The Influence of Haematocrit and Fibrinogen concentration on vascular resistance

William W.Barrie M.B., Ch.B., F.R.C.S.

A Thesis submitted to the
University of Glasgow for
the Degree of M.D.

Research carried out at Department of Surgery,
State University of New York at Buffalo, U.S.A.,
and Department of Surgery, University of Leicester.

Submitted August 1979

CONTENTS

CONTENTS

Figures

CONTENTS

TABLES

ACKNOWLEDGEMENTS

I am indebted to Professor W.G.Schenk, Department of Surgery, State University of New York at Buffalo, and Professor P.R.F.Bell, Department of Surgery, University of Leicester, for their help and encouragement.

Mr. Mark Falzone, B.Sc., provided invaluable assistance with the construction and operation of the equipment used, as did Mr. C.Shallala and Mr. D.Amato.

The diagrams and illustrations were mainly the work of the Department of Medical Illustration, Leicester Royal Infirmary.

The secretarial work of this thesis has been carried out by Mrs. Rosalind Aldwinckle to whom I am most grateful.

SUMMARY

Obstructive arterial disease is common and is a major source of morbidity. It is frequently not amenable to direct surgery, but there is no effective form of medical therapy. The use of vasodilator drugs to lower vascular resistance has been discredited.

Although in theory flow should be increased by reducing blood viscosity,the flow properties of blood are complex and unpredictable in vivo. In vitro blood viscosity increases enormously at very low shear rates and a yield stress exists (that is a finite minimum force is required for flow to start). These properties are related both to haematocrit and the presence of fibrinogen, and would be of great therapeutic potential if reflected in vivo, since plasma fibrinogen concentration, and to some extent haematocrit can be safely manipulated.

Initially a simple canine model was used to investigate the effect of defibrinogenation on blood flow. A critical arterial stenosis was created in one femoral artery and electromagnetic flowmeters used to measure flow through both femoral arteries and cardiac output. In both limbs of the control animals and in the non ischaemic limb of the defibrinogenated animals flow decreased as did cardiac output. However, in the ischaemic limb of the defibrinogenated animals flow relative to cardiac output increased significantly after defibrinogenation suggesting that fibrinogen might be important in low flow states. These experiments were not conclusive however as the influence of changes in vasomotor tone or coagulation

could not be excluded.

To try to exclude influences other than blood viscosity, an isolated canine hind limb preparation was devised in which fresh anticoagulated canine blood at 37°C was oxygenated and perfused in a pulsatile fashion, while accurate measurements of pressure and flow were made. Pressure/flow curves were constructed using blood of haematocrit ranging from less than 20 to more than 70, at mean perfusion pressures from virtually zero to more than 150 mm Hg. The effect of fibrinogen was studied by using blood of varying natural fibrinogen concentration, defibrinogenated blood (by pre-treatment with Ancrod) and blood whose fibrinogen concentration had been increased by the addition of purified autologous canine fibrinogen. Protein free suspensions of red blood corpuscles/ Ringer Lactate and red blood corpuscles/dextran were also studied, as was the effect of temperatures lower than 37°C. Thirty separate limb perfusion models were used to study the pressure/flow characteristics of ninety samples of blood, approximately one litre of blood being required for each perfusion experiment.

Dextran 40/10% saline was perfused in a similar fashion to blood in each experiment and used as a standard to compare one experiment to another. Blood viscosity was measured in vitro at six shear rates ranging from 5.75 sec^{-1} to 230 sec^{-1}, using a Wells Brookfield microviscometer. Haematocrit, total plasma protein concentration and plasma fibrinogen concentration were measured by standard laboratory methods.

It was found that in vitro viscosity was determined largely by haematocrit, the relationship between log viscosity and haematocrit being virtually linear. Blood viscosity increased at low rates of shear, the magnitude of the increase, and the shear rate below which it took place being related to haematocrit. The slope of the haematocrit/log viscosity relationship was a function of shear rate.

Vascular resistance, derived from the pressure/flow data in the isolated limb preparation was an index of 'in vivo viscosity'. At mean perfusion pressures greater than 80 mm Hg it was determined mainly by haematocrit. The relationship between resistance and haematocrit was approximately linear in the range haematocrit 30 - 70, below which resistance was much less influenced by haematocrit.

Vascular resistance in the limb perfusion model was not constant at a given blood viscosity but below a certain perfusion pressure increased with decreasing perfusion pressure. The magnitude of the increase, and the perfusion pressure below which the increase took place varied with haematocrit.

Although vascular resistance in the isolated limb preparation correlated well with in vitro viscosity, the magnitude of the change in vascular resistance with haematocrit or perfusion pressure was considerably less than that of the change in in vitro blood viscosity.

Neither fibrinogen nor total plasma protein in the concentrations studied, had a significant effect on either blood viscosity in vitro, or vascular resistance in the isolated limb.

Suspensions of red blood corpuscles in protein free solutions (dextran 40/normal saline, Ringer Lactate) had similar non Newtonian properties to whole blood, while Dextran perfused alone was almost Newtonian. (A Newtonian liquid, for example water, is one whose viscosity is independent of shear rate).

The effect of cooling the perfused blood in the isolated limb preparation was to increase vascular resistance, and probably to increase blood's non Newtonian behaviour.

It is concluded that a therapeutic reduction in in vivo viscosity would not be achieved by lowering plasma fibrinogen concentration, since the large in vitro fibrinogen dependent increase in viscosity at low shear rates does not seem to occur, even at very low rates of flow, in the intact circulation. Vascular resistance does increase considerably at low flow rates in this experimental model but this is related to haematocrit concentration. It may be that patients with arterial disease whose haematocrit is at or above the upper limit of normal will benefit from venesection, but it seems most unlikely that minor changes in in vitro viscosity will achieve a significant alteration in flow in the intact circulation.

