BLOOD VISCOSITY IN HEART DISEASE AND CANCER

Editors:
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BLOOD VISCOSITY IN HEART DISEASE AND CANCER

Based in part on the proceedings of a conference held under the auspices of The University of Sydney in the Stephen Roberts Lecture Theatre, The University of Sydney, Sydney, Australia, 29th May 1978 and updated through April 1981

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PERGAMON PRESS

OXFORD · NEW YORK · TORONTO · SYDNEY · PARIS · FRANKFURT

U.K.	Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England
U.S.A.	Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.
CANADA	Pergamon Press Canada Ltd., Suite 104, 150 Consumers Rd., Willowdale, Ontario M2J 1P9, Canada
AUSTRALIA	Pergamon Press (Aust.) Pty. Ltd., P.O. Box 544, Potts Point, N.S.W. 2011, Australia
FRANCE	Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France
FEDERAL REPUBLIC OF GERMANY	Pergamon Press GmbH, 6242 Kronberg-Taunus, Hammerweg 6, Federal Republic of Germany

First edition 1981

Library of Congress Cataloging in Publication Data

Main entry under title:
Blood viscosity in heart disease and cancer.
"Based in part on the proceedings of a conference held under the auspices of The University of Sydney in the Stephen Roberts Lecture Theatre, The University of Sydney, Sydney, Australia, 29th May 1978 and updated through April 1981."
1. Blood—Diseases—Congresses. 2. Blood—Vicosity— Congresses. 3. Heart—Diseases—Congresses. 4. Cancer—Congresses.
I. Dintenfass, Leopold, 1921 II. Seaman, Geoffrey V. F. III. University of Sydney.
[DNLM: 1. Blood viscosity—Congresses. 2. Heart diseases—Blood—Congresses. 3. Neoplasms—Blood—congresses. WG 106 B653 1981]
RC633.B556 616'.1181 81-15369

British Library Cataloguing in Publication Data

Blood viscosity in heart disease and cancer.
1. Blood flow
2. Rheology (Biology)
I. Dintenfass, Leopold
II. Seaman, Geoffrey V.F.
612'.1181 QP105

ISBN 0-08-024954-X

In order to make this volume available as economically and as rapidly as possible the authors' typescripts have been reproduced in their original forms. This method unfortunately has its typographical limitations but it is hoped that they in no way distract the reader.

Printed in Great Britain by A. Wheaton & Co. Ltd., Exeter

Dedicated to

Professor Sir John Loewenthal, KCMG

whose enthusiastic and wholehearted support of haemorheological research for many years has earned our gratitude

PREFACE

The purpose of this book is to provide an update on the status of blood rheology (haemorheology) in diagnostic, prognostic and preventive medicine especially as applied to the problems of cancer, heart disease and thromboembolism. It can be seen from the material included in this book that rheological tests on blood or its components are becoming of increasing importance in patient care. It is to be hoped that hospital laboratories will be persuaded eventually to include the determination of blood viscosity factors as a part of their routine screening procedures.

One of us (L.D.) is the author of two books on haemorheology; one of these, Rheology of Blood in Diagnostic and Preventive Medicine - an Introduction to Clinical Haemorheology, covers previous work on clinical haemorheology; the other, Blood Microrheology, Viscosity Factors in Blood Flow, Ischaemia and Thrombosis treats the more basic aspects of the subject.†

Since the pioneering work of one of us (L.D.), which began in 1961, the microrheology of blood, that is, the effect of blood components on various aspects of its viscosity has progressed to the point where it is now evident that blood viscosity factors are of great importance in clinical medicine in such areas as our understanding of the flow mechanics of ischaemia or shock and the realization of proper therapy and appropriate preventive measures.

The aim of the conference, the proceedings of which form the original basis of this book, was to bring together those in Australia interested in haemorheology in order to describe and review the state of the art of blood rheology. The conference was opened by the late Professor Sir John Loewenthal, President of the National Heart Foundation of Australia and Chairman of the Department of Surgery of The University of Sydney (and formerly Chairman of the National Health and Medical Research Council of Australia), who had long recognized the importance of the field and supported haemorheological research vigorously. Although this book is based in part on the original conference in Sydney, the papers have been updated through April 1981 and some recent additional papers have been included. We express our thanks to all the contributors and session chairmen.

