent and fulminant haemorrhage remains exceedingly rare. Nevertheless, this complication is potentially lethal. Recent reviews have stressed the necessity to avoid delay should surgical intervention be contemplated (Wong, 1978; Wig et al, 1981). A quarter of the patients in this present study had bloody diarrhoea on admission, but in none was this sufficiently serious to

warrant any form of surgical management. The complication settled with appropriate medical therapy.

Louis in 1841 and Sir William Osler later in the nineteenth century, commented upon generalised bleeding in typhoid (recorded in Harvey & McKusick, 1967). In 1927, Hartmann reported a relationship between thrombocytopenia and the haemorrhagic manifestations of this disease. In their series of 400 cases, the French workers Destaing and Grangard (1965), noted an 11% incidence of haemorrhage and purpura in thrombocytopenic patients. However, the first report of D.I.C. and typhoid fever appeared comparatively recently (Allen et al, 1969). A number of confirmatory reports have subsequently appeared.

Colman's work on volunteers (1978), and the studies of Butler and co-workers (1978) suggest the relative frequency of a subclinical, laboratory confirmed, D.I.C. Five of the thirteen patients in this study viz. 38,5%, fulfilled the criteria of a D.I.C. and support the findings of the above workers. Two of these patients had features of a clinical D.I.C. In none was the condition life-threatening. Spontaneous improvement occurred with treatment of the underlying infective state.

Mediation of the coagulation disturbance via activation of Hageman factor has been implied following the reduction in serum levels of functional prekallikrein and the kallikrein inhibitor (Colman et al, 1978; Beard & Hickton, 1981). As confirmed in the present study, both the above

groups also noted no reduction in factor XII levels in their subjects. Colman has postulated that the concentration of active factor XII fragments may be quantitatively too low to be measured. It remains a possibility that prekallikrein activation may have occurred independently of Hageman factor activation. In the present study both patients with clinical evidence of a D.I.C. showed significantly reduced prekallikrein levels.

Hornick and Greisman (1978) have suggested that endotoxin may be the initiator of the pathological sequelae: Limulus testing for systemic endotoxin in typhoid patients has nontheless remained negative. These authors have therefore postulated a tissue-based 'priming' role for endotoxin. Endotoxin is active within inflamed tissues without significant systemic release, although capable nevertheless of generalised changes in the subject. Indeed, the injection of endotoxin into the skin of typhoid subjects has been demonstrated to elicit local lesions histologically indistinguishable from the classical 'rose-spots' of this disease. Endotoxin as discussed earlier, is capable of activating factor XII and promoting tissue factor release from endothelial cells.

Hoffman and co-workers (1984) have recently drawn attention to the favourable outcome in severely-ill typhoid patients of the use of high-dose dexamethasone. An endotoxin-mediated role for activation of macrophages and mononuclear cells is postulated. The release of

procoagulant enzymes, tissue factor and interleukin-1 from mononuclear cells has been discussed above. Hoffman has suggested a steroid-induced blocking effect upon the release of these "toxic macrophage products". A similar mechanism was discussed above in the section dealing with Dengue Haemorrhagic Fever and Halstead's "Immune Enhancement Theory" (page 49); immune complexes and IgG interaction with the mononuclear cell has been suggested as the activating factor in this syndrome.

A number of studies have demonstrated immune-complex deposition and the reduction of various complement components in typhoid (Faierman et al, 1972; Sitprija et al, 1974; Dan et al, 1982; Khajehdehi et al, 1984). It remains possible that several mechanisms may be operative, in which the activated macrophage may be implicated as the ultimate focus of the pathological sequelae.

Possibly supportive of this hypothesis, is the finding of monocytopenia in the subjects of the present study. The writer has been unable to find confirmatory reports of monocytopenia in the recent literature on typhoid fever. Sequestration and activation of monocytes within the reticuloendothelial system is possible. The persistent fever of typhoid may result from macrophage release of interleukin-1; the intracellular situation of the organism forms a protective barrier to the activity of antimicrobial agents. Utilization of more than one antibiotic with acknowledged effectiveness in typhoid fever has been noted

in several of the cases reported above. Antibiotic 'changes' were necessitated by the apparent unresponsiveness and persistence of fever in a number of patients.

Statistically significant elevations of alpha-1 antitrypsin and C1-inhibitor were recorded in this study. Colman (1978) excluded a kallikrein inhibitory role for the former and concluded that elevated levels conformed to an 'acute phase' reaction.