CHAPTER ONE

INTRODUCTION

Obstructive arterial disease comprises a group of conditions characterised by lengthening, hardening and narrowing of the arteries, resulting in limitation of blood flow. In its most common form it represents an exaggeration of the normal ageing process in which the arterial wall is progressively thickened and calcified and the lumen acquires a lining of soft fatty material (atheroma) on which clot forms readily. The condition is becoming increasingly common in Western society, the incidence in the middle aged and elderly population being in the order of 7 per cent (Eastcott, 1974). Any of the arteries of the body may be affected but in clinical practice the regions most affected are the cerebrovascular system, the coronary arteries and the peripheral arterial circulation. It is to this last system that this work is applied, although observations on blood flow in one system are likely to be of relevance, at least in part, to the others.

The most commonly affected peripheral arteries are those supplying the lower limbs. The efficiency of the arterial circulation is such that apparently severe narrowing of these arteries may produce minor or no symptoms, depending on the availability of alternative ('collateral') pathways and the demand for blood by the tissues. When symptoms occur the most common complaint is of cramping pain in the calf, or less frequently thigh, muscles after a certain distance is walked. The pain always disappears after a short rest and the diagnosis

of 'intermittent claudication' is easily made by the characteristic history. If the disease progresses the distance which the patient can walk, the claudication distance, decreases until eventually he is restricted to only a few yards. At this point a much more serious development may occur, the onset of ischaemic rest pain. This is a debilitating and intolerable complaint which unless effectively treated necessitates amputation of the limb. Fortunately, intermittent claudication very often does not progress: indeed frequently it runs a benign course, remaining static or even improving over the years. However, particularly in the elderly, ischaemic rest pain or gangrene may be the first manifestations of peripheral vascular disease, there being little or no antecedent history of intermittent claudication. Thus obstructive arterial disease is extremely common but often causes few symptoms. In its most severe form however, loss of life or limb is likely and it is a major cause of severe disability in the middle aged and elderly.

The fact that obstructive arterial disease tends to be patchy in distribution may make it possible surgically to rebore or bypass the diseased segment from relatively healthy artery above to relatively healthy artery below the obstruction. Clinically three main patterns of disease are recognised:aorto-iliac, femoro-popliteal and tibial vessel disease (distal disease). Although pure examples of each type are encountered it is common to have more than one region involved, and often all three. The techniques of reconstructive surgery for obstructive arterial

disease have advanced rapidly in the past two decades and vascular surgery is now an established surgical routine. However, of the many patients presenting with claudication, rest pain, or gangrene, only a minority are suitable for surgical reconstruction. The main reason for this is that surgery becomes progressively less successful the more distal and smaller the vessels which are involved. When arterial disease affects mainly the aorto-iliac segment the results of reconstruction are excellent, and for the femoro-popliteal segment reasonably good, but for disease more distal to this surgery is often disappointing, (Watt, 1974).

Unfortunately it is just this last group of patients with severe distal (or multi-level) disease who, on account of rest pain or gangrene, are most at risk of amputation. In addition a further substantial group of patients with otherwise operable arterial disease will prove unfit for surgery often because of associated coronary or cerebrovascular insufficiency. There is therefore a major need for a non-surgical method of increasing the blood supply to the ischaemic limb, particularly as it seems likely that in terms of blood flow the difference between viability and non-viability is slight.

When obstruction of a main artery occurs arterial pressure distal to the obstruction falls and the direction of flow in certain side branches distal to the obstruction may reverse (Fig. 1). The arterial territory distal to the block is now supplied from the network of interconnecting vessels which form the collateral pathway. The adequacy of this pathway in meeting