The importance of blood rheology in clinical medicine is now firmly established, but this does not prevent research from continuously opening up new vistas for study, thus providing a focus for further studies for decades to come.

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†Both books may be obtained from The Interfacial Dynamics Corporation, Books and Publications Division, P.O. Box 40306, Portland, Oregon 97240, USA.

ACKNOWLEDGEMENTS

A large part of this book is based upon the proceedings of a conference on blood viscosity in heart disease, thromboembolism and cancer held under the auspices of the University of Sydney in the Stephen Roberts Lecture Theatre, the University of Sydney, Sydney 2006, Australia, 29th May 1978. We are indebted to Boehringer Ingelheim, Pty Ltd., Artarmon, N.S.W.; Upjohn Pty Ltd., Rydalmere, N.S.W.; Astra Chemicals Pty Ltd., North Ryde, N.S.W.; Wellcome Australasia, Concord, N.S.W.; and Imfra Electron Tube Products, St. Leonards, N.S.W. for support of the conference. We are also greatly indebted to the following distinguished members of the Australian academic community who acted as session chairmen: Professor C.R.B. Blackburn, Head of the Department of Medicine, University of Sydney; Professor G.W. Milton, Professor of Surgery, Head of the University Department of Surgery at the Sydney Hospital and Head of the Melanoma Clinic; Professor G.D. Tracy, Professor of Surgery, University of New South Wales, and Head of the University Department of Surgery at St. Vincent's Hospital; and Professor R.B. Walsh, Professor of Human Genetics and Dean of the Faculty of Medicine, University of New South Wales.

Thanks are due to Ms Janet Cowan and Ms Pamela Ewing for their careful preparation of the camera-ready typescript. A special acknowledgement is given to Ms Ewing for editorial assistance. Much of the hard work involved in the lay-out of the book fell upon her shoulders.

INTRODUCTION

Professor Sir John Loewenthal[†]

Department of Surgery, University of Sydney, Sydney 2006, Australia

It is a great pleasure and an honour to be invited to open the first conference on the role of blood viscosity in heart disease, thromboembolism and cancer. We in Sydney are delighted that the conference is being held here. The fact that we are so favoured is due in large measure to the tremendous drive and enthusiasm of Dr. Leopold Dintenfass. I need scarcely tell this audience of the interest and scientific dedication that he has put into the subject and the organization of this conference. At the same time I would like particularly to welcome Professor G.V.F. Seaman who is a distinguished authority in this field. We wish to make our guest feel that his time and effort in coming this very long distance is much appreciated and that he is very welcome.

In the past the field of rheology has largely, of necessity, confined itself to the physical sciences. Its application to biology and particularly to clinical medicine has always been difficult and complex. However it is in this latter field that its most important contributions may yet develop. Those of us involved in vascular surgery, which itself is largely predicated by a need to relieve obstructed arteries, are increasingly aware of the significance of the physical factors concerned, especially in vessels which are already predisposed to intravascular obstruction. Haemorheology, whilst modern in its development, may possibly be central to further scientific investigation in a field which is full of queries and as yet unproven hypotheses.

May I wish you fruitful discussions which will lead to exciting and attractive newer knowledge in your chosen field of study. At the same time, may I once again bid you welcome to The University of Sydney in particular and to the city of Sydney in general.

[†]Professor Sir John Loewenthal, K.C.M.G., M.S.Melb., F.R.C.S., F.R.A.C.S. died of a heart attack on August 25, 1979, just two days after preparation of this introduction.

CONTENTS

ROLE OF BLOOD RHEOLOGY IN MEDICINE: CLINICAL HEMORHEOLOGY

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ABSTRACT The role of clinical hemorheology in medicine is reviewed. Clinical hemorheology programs evaluate rheological parameters as diagnostic indices for diseases related to or produced by altered blood flow. A requirement for a successful program is a close working relationship between basic research scientists and practicing physicians in a clinical setting where the specialized equipment for the measurement of blood viscosity factors is available. Clinical hemorheology is being applied in depth to four areas where significant findings have already been obtained, namely a) examination of patients with sickle cell disease, b) study of patients with markedly elevated plasma viscosities, c) screening of patients with vascular disease, and d) evaluation of rheological changes in diseases such as diabetes mellitus. Blood viscosity related factors normally examined include i) viscosity of anticoagulated blood, washed red blood cells and plasma as a function of rate of shear, ii) deformability of the red blood cells, iii) internal viscosity of the erythrocytes, iv) quantitative measure of red cell and platelet aggregation under specific conditions, v) hematocrit and mean cell hemoglobin concentration, vi) erythrocyte sedimentation rate, vii) plasma protein levels, and viii) concentrations of plasma fibrinogen, fibrin monomer and fibrinogen breakdown products. Precise knowledge of blood viscosity related factors should ultimately enable the design of improved diagnostic tests and therapeutic manipulations to produce selective modification of deviant rheological parameters.