The patients in the present study were evaluated as regards hepatic, renal and bone marrow function in order to assess the contribution of these parameters to a coagulation disturbance:

Alterations in liver function as determined by increase in hepatic enzymes occurred in 87%. Parameters of liver synthetic function suggest a significant disturbance in many patients. Further, the normalization of biochemistry in the majority of patients at the time of discharge, suggest typhoid as the aetiological agent. Greig and Naidoo (1981) have described a patient in whom factors II, VII, IX and X were exceedingly low and in whom spontaneous bleeding occurred. This young lady also had evidence of both a hepatitis and D.I.C.

The present study indicates that 'typhoid hepatitis' is a frequent complication. Furthermore, impaired hepatic synthesis of coagulation factors may at times contribute towards a coagulation disturbance, and may accompany a sub-clinical D.I.C.

Typhoid hepatitis has received increasing attention in recent reports (Faierman et al, 1972; Ayhan et al, 1973; Ramachandran et al, 1974; Pais, 1984). The hepatitis seldom presents with hyperbilirubinaemia and jaundice. Histologically, the liver may show the presence of typhoid nodules: these are areas of focal hepatocyte necrosis with mononuclear cell infiltrates. Sinusoids are dilated and congested, and the portal tracts are infiltrated with mononuclear cells. Electron and light microscopy of liver tissue has failed to show the presence of bacteria in damaged cells (Dan et al, 1982). The pathogenesis of the hepatitis is unknown although endotoxin and immune mechanisms have been suggested.

This study records the highest levels of LDH in those patients most severely ill. This enzyme may be released from a large number of inflamed tissues in addition to the liver, and may therefore reflect the widespread toxic effects of typhoid. While low albumin levels probably reflect inadequate synthesis, other mechanisms may operate. Khajehdehi and co-workers (1984) report significant proteinuria in Iranian patients with typhoid, many of whom had concomitant glucose-6-phosphate dehydrogenase (G6PD) deficiency. Nutritional protein deficiency and protein leakage may have contributed to the hypoalbuminaemia in some of our patients. Case 4 discussed above, had a documented nephrotic syndrome. A review of the cases in this study suggests that a depressed level of albumin together with a

five-fold or more increase of LDH tends to correlate with the severity of disease. Four of the five patients with D.I.C. fulfilled these criteria of morbidity: The fifth patient who had marginally deranged levels on admission, showed significant deterioration in albumin and LDH levels later in her first hospital week thereby confirming the impression of a relationship between these parameters and morbidity in typhoid fever.

Renal dysfunction was noted in a small number of patients but did not correlate with haemostatic abnormalities. Low serum electrolytes were commonly found: These results probably reflect gastrointestinal losses. Several patients reported to be confused at the time of admission were also noted to be hyponatraemic. Adams (1983) comments that the neuropsychiatric manifestations of typhoid are of unknown aetiology, although thrombosis of small cerebral vessels has been described. As these features are rarely persistent, it would appear more likely that confusion and neuropsychiatric signs relate in the majority to metabolic changes and the hectic fever frequently exhibited on admission. Mandal (1979) has commented upon the frequency and dominance of neuropsychiatric features in patients seen in India and Africa. Following the description of a perivenous demyelinating leucoencephalitis in a fatal case, Mandal has suggested that immunological mechanisms may play a role. Very occasionally this syndrome may persist for several weeks and may be accompanied by

electroencephalographic changes.

While thrombocytopenia occurred commonly, this was too frequent to be explained on the basis of a D.I.C. alone and was quantitatively too mild to cause bleeding. Anaemia and leucopenia were likewise not unusual. Primary bone marrow failure has not been excluded in this study. Nontheless this would seem unlikely in view of the lack of a correlation in the low neutrophil and platelet counts. Allen et al (1969) noted a hypercellular marrow with normal megakaryocytes and increased erythroid elements in their patient with D.I.C. A similar active marrow was noted in one of the patients in this study. Foote and Hook (1979) have however reported bone marrow failure as a possible consequence of the toxaemia of the illness or the use of antimicrobials particularly chloramphenicol. Patients in the present study were evaluated prior to the commencement of antibiotic therapy thereby excluding a drug-related effect upon the marrow.