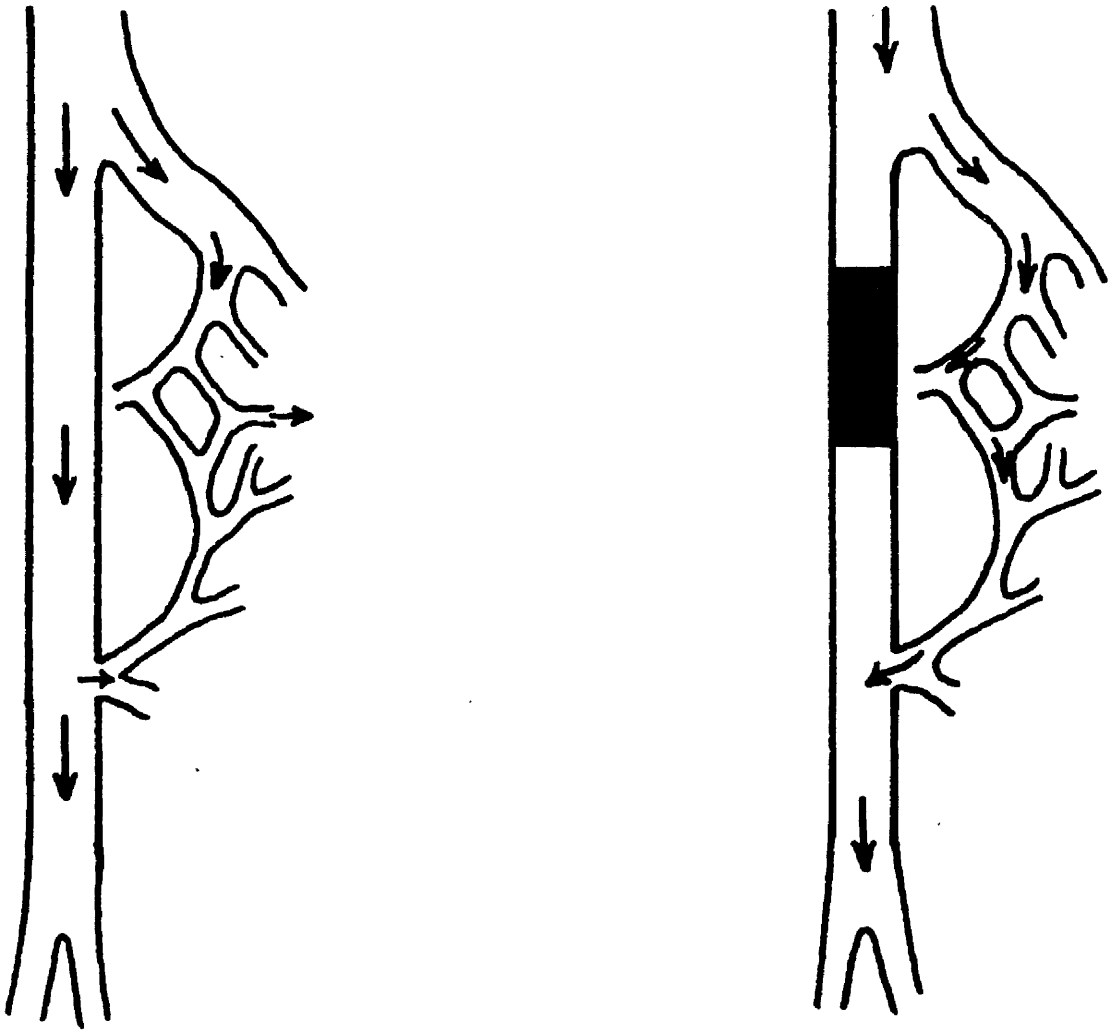


FIG 1

the demands of the dependant tissues varies with the abundance and size of vessels available and their own involvement with atheroma. In the lower limb collateral pathways usually offer a considerably higher resistance to flow than the unobstructed artery. The components of vascular resistance in a collateral bed, or other vascular system are the constricting tone of the resistance vessels, mainly the arterioles, and the viscosity of blood. Theoretically, improvement in flow can be obtained by reducing either arteriolar tone or blood viscosity.

Arteriolar constriction can be diminished, and thus the size of the vessels increased, by reducing constricting sympathetic tone. This may be done either by surgical or chemical destruction of the sympathetic chain, or by alpha receptor blockade with drugs. Localised sympathectomy of the affected limb has been claimed to palliate about 50% of patients with early rest pain, whether the sympathectomy is carried out by a formal operation (Strand, 1969) or by percutaneous injection of phenol into the lumbar sympathetic chain (Reid, 1970). Drugs which vasodilate by blocking sympathetic nerve transmission in various ways have enjoyed a vogue in treating peripheral vascular disease and have been widely employed, particularly in continental Europe. Whether these drugs are given orally, intravenously or intra-arterially they result in generalised vasodilatation. The inherent defect in any form of sympathectomy particularly alpha blockade, is that in a severely ischaemic limb the collaterals are either incapable of dilating (on account of arterial disease) or are already maximally dilated. Any increase in total limb blood flow from vasodilatation may be achieved by

increasing the flow to more healthy areas, (De Bakey, 1947), while the ischaemic area is either not affected at all or is adversely affected by the shunting of blood to more healthy areas capable of vasodilatation. The work of Gillespie (1959) has demonstrated that vasodilatation has little, if any, part in the treatment of obstructive arterial disease.

It is surprising that drugs aimed at increasing blood supply to the ischaemic limb by vasodilatation have been, and continue to be so widely used given the weight of theoretical and clinical evidence against them. The alternative non surgical approach to increasing blood flow to the inoperable ischaemic limb, by manipulating the flow properties of blood itself has been comparatively little explored.

Increased blood viscosity has long been recognised as the cause of symptoms in such relatively uncommon conditions as polycythaemia vera, thrombocythaemia and macroglubulinaemia. In these conditions blood viscosity is very greatly elevated and symptoms are relieved upon restoration of blood viscosity to normal. It seems reasonable, however, that lesser elevations of blood viscosity - insufficient to be the sole cause of symptoms - might assume clinical significance when combined with otherwise minor obstructive vascular disease. It is a common observation among surgeons dealing with peripheral vascular disease that an undue number of patients, while not frankly polycythaemic, have haematocrit levels toward the upper limit of normal and that these patients tend to fare badly.

In a group of patients with intermittent claudication Dormandy (1973a,b) demonstrated a correlation between whole blood

viscosity, plasma fibrinogen concentration and severity of symptoms, and subsequently reported improvement with treatment aimed at lowering elevated plasma fibrinogen concentration and hence viscosity (Dormandy, 1974). Other reports have linked the incidence of post-operative deep vein thrombosis to raised pre-operative blood viscosity levels(Dormandy, 1973c), and complications after vascular surgery to elevated haemoglobin concentration, and therefore increased whole blood viscosity (Morris, 1975). In addition sufferer's from Raynaud's syndrome have been shown to have greater than normal blood viscosity, (Pringle, 1965; Tietjen, 1975), although this has been disputed.

As yet there has been no convincing success in treating obstructive arterial disease by reducing blood viscosity but there is sufficient indirect evidence that blood flow properties have been under-emphasised to make a study of the factors influencing blood viscosity worthwhile.

CHAPTER TWO

FLOW PROPERTIES OF BLOOD IN VITRO

In contrast to our meagre knowledge of blood flow in vivo there is a substantial volume of in vitro work on the experimental and theoretical aspects of blood flow. In the main, this has been carried out by physiologists, mathematicians and engineers with an interest in biology and fluid mechanics and, while of undoubted academic interest, it is difficult for the clinician to assess its significance in relation to human pathology. However, as a starting point in any investigation into blood flow in vivo, it is essential to be familiar with the main properties of blood flow in vitro.