INTRODUCTION

Blood rheology or hemorheology, the latter term first coined by Copley (1), is concerned with studies on the flow properties of blood and its constituents and with the interactions between blood and the vessels in which it is contained. There is an increasing awareness of the role of blood rheology or blood viscosity factors in medicine. This has led to a transition from basic hemorheological studies conducted in vitro to investigations of the flow properties of blood in vivo in health and disease. Thus the field of clinical hemorheology has been born.

The foundations for clinical hemorheology have been laid over the past several decades. Notable reviews on blood rheology include contributions by Merrill (2), Whitmore (3), Dintenfass (4), Schmid-Schönbein and Wells (5), Cokelet (6), Chien (7), and Cokelet, Meiselman and Brooks (8). Major contributors to the field of clinical hemorheology are Chien (9,10), Dintenfass (11) and Dormandy and coworkers (12,13). The critical hemorheological parameters which give rise to significant

and detrimental clinical manifestations in specific diseases have been established in some instances, for example sickle cell disease (9) and macroglobulinemia (14). Application of hemorheology to clinical studies results in a fuller understanding of the rheological changes which occur in disease states, surgical trauma or shock (11). Dintenfass (11) has introduced the term blood viscosity factors to describe those variables which control the flow properties of blood in the organism. These blood viscosity related factors include the apparent viscosity of whole blood at a given rate of shear, the deformability of the erythrocytes, the internal viscosity of the red blood cells, the hematocrit, the viscosity of the blood plasma and serum, the extent of reversible and irreversible aggregation of red blood cells in a given shear regime and the geometry and rheological properties of the blood vessels. These variables are in turn influenced by other properties of the system such as the mean cellular hemoglobin levels in the red blood cells or the levels of the various plasma proteins.

Rheological studies on blood had as their original objective the prediction of microscopic flow behavior of blood in any part of the circulatory system from the macroscopic rheological data. This particular approach has proved to be less satisfactory than had been hoped because of the continuous changes in composition of the blood which occur from one part of the circulation to another (15) and from one time to another (16). Subsequently emphasis has shifted towards the physicochemical properties of the constituents of blood and the nature of the interactions between these constituents. This has resulted in an awareness of which variables are of primary importance in determining the flow properties of blood.

It has become apparent that the rheological properties of blood can be examined at three levels of organization.

i) Phenomenological hemorheology (macrohemorheology) which treats the system as a continuum which is characterized by experimental rheological parameters without consideration of structure at the cellular or molecular level. An example of this approach would be the measurement of a variable such as apparent whole blood viscosity.

ii) Microhemorheology (cellular hemorheology) which examines the properties of blood at the cellular level and considers the averaged properties of whole cells or plasma, and interactions between the various types of blood cell and blood cells and plasma. Examples of microhemorheology would be the measurement of the electrokinetic charge of cells or the deformability of individual cells. Microhemorheology analyzes phenomenological hemorheology at the cellular level.

iii) Molecular hemorheology which analyzes the molecular basis for properties observed at the cellular or phenomenological level. Examples of molecular hemorheology would include identification of the specific charge groups or antigenic sites involved in specific cellular aggregation such as agglutination induced by antigen-antibody interaction, or the role of conformation of immunoglobulins or fibrinogen as a determinant of apparent viscosity of solutions of these macromolecules.