Foote and Hook (1979) remark on the presence of a leucocytosis during the first week of the illness followed by a leucopenia, predominantly a neutropenia, in the subsequent second and third week of untreated disease. The majority of admission bloods in this present series revealed a generally normal range of absolute neutrophil counts. Nevertheless neutrophil and lymphocyte counts tended to aggregate within the lower ranges, with monocyte values being significantly low. The possible pathogenetic role of

the mononuclear cell in typhoid fever has already been alluded to and reviewed previously in a number of reports (Hornick et al, 1970a,b; Hornick & Greisman, 1978; Butler et al, 1978). Immunological mechanisms may operate in the thrombocytopenia and in bone marrow failure, though this latter complication must be extremely rare. Immune complex deposition has been described in hepatic (bile duct) and renal sites. Peripheral destruction and possibly splenic sequestration remain as further explanations of the low platelet count.

3.1.5 Conclusions

This study confirms the finding of Coleman et al (1978) and Butler et al (1978), of a relatively frequent subclinical D.I.C. in typhoid fever. However the aetiology of the coagulopathy is diverse. It would appear that the contribution of hepatic dysfunction may have hitherto been overlooked. Prolonged prothrombin ratios and partial thromboplastin times, together with marked derangement in hepatic enzyme levels and depressed albumin and protein C values, suggest significant liver dysfunction. Levels of antithrombin III and various protease inhibitors were unfortunately not assessed in this study. The possible role of hepatic debris as pro-coagulant stimuli has been addressed in the text of this thesis, but remains speculative in the present context. It appears highly probable that the haemostatic disturbances noted in typhoid fever are at least a combination of factors promoting

intravascular coagulation and factors reflecting hepatic dysfunction.

Furthermore, it would seem unlikely that metabolic alterations such as renal dysfunction or the thrombocytopenia per se, contribute significantly to the coagulation disturbance. While thrombocytopenia is indeed common, absolute platelet counts were generally only modestly depressed. In the thirteen patients in whom coagulation tests could be performed, bleeding times were never extremely prolonged. Peripheral sequestration of leucocytes and thrombocytes may have contributed to the low absolute counts recorded in this study.

The pathogenesis of the D.I.C. in typhoid remains uncertain. While endotoxin has frequently been suggested, a systemic role has proved difficult to sustain (Butler et al, 1978). At the same time the local, tissue priming role of endotoxin as suggested by Hornick and Greisman (197Ø a,b), has still to be confirmed. Endotoxaemia has however, been implicated in fatal coagulopathies associated with non-typhoid, salmonella infections (Glover et al, 1982). Direct invasion of vascular endothelium in typhoid does not appear to occur although this event is described in infection with other salmonellae (Cohen et al, 1978). Hornick and Greisman (197Ø b) noted 'hyper-reactivity' of small vessels to local catecholamine infusions in subjects with typhoid fever: Intradermal injection of catecholamines produced fibrinoid arteriolar necrosis in some subjects. These authors

suggested that such vascular 'hyper-reactivity' might be incriminated in some of the focal manifestations of disease.

In view of the description of immune complex deposition and utilisation of complement in this condition, it remains feasible that activation of the coagulation cascade may occasionally follow. As yet however this has not been confirmed. An immunologically mediated thrombocytopenia also remains to be confirmed.

The generally low mononuclear cell counts recorded in this study may support the suggestion of Hoffman et al (1984) that the tissue-sequestered mononuclear cell e.g. macrophage, participates in the pathogenesis of the disease through the release of monokines and various activated substances. The role of the leucocyte in the promotion of coagulation has already been noted above (Harlan, 1985), as has the similar effect of interleukin-1 in laboratory studies (Berilacqua et al, 1984). Nevertheless a definitive role for the mononuclear cell in the coagulation disturbance of typhoid fever is still unproven.

Fortunately the haemostatic disturbances of typhoid would appear to be seldom life-threatening. In those patients with such complications, all in this study recovered without long-term sequelae and without requiring specific therapy directed towards the coagulopathy. Following the report of Hoffman (above), Hook (1984) has endorsed the use of steroids in severely ill patients and has drawn attention to earlier reports of steroid-related

responses. Supportive and antimicrobial treatment clearly form the mainstay of therapy. At times the protean clinical manifestations of typhoid make the evaluation of severity extremely difficult. The present study suggests that significantly deranged levels of albumin and lactate dehydrogenase may aid in the clinical assessment of patients with more severe disease. Where available, these biochemical parameters may be helpful indices of toxicity particularly where steroid therapy is contemplated.

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