Poiseuille (1799-1869), a physician as well as physicist by training, originally intended his studies to be of the flow properties of blood, but because of coagulation problems (there were no available anticoagulants) turned to the study of "pure" liquids flowing in rigid tubes, and studied distilled water and alcohol flowing through horizontal capillary glass tubes to devise his well known equation relating pressure and flow (Poiseuille, 1847). There was a lapse of several years before Poiseuille published his results and in the meantime Hagen (Hagen, 1839) had independently derived the same equation. The formula is thus sometimes referred to as the Hagen-Poiseuille equation.

$$Q = \frac{P\,d^{4}}{K\,L}$$

Where Q equals volume flow

P = pressure drop

d = inside tube diameter

L = tube length

K = a constant depending on fluid viscosity

Poiseuille's law that the head of pressure is directly proportional to tube length, rate of flow and viscosity and inversely proportional to the fourth power of the internal radius also implies that viscosity is a constant independent of flow rate or applied force.

The viscosity of a liquid represents its internal resistance to flow(in Newton's expression "lack of slipperiness"). When a liquid flows in a cylindrical tube it does so in concentric layers the flow being fastest in the centre of the stream and decreasing progressively to zero at the edges of the tube, it being assumed that there is no flow, or 'slip' between the outermost layer of fluid and the tube wall. As the layers of fluid slide over each other the molecular attraction between the layers of fluid resists flow, the magnitude of this force determining the viscosity of the liquid.

Consider two very thin parallel laminae of fluid a distance dr apart (Fig 2). A force is applied to one lamina causing it to move over the other lamina in the direction of the applied force.

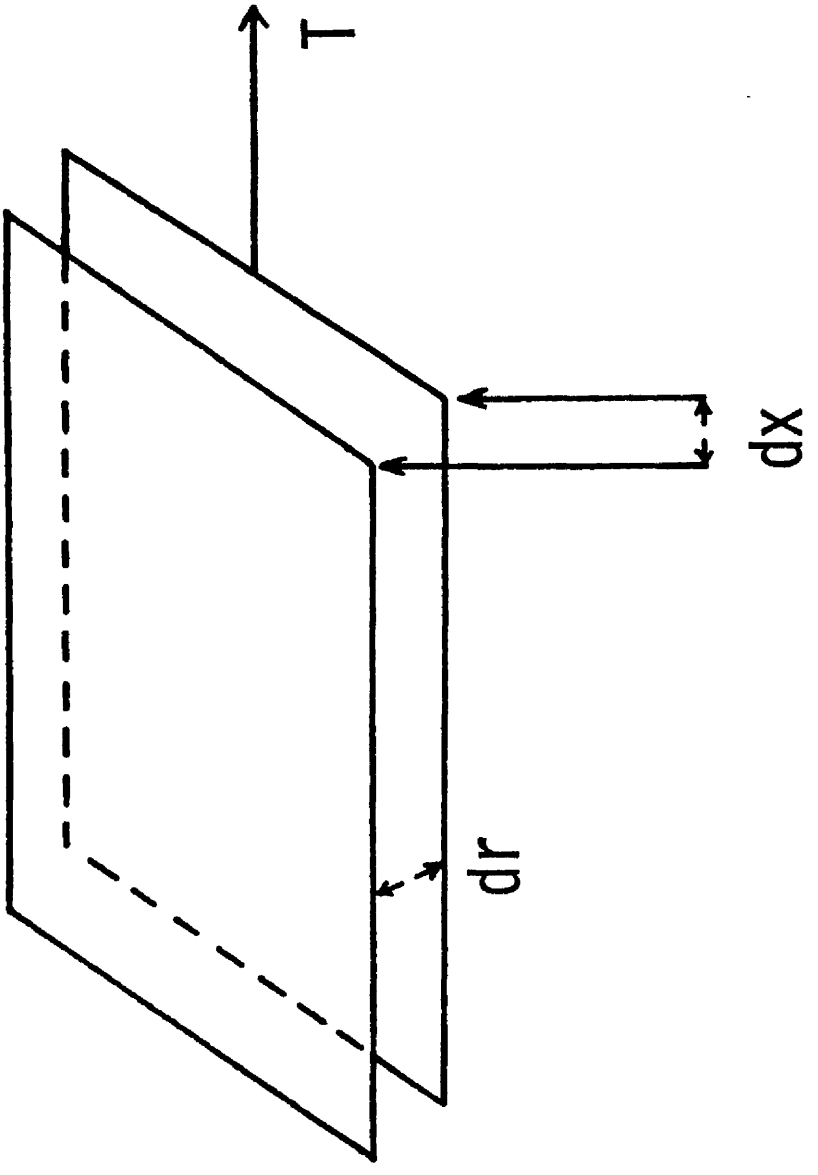


FIG 2

This force is the shear stress, T. If in unit time the displacement of one lamina relative to the other is dx and its relative velocity is dv, then the shear rate D, is $\frac{dv}{dr}$. The shear rate, D, is thus the change in velocity across successive laminae of fluid, that is the velocity gradient. The coefficient of viscosity is, by definition, the ratio between the shear stress T and the shear rate D

$$u = \frac{T}{D}$$

Since shear rate

$$D = \frac{dv\ (cm/sec)}{dr\ (cm)} \quad *$$

the dimensions of shear rate (after cancelling) are sec^{-1} or inverse seconds. Shear stress, T, is a force per unit area thus its dimensions in the c.g.s. system are dynes. $centimetre^{-2}$. The dimensions of viscosity, $u = \frac{T}{D}$, are dyne. seconds. $centimetres^{-2}$. One dyne. seconds. $centimetre^{-2}$ is equal to one Poise. For blood rheology purposes the unit normally used is the Centipoise, 0.01 Poise, since the viscosity of normal blood is in the order of 0.04 Poise, and that of plasma 0.015 Poise. A liquid whose viscosity, at a given temperature, is independent of shear rate or shear stress is known as Newtonian since Newton, in Principia (1686) considered the force necessary to move a solid through a liquid at a given velocity and assumed as a

* All dimensions expressed here are in the centimetre.gram. second (c.g.s.) system.

hypothesis that the one was directly proportional to the other. If this is so then viscosity must be independent of velocity. This was however merely a hypothesis until the experiments of Poiseuille confirmed its veracity for simple liquids. Blood of course is not a simple liquid but a suspension of complex particles and it is not surprising that it has decidedly non Newtonian properties. It is therefore necessary to consider blood viscosity in relation to the conditions under which it is measured, as well as its constituent parts.