Blood displays shear thinning with increasing rates of shear (17,18). If this decrease in apparent viscosity with increasing rate of shear is independent of time under the measurement conditions used, the system is said to exhibit pseudoplastic behavior and if the decreases are time dependent the system is described as being thixotropic. Usually in instrumentation used for viscometric measurements anticoagulated blood exhibits pseudoplastic behavior. The dependence of the apparent blood viscosity on rate of shear indicates that blood will behave differently in various parts of the vascular system since there is appreciable variation in the rate of shear from one part of the vascular system to another (see Table 1). Under

TABLE 1. Rates of shear of blood in various parts of the vasculature

Vessel	Radius (cm)	Flow Velocity (cm sec^{-1})	Shear Rate (sec^{-1})
Aorta	< 2	∿ 100	100-200
Large arteries	0.15	17	400-600
Capillaries	∿ 0.0004	∿ 0.04	500-1000
Vena cava	∿ 2	< 30	30-60
Large veins	0.1-0.3	2-4	50-200

conditions of near zero or stopped flow as may arise during stasis or vessel occlusion the blood will develop extensive three dimensional networks of aggregated red blood cells (11,19). The presence of the three dimensional network will confer some structural rigidity to the system such that the applied shear stress will have to exceed a certain minimum value before flow will recommence (20). The applied stress at which flow recommences is termed the yield value or yield stress, which represents the breakdown point of the continuous three dimensional structural network which had formed on cessation of flow.

The four major interrelated factors which regulate the rheological properties of blood are:

a) the composition of the plasma;
b) the concentration of suspended particles, especially red blood cells;
c) the deformability of the red blood cells;
d) the extent of cellular aggregation.

Changes in the apparent viscosity of blood under both normal and pathological situations involve more than one of these factors. Increases in the apparent viscosity of blood or red cell suspensions at a given rate of shear could result from increasing the volume concentration (hematocrit) of red cells, the asymmetry of the erythrocyte, its rigidity, or the degree of cellular aggregation. At various rates of shear the effects of one or another of these factors may predominate. For example, at very low rates of shear < 0.3 sec^{-1} the formation of asymmetric rouleaux or aggregates in whole blood is mainly responsible for the elevated viscosity (21,22). The forces needed to disaggregate erythrocytes have been estimated from rheological data to be comparable in magnitude to the stresses required to locally deform red cells (22,23). Shear induced disaggregation of cells normally takes place largely over the range 0.2 to 50 sec^{-1} and is essentially complete at a shear rate of 50 sec^{-1} (22). At intermediate rates of shear 1 to 40 sec^{-1}, aggregates are at various stages of breakdown and the shape and deformability of individual red blood cells assume more importance. Some aspects of the rheological contribution of deformbility may be examined by studies on suspensions of red blood cells which have been fixed by aldehydes (24,25). Thus during the past couple of decades the dependence of the flow properties of blood on parameters such as, volume concentration of red blood cells, plasma protein composition, deformability of cells, osmotic pressure, pH, status of cell membranes and so forth has been relatively well worked out.

It has long been suspected that the net negative charge carried by all of the blood cellular elements as well as the vessel walls is an important factor contributing to the suspension stability of blood. Electrophoretic mobility measurements have shown that under approximately physiological conditions red blood cells carry a net negative charge (26). Various surface groups contribute to the electrokinetic charge of cells; a widespread major contributor is sialic acid, most frequently as

the N-acetyl derivative (27). The role of the electrokinetic charge of blood cells as a factor in the flow properties of their suspensions was first investigated in depth by Seaman and Swank (18). It was recognized that alteration of the electrokinetic charge of red blood cells in suspension would have a marked effect over molecular dimensions on the intercellular interactions, which however was not reflected at the macroscopic level as a change in the rheological characteristics of the system at intermediate and high rates of shear. Electrophoretic mobility measurements and rheological studies conducted in parallel suggest that decreases in surface charge of cells brought about by enzymatic means will enhance cell aggregation (29). An increase in red cell aggregation and blood viscosity has also been found in a patient with reduced red cell surface charge (30). A biochemical approach to the control of blood viscosity is illustrated by the studies of Rosato et al. (31) who found that treatment of whole blood with neuraminidase produced a marked elevation in blood viscosity especially at low rates of shear. Unfortunately the experimental conditions used do not permit a comparison of the relative roles of desialylation of red blood cells and other blood cellular elements as opposed to desialylation of the plasma proteins.

Detailed studies of the interaction of neutral polymers such as the dextrans with red blood cells have shown that dextran adsorption depends linearly on the bulk polymer concentration and that as a result of this adsorption some expansion of the diffuse double layer associated with the cell surface bearing the fixed charge occurs (32-35). This double layer expansion brought about by exclusion of counterions because of the volume occupied by the adsorbed dextran produces an increase in the zeta potential of the erythrocytes. Intererythrocyte bridging by dextran molecules simultaneously adsorbed to adjacent red cell surfaces can occur leading to aggregation (35). The attractive force due to bridging is opposed by electrostatic repulsion with the repulsion increasing faster than the effective attraction with increasing dextran concentration. At a critical concentration of dextran which is an increasing function of molecular weight the red cells disaggregate (35). The sensitivity of the critical disaggregation concentration to ionic strength provides supporting evidence for the electrostatic repulsion mechanism (36). This dextran-red cell system, in which electrostatic effects regulate the aggregation-disaggregation behavior of cells both in static suspensions and under externally applied shear gradients, is probably the first example of such an electroviscous effect in hemorheology (37).

BLOOD RHEOLOGY IN DISEASE: CLINICAL HEMORHEOLOGY

As a consequence of some of the basic studies it is now appreciated that many diseases may involve changes in one or more rheological components. The rheological aspect of the disease may vary from one of prime importance as in the case of sickle cell disease (38) or hyperviscosity syndromes (39) to one of lesser significance as in the cases of hereditary spherocytosis (40) or polyagglutinability arising from a partial deficiency of sialic acid at the surface of the red cells (41). A study of the rheological behavior of red blood cells in the latter case has shown an increase in blood viscosity especially at low shear rates (30). Abnormal flow properties of blood in disease have been discussed by several prominent hemorheologists (7,11,42). Dintenfass (11) has emphasized the relevance of blood rheology to the study of disease and reports that patients with myocardial infarction and arterial thrombosis have apparent blood viscosities at low rates of shear which are markedly elevated above normal values.

Flow properties of blood in hematological disorders.

Hematological diseases involve abnormalities in the physicochemical properties of blood or one or more of its components. Usually the pertinent rheological variable is easily identifiable.

i) Changes in the volume concentration (hematocrit) of red blood cells.

In anemias a low value for the hematocrit is present as a result of reduction in the total red cell volume either from a decrease in the rate of erythropoiesis (hypoplastic anemia) and/or an increase in the rate of red cell destruction (hemolytic anemia). The low volume concentration of red blood cells will give rise to lowered values in the apparent blood viscosity. Conversely in polycythemia the increase in the apparent blood viscosity which has been reported at high rates of shear (4) is to be expected on the basis of the increased hematocrit. The increase in blood viscosity at low rates of shear is less than that expected, probably reflecting a decrease in red cell aggregation or rouleaux formation which is consistent with the lower than normal viscosity values reported for sera from polycythemic patients (7).

ii) Changes in red cell deformability.

Changes in deformability of the erythrocyte can arise from several sources including increase in the viscosity of the cellular contents, reduction in membrane deformability, change in cell shape, etc. In many hemoglobinopathies changes in the physicochemical properties of the hemoglobin often result in an increase in internal fluid viscosity with as a consequence hindrance of passage of such cells through the microcirculation. In sickle cell disease the limited solubility of the abnormal hemoglobin S results in gelation of the intracellular contents resulting in rigidified cells of abnormal shape frequently sickle in form. These cells impart a markedly increased apparent viscosity to whole blood in vitro (4,7). Heinz body disorders involve the denaturation of hemoglobin which also produces an increase in the internal viscosity of the red cells (4). In hereditary spherocytosis changes in composition of the red cell membrane (spherocyte) produce decreases in membrane flexibility as evidenced by hindered passage of the spherocytes through narrow channels (40).

iii) Changes in plasma or serum proteins.

Plasma cell dyscrasias are of relatively infrequent occurrence. The basic feature of these disorders is the uncontrolled and usually excessive proliferation of a single clone of immunoglobulin producing cells (39). Multiple myeloma is the most common form of plasma cell dyscrasia. The two most common varieties of myeloma, IgG and IgA result in elevated serum viscosities and a preferential rise in blood viscosity at low rates of shear (4). Waldenströms primary macroglobulinemia is a relatively infrequent neoplastic disorder of the lymphoreticular system accompanied by an increase usually marked in the serum IgM (macroglobulin) concentration. The excess of macroglobulins produces an increase in serum viscosity (39). Cryoglobulinemia describes a condition in which serum proteins undergo gelling or precipitation in the cold. The cryoproteins consist most frequently of mixtures of immunoglobulins and components of complement. Quite often cryoproteins are observed in sera from multiple myeloma and macroglobulinemia patients where usually the IgG or IgM proteins are implicated. The blood from patients with cryoproteins exhibits increased plasma and apparent whole blood viscosities at low temperature.