Relationship to tube size

In 1906 Denning and Watson described, to the Royal Society, studies of blood flowing through small tubes, the project that Poiseuille had been unable to carry out seventy years before. Using a modification of the Ostwald viscosimeter they found that the viscosity of anti-coagulated blood (from horses) was not constant but varied with the diameter of the tube in which it was measured, as well as with temperature and corpuscular concentration. The relationships they showed between viscosity and temperature, and viscosity and haematocrit concentration have subsequently been shown to be approximately correct. Paradoxically, the discovery with which they have been credited, that blood viscosity may vary with the size of the tube in which it flows, has been confirmed but the relationship is now known to be the opposite of that which they showed. Denning and Watson found that blood viscosity increased as tube diameter decreased from 2 mm to 0.3 mm whereas more recent work has established that with tubes greater than about 1 mm in diameter, viscosity is independent

of tube diameter, but with decreasing size of capillary tube viscosity decreases (Fahraeus, 1931). The explanation of this behaviour may lie in the fact that as blood flows in a tube there is a tendency for the red cells to gravitate to the centre of the tube so that the outer layers are relatively cell free. This introduces several complexities into blood flow including the probability that in a non uniform velocity gradient, as in a tube, the red cells will have a component of velocity directed towards the axis (Saffman, 1956). Fahraeus and Lindqvist considered that the decreasing viscosity of blood in very small tubes might be attributable to the increasing relative importance of the cell free zone as tube diameter decreases. They showed that when blood flowed from a reservoir through a small bore tube, the haematocrit of the emerging blood was lower than that of the reservoir. Since they were the first to report this in detail the phenomenon of decreased blood viscosity in small tubes has become known as the Fahraeus-Lindqvist effect.

There has been considerable debate over this cell free or "plasmatic zone" and to what extent it exists. Certainly the layers immediately adjacent to the tube wall cannot have exactly the same concentration of red cells as the more central layers since the individual red blood cell, has a bulk of its own and no cell can have its centre immediately adjacent to the wall. (Fig. 3). Some of the controversy has arisen because high speed photographs of blood flow in the micro-circulation can be highly misleading on account of lighting artefacts and it has been shown

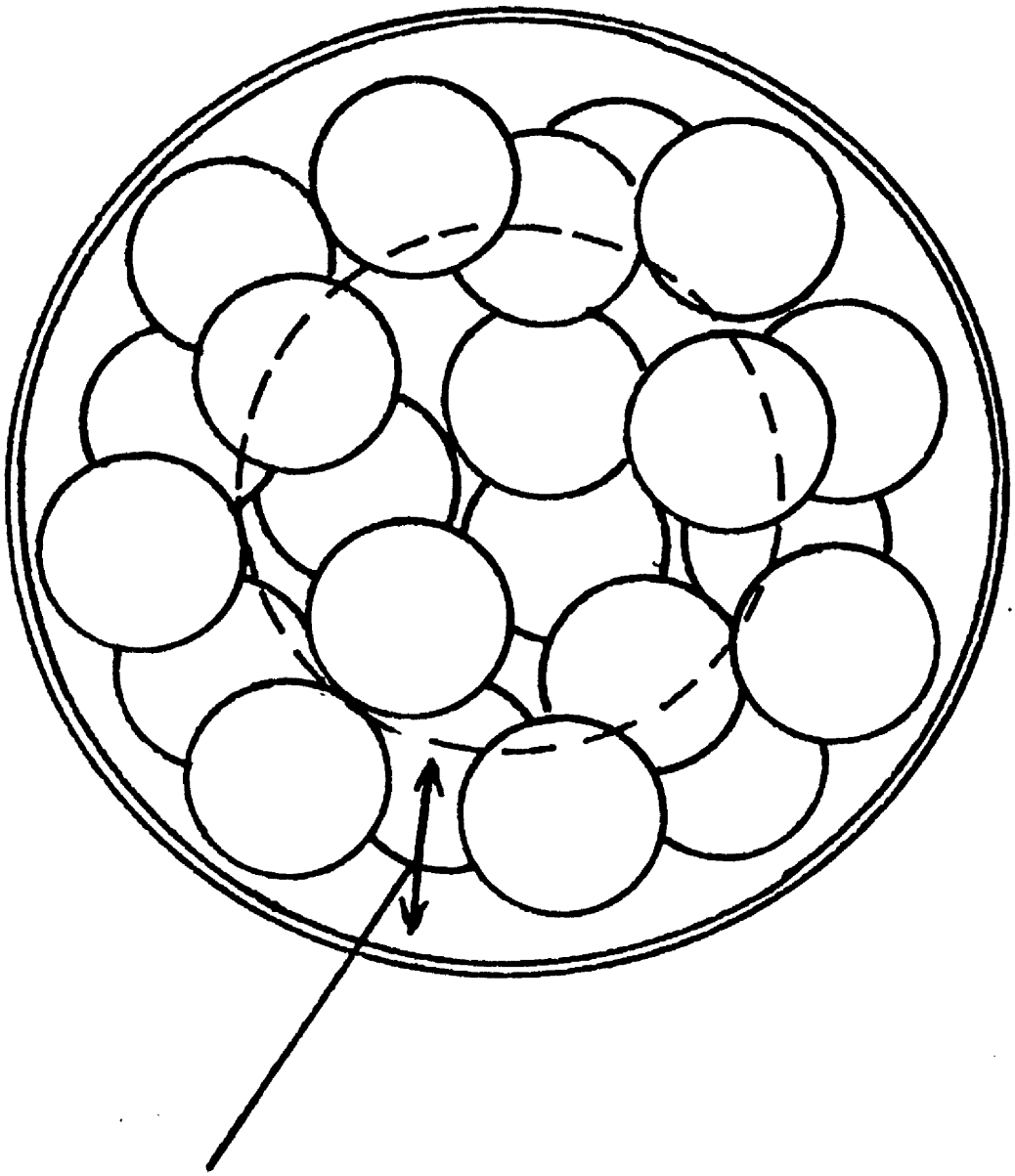


FIG 3

(Wiederhielm, 1967) that varying the angle of illumination can make the same vessel appear to have different widths of cell free layer. In an attempt to overcome this difficulty experiments have been performed in which blood flowing in small vessels has been very rapidly frozen in an attempt to maintain the cell distribution for examination but again there is uncertainty about the extent of artefact. Bayliss (1963), in a review of this difficult subject, considers that the cell free zone is in the order of 1-4μ which, according to Wayland (1967) is roughly that required by simple volume exclusion alone.

Another observation which may be of relevance to the work of this thesis is that pulsatile flow seems to accentuate the axial concentration of suspensions and thüs perfusion experiments may have to take this factor into account.(Goldsmith 1965).

Whatever the mechanism of the reduction in viscosity as tube radius falls to within about 100 times the radius of the red cell, the effect is real enough. Surprisingly there have been few studies which have actually recorded the reduction in viscosity obtained and the scatter of results is fairly wide. From the review article by Bayliss(1963) it seems that in a tube of radius 20 μ the asymptotic apparent viscosity of blood is about two thirds of the value obtained in large tubes. However, Dintenfass (1971) using specially developed parallel plate slit viscometers,which he considers permit determinations of blood viscosity under conditions simulating the micro-capillary bed,describes an inversion phenomenon, that is the existence of a critical radius below which there is a sudden and dramatic increase in resistance to flow. This seems to depend on the internal viscosity of the

red blood cells and aggregrates of these cells, and is very sensitive to blood pH levels.

Relationship to Haematocrit

When blood is flowing at high (over 200 sec^{-1}) shear rate the main determinant of blood viscosity in vitro is haematocrit. Viscosity increases with increasing haematocrit, the traditional view being that the increase becomes progressively steep with haematocrits above 50 or so. Several workers however, demonstrated an approximately linear relationship between haematocrit and the logarithm of viscosity and there is now general agreement that a relationship such as that shown in figure 4 applies (Haynes, 1960; Peric, 1963; Gregersen, 1965; Chien, 1966;Merrill, 1969; Dormandy, 1970).

Relationship to Shear Rate

When blood starts to flow its viscosity is initially very high but decreases very rapidly as shear rate increases (Fig. 5). At shear rates greater than about 200 sec^{-1} blood viscosity reaches a constant minimum, or asymptotic value. Thus blood can be regarded as more or less Newtonian at rapid (but not so rapid as to cause turbulance) flow rates in large bore (over 1 mm) tubes. As shear rate is reduced to about 50 sec^{-1} the increase in viscosity (compared with the asymptotic value) is fairly modest, perhaps in the region of 20-50% but at very low rates of shear, less than 10 sec^{-1}, the increase becomes much greater. For blood of haematocrit 40,Merrill(1969) has shown a 40 fold increase in viscosity as shear rate falls from 1 sec^{-1} to 0.01 sec^{-1}.