Plasma viscosity increases with decreasing temperature and the ratio of the viscosity of normal plasma to water is independent of temperature over the range 20 to 37°C with a normal value of about 1.7 (43). The viscosity of macromolecular solutions such as plasma or serum will depend upon the concentration of individual components and their molecular characteristics (size, shape, flexibility, solvation and electrical charge). The most decisive factor among the determinants of plasma viscosity is the shape of the protein molecules in solution (44). The effect of experimental conditions on the determination of plasma or serum viscosity is intimately connected with the question of the possible non-Newtonian behavior of

these solutions. Although plasma or serum from healthy (normal) individuals is accepted to be Newtonian (39,44) there is considerable evidence that pathological plasma with elevated apparent viscosities does in certain circumstances exhibit non-Newtonian and thixotropic properties (39,45). This also means that ideally the determination of the viscosities of plasma and serum should be made with viscometers capable of quantitating shear stress and rate of shear.

Flow properties of blood in cardiovascular disease.

A number of studies have demonstrated an increase of blood viscosity in patients with coronary heart disease (7). In blood samples from patients who have suffered an acute myocardial infarction intensified aggregation is seen. These aggregates are not dispersed until shear rates in excess of 200 sec^{-1} are reached. At lower rates of shear the aggregates are larger at any given rate of shear than in control blood samples (5). Ohshima (46) has confirmed the presence of an elevated apparent blood viscosity in patients who have had a recent myocardial infarction. The yield stress of the blood reached a maximum three days after the infarction. The increase in yield stress of the patient's blood paralleled the clinical symptoms and in three cases where a large increase in the yield stress occurred, the patients died within two weeks. In a study by Jan et al. (47) on a series of patients immediately post acute myocardial infarction it was found that the observed increase in blood viscosity over the first 2 to 3 days after infarction could be attributed to increases in hematocrit. Fibrinogen and α_2-globulin were elevated within the first 24 hours and continued to rise during the first week after infarction and then decreased gradually thereafter.

Various approaches have been used to offset the abnormalities produced in blood rheology by elevations in the levels of fibrinogen in the plasma. Ehrly and Lange (48) reduced the level of fibrinogen in the plasma by use of a streptokinase treatment regimen. The decrease in plasma fibrinogen concentration was accompanied by decreases in blood viscosity especially at low rates of shear as well as reductions in plasma viscosity, degree of red cell aggregation and erythrocyte sedimentation rate (ESR). Ehrly (49) has also used sodium oleate to decrease blood viscosity. Sodium oleate is able to produce disaggregation of erythrocytes under no flow conditions and therefore has clinical potential as an agent for ameliorating blood flow problems which arise during shock, hypotension or other low blood flow states such as thromboembolism. Dormandy et al. (12) have treated a series of patients who had intermittent claudication associated with peripheral arterial disease with daily doses of clofibrate for extended periods. Clofibrate produces a fall in plasma fibrinogen. Dormandy et al. (50) have also used the relationship between apparent whole blood viscosity measurements and fibrinogen levels to predict the probable outcome of reconstructive arterial surgery, i.e., graft patency. It is becoming increasingly evident that the apparent viscosity of blood measured under suitable conditions over a wide range of shear rates represents an important and at times a critical indicator of peripheral blood flow (12,51).

Abnormalities in the adhesiveness and aggregation properties of platelets have been shown to be of clinical relevance in many patients with thrombotic disorders (52). Analyses of platelet adhesiveness, blood viscosity and microvascular flow in conjunctival vessels were made on normal subjects and a group of patients with thrombotic disease. Platelet adhesiveness was increased significantly in patients with acute myocardial infarction or major arterial occlusions when compared with controls. Blood viscosity was increased in all patients with arterial or venous thrombotic disease (52). In another study platelet adhesiveness was found to have undergone about a twofold increase in patients with hypertensive cardiovascular disease compared with normal subjects (53). The extent of platelet aggregation induced by adenosine diphosphate (ADP) has also been found to increase after myocardial infarction (54). In addition it has been reported that there is up to