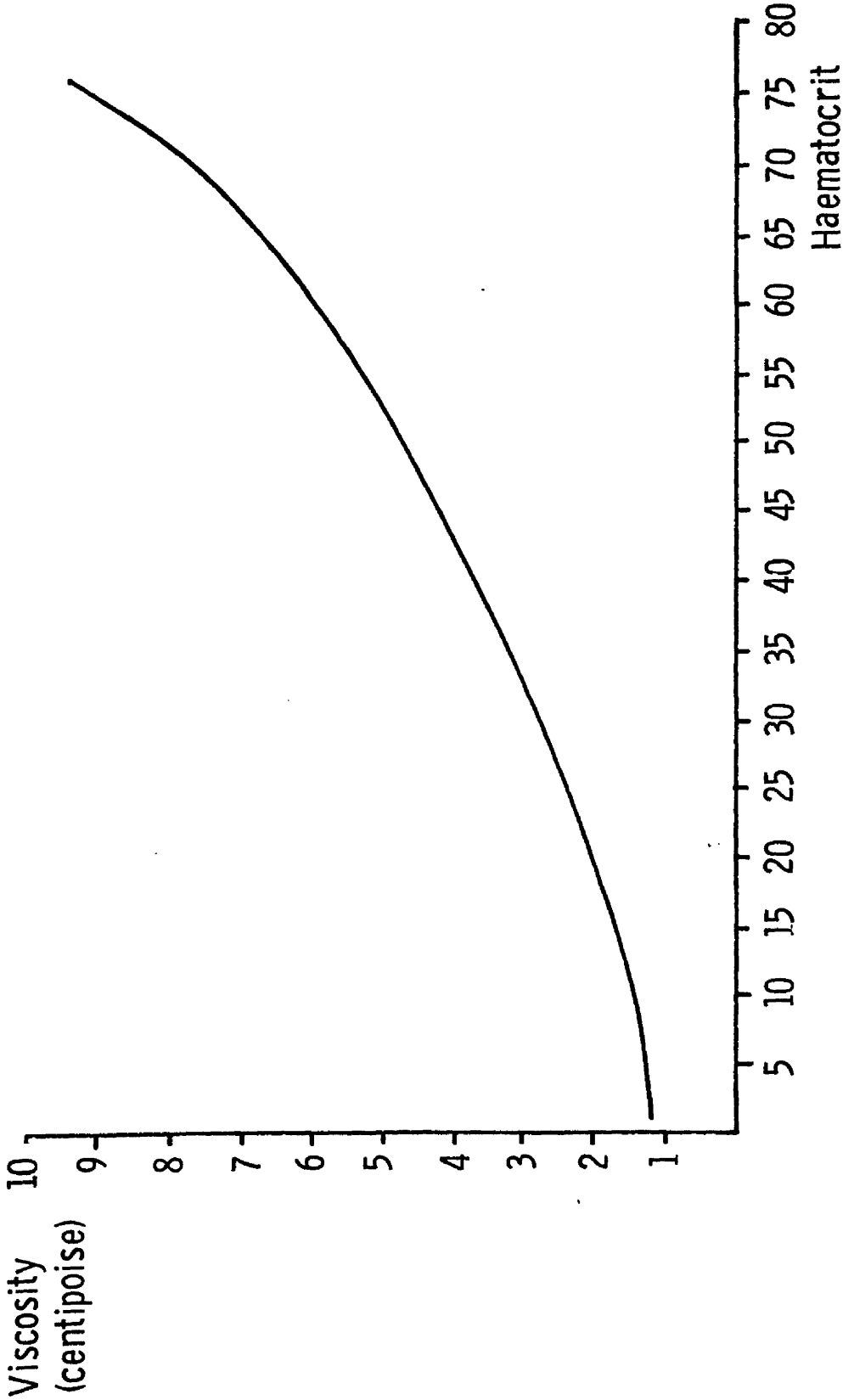

Viscosity
(centipoise)
10
9
8
7
6
5
4
3
2
1
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Haematocrit

FIG 4

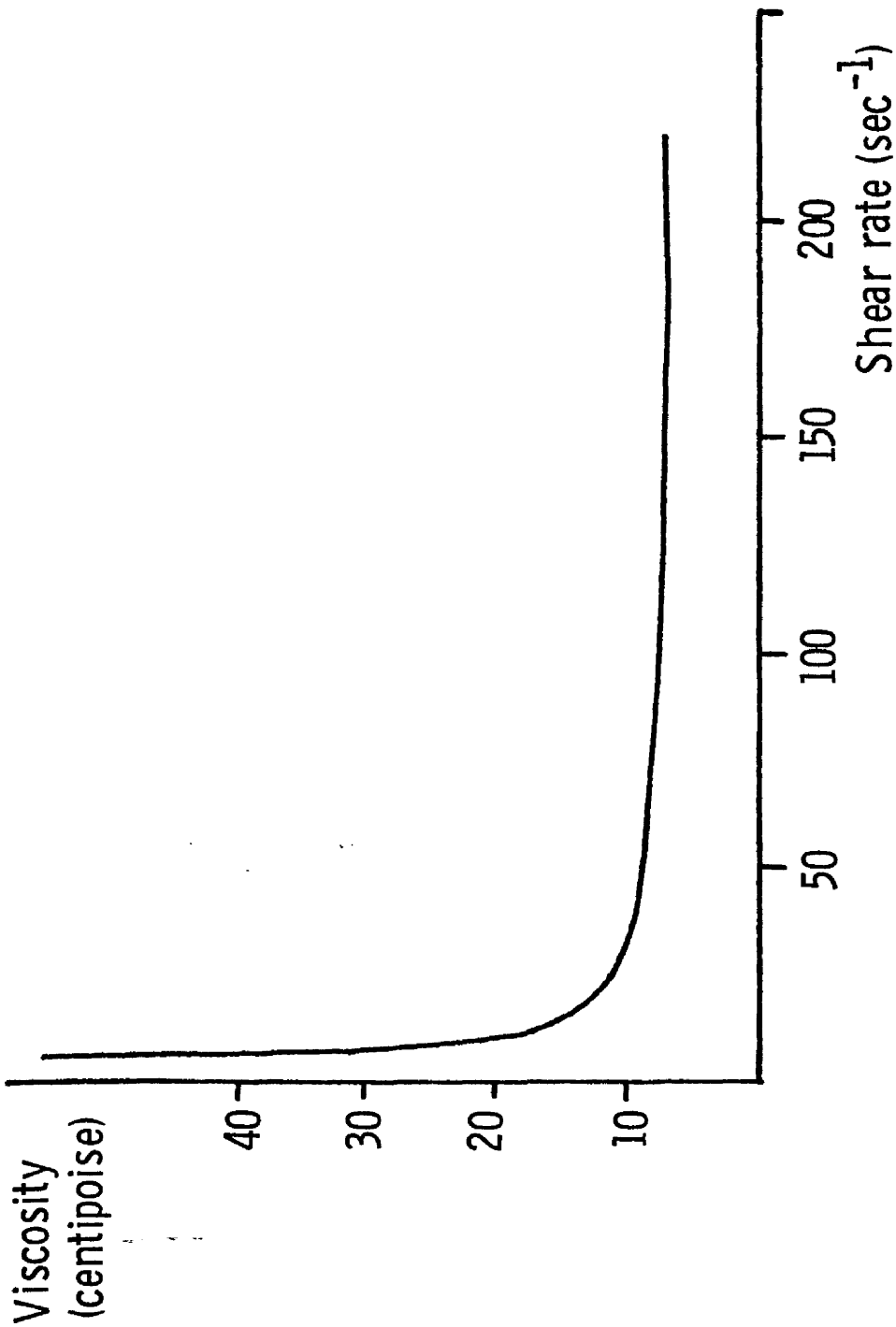

Viscosity
(centipoise)
40
30
20
10
50
100
150
200
Shear rate (sec^{-1})

FIG 5

This anomalous behaviour increases with increasing haematocrit, blood of haematocrit less than 15-20 exhibiting little variation in viscosity with changing shear rate. The variation of viscosity with rate of flow,or "shear dependence", is also related to the composition of plasma, in particular plasma fibrinogen concentration. It is reported that defibrinated blood of haematocrit less than 30, or suspensions of red blood cells in Ringer's Solution are virtually Newtonian (Merrill,1969), which implies that plasma fibrinogen is a most important factor in the æetiology of the anomalous flow properties of blood.