a hundredfold increase in the sensitivity of the electrophoretic response of human platelets to ADP in patients who have suffered a myocardial infarction or undergone extensive surgical procedures (55). The sensitivity is measured by the concentration of aggregating agent required to produce the maximum increase in the electrophoretic mobility of the platelets. Studies by Seaman and Vassar (56) and Seaman and Brooks (57) have shown that there is a parallelism between the induction of platelet aggregation with agents such as ADP and the decrease in platelet electrophoretic mobility (charge). Note that the decreases in platelet mobility occur at higher (aggregating) doses of ADP in contrast to the increases in mobility which occur at very low doses of ADP. The nature of these concentration dependent biphasic responses of the platelet electrophoretic mobility to addition of ADP was examined by Betts et al. (58) who found that ADP and fibrinogen together induce the increase in platelet mobility whereas the decrease is an appreciably more complicated phenomenon. The studies relating to the increase in platelet mobility at very low levels of ADP have proven difficult to reproduce (59). This appears to be due to the problems involved in the preparation of platelet suspensions (59,60). Difficulties include the choice of anticoagulant for the blood, temperature, surface properties of containers in which the studies are conducted, pH, calcium ion concentration and so forth.

Flow properties of blood in diabetes mellitus.

A number of studies have suggested that the erythrocytes in diabetic patients are rheologically abnormal (61). In diabetics red cell aggregation is frequently observed in the retinal and conjunctival venules, and even arterioles, under shear rates which normally produce a monodisperse suspension of red blood cells. The extent of red cell aggregation can be correlated with the appearance of retinopathy and neuropathy (62). The enhanced red cell aggregation has been attributed partially to elevations of α- and β-globulins and fibrinogen levels. The usual abnormal plasma protein pattern probably accounts for the reported increases in plasma viscosity (63). Skovberg et al. (37) have shown that the viscosity of whole blood in adults with diabetes is higher than for normal adults. It is possible that this increase in apparent blood viscosity may signal or be associated with the development of cardiovascular complications, the incidence of which is high in diabetic animals (64).

Flow properties of blood during circulatory shock.

During shock, hypotension or other low blood flow states, low rates of shear are liable to occur in the vasculature. For example, in the center of the parabolic velocity curve of major arteries and veins the shear rate will be very low as will also be the case in capillaries and post capillary venules (65). The decreases in rate of shear which occur in these situations favor the development of red cell aggregation and also reduce shear stress which normally would produce cell deformation. These two changes will result in an increase in blood viscosity at a given hematocrit. In experimental shock rheological and electrokinetic studies have been made on canine blood under standardized conditions of hemorrhage, trauma and administration of histamine (66,67). The decreases in the electrokinetic charge of dog erythrocytes and platelets which occur in shock favor the development of blood cell aggregates which could account for the stagnation which arises in the microcirculation and the decrease in circulating blood volume. Reduction in red cell deformability in the stagnant regions of low pH and pO_2 probably also exacerbates the general blood flow problem.

Flow properties of blood during surgical procedures.

Major surgery usually results in a decreased hematocrit with diminished apparent blood viscosity and an elevated level of fibrinogen. Comparisons made at equivalent

hematocrits indicate that the postoperative blood viscosity is higher than for normal controls since the increase in fibrinogen will enhance both red cell aggregation and plasma viscosity (7). Human erythrocytes are known to be damaged when pumped through extracorporeal cardiopulmonary bypass circuits, hemodialysis units or prosthetic valves (68). The extent of the problems which arise from interfacial phenomena such as denaturation of plasma proteins will vary with the type of equipment used. General anesthetic agents at concentrations in blood comparable to those used during clinical anesthesia produce no detectable influence on the apparent viscosity of whole blood. Agents which have been tested include halothane, cyclopropane, nitrous oxide, diethyl ether and methoxyflurane (69).

Flow properties of blood in cancer.

Dintenfass (11) has pointed out that plasma viscosity, blood viscosity and the extent of red cell aggregation may be elevated in neoplastic disease. While approaches to effect a decrease in the blood viscosity related factors cannot reverse the course of the disease, amelioration of some symptoms can result. A study by Dintenfass (70) of blood and plasma viscosities and of aggregation of red blood cells in 125 patients with the diagnosis of malignant melanoma showed that patients who died subsequently of metastases exhibited a significant elevation of plasma viscosity and of aggregation of red blood cells. Plots of plasma viscosity against globulin level and plots of aggregation of red cells against albumin/fibrinogen ratio showed significant differences between the slopes of the linear regressions for the survivors compared with the deceased group.

The changes which occur in blood viscosity factors in cancer appear to stem largely from changes in the plasma proteins. The plasma proteins comprise about 25 major identifiable and quantifiable components. The serum protein changes which occur in malignant neoplastic disease have been summarized and for the most part the changes are a reflection of the activity of malignant cells or the response of the host (71-73). Elevation of the serum glycoproteins is frequently especially pronounced in malignant disease (74). However efforts to use the increases in serum glycoprotein levels as a screening procedure for cancer have been largely unsuccessful. Cleve and Strohmeyer (75) have suggested that the quantitation of α_1-acid glycoprotein levels may be of value in screening for malignant neoplasms, and elevations in seromucoid levels have been found in patients with a variety of tumors (76). Rosato (77) has reported elevations of protein-bound glucose in carcinoma of the breast and Harshman et al. (78) have noted that a high seromucoid level following corrective surgery for malignancy was indicative of the continued presence of disease whereas a decrease to normal levels correlated well with remission.

In studies involving the examination of sera from 70 clinically well persons, 120 subjects with active malignancy and 220 patients who had received surgery for cancer we found that:

i) the levels of serum protein-bound carbohydrate, hexosamine and sialic acid are significantly above normal in cases with evidence of malignant disease (Table 2).

ii) the more advanced the stage of development of the malignant disease the higher the mean levels which are found for hexosamine and sialic acid in the serum (Table 3).

iii) the probability of reappearance of malignant disease in persons who have received surgery correlated with continuing elevations in the levels of serum protein-bound carbohydrate, hexosamine and sialic acid, those cases with the highest levels being most likely to display a recurrence.

TABLE 2. Comparison of levels of protein-bound carbohydrate, hexosamine and sialic acid for cancer patients, patients who have received corrective surgery, and normal control subjects. Values expressed per 7 g of total protein in mg% ± standard error of the mean, number of persons in brackets.

Category	Carbohydrate	Hexosamine	Sialic Acid
Normal	129.4 ± 1.65 (70)	93.4 ± 1.91 (39)	64.0 ± 1.10 (69)
Cancer	158.9 ± 1.99 (114) p<0.001	116.9 ± 2.29 (113) p<0.001	78.8 ± 1.59 (114) p<0.001
Surgically treated No recurrence	148.2 ± 1.21 (217) p<0.001	107.6 ± 1.17 (217) p<0.001	71.3 ± 0.81 (217) p<0.001

TABLE 3. Levels of protein-bound carbohydrate, hexosamine and sialic acid in relation to the cancer stage. Values corrected to 7 g% of total serum protein and expressed in mg% ± standard error of the mean. Number of persons in brackets, and p values with respect to the normal control.

Stage	Carbohydrate	Hexosamine	Sialic Acid
Control	129.4 ± 1.65 (70)	93.4 ± 1.91 (39)	64.0 ± 1.10 (69)
Localized	157.2 ± 3.36 (22) p<0.001	106.0 ± 3.69 (21) 0.01>p>0.001	72.2 ± 1.94 (22) p<0.001
Regional	153.6 ± 2.96 (41) p<0.001	111.6 ± 3.30 (41) p<0.001	78.2 ± 2.59 (41) p<0.001
Disseminated	165.4 ± 2.98 (58) p<0.001	124.5 ± 3.54 (57) p<0.001	83.5 ± 2.56 (58) p<0.001

Total serum proteins were determined according to the biuret method of Weichselbaum (79). Protein-bound carbohydrate was estimated using the phenol-sulfuric acid colorimetric method of Dubois et al. (80) on the trichloroacetic acid insoluble fraction of serum. Serum protein-bound hexosamine was assayed by the procedure of Winzler (81). Protein-bound sialic acid was estimated on the trichloroacetic acid insoluble fraction of serum using the resorcinol reaction (82).

While these analytical procedures have been successful in identifying the elevation in serum or plasma of glycoproteins containing specific chemical moieties, attempts to use plasma viscosity as an umbrella measure of general increases in the levels of glycoproteins have been unsuccessful. This is probably because while the levels of some plasma components increase others decrease (73). Plasma viscosity is a measurement which depends upon the averaged contributions from all of the