Yield Stress

When shear stress is plotted against shear rate (Fig. 6) and measurements are made at very low rates of shear, as is possible with specially designed rotating viscometers, it is seen that the graph intercepts the shear stress axis at a finite point. A force, or 'yield stress' is required to initiate flow. For blood this force is small, less than 0.1 dynes/cm^2, but is said to be dependent on the presence of fibrinogen, a minimum concentration of 200 mg% being necessary for the existence of a yield stress (Merrill,1963a). Wells (1971), however, demonstrated red cell aggregation and a yield stress in the absence of fibrinogen.

The establishment of a mathematical formula relating shear stress and shear rate (broadly analogous to pressure and flow in vivo) has attracted the efforts of rheologists for the past few decades. There has been much esoteric debate as to which of the established rheological formulae (devised for the flow of

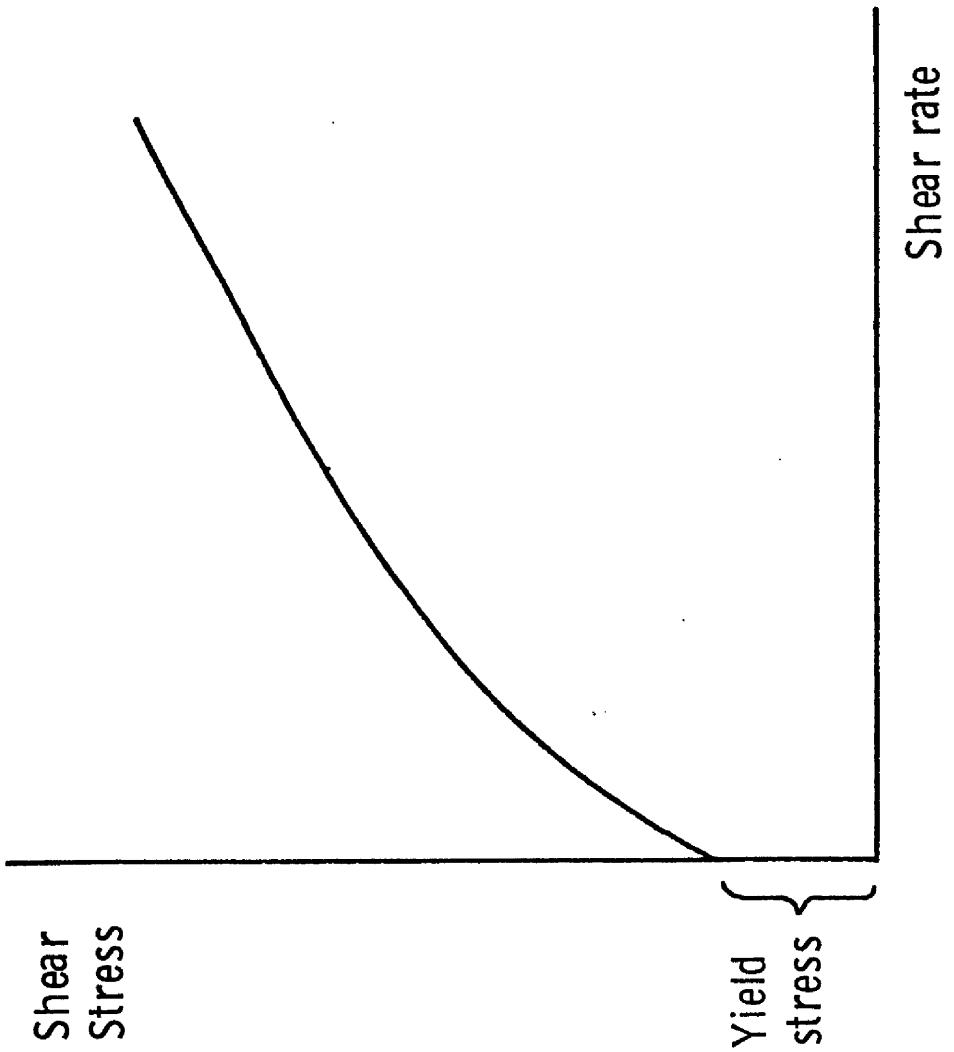


FIG 6

FIG 6

paints, inks, dairy products etc) achieves the best 'fit' for blood. It would seem that a power low relationship is inapplicable since when log shear stress is plotted against log shear rate a linear relationship is not achieved (Whitmore, 1968). However, the Casson equation, (Casson, 1959), originally proposed to describe the flow properties of certain printing inks, seems to apply reasonably well to blood.

If Kc is a measure of the consistency of the material

$$Kc = \frac{\sqrt{T} - T_y}{\sqrt{D}}$$

where T = shear stress

T_y = yield stress

D = shear rate

Charm et al (1965) demonstrated a reasonable fit of the equation to blood bank samples, over a very wide range of shear rate.

Fibrinogen

Although the Casson equation is best regarded as empirical it is of interest that it was devised to describe a fluid which exhibits reversible aggregation at low shear rates, the suspended particles forming rod like aggregates of increasing length as shear rate falls.

It is tempting to relate this to the formation of rouleaux by the red cell corpuscles of the blood, and thereby provide a physical basis for the 'fit' of the Casson equation. Certainly it seems likely that red cell aggregation is a factor affecting blood's anomalous viscous properties, the yield stress being the force required to separate the cells sufficiently to start blood moving

and the decrease in viscosity as flow increases being attributable to the progressive dispersion of cells, until when these cells are fully dispersed blood viscosity reaches its asymptotic value and is independent of shear rate.

Fibrinogen is known to have a profound influence on red cell aggregation (e.g. in most blood samples the erythrocyte sedimentation rate (ESR) and fibrinogen content are directly related); the higher the fibrinogen level the greater the degree of aggregation. Thus it seems reasonable that fibrinogen should greatly influence the low flow properties of blood. Merrillhas shown that yield stress in blood is related to the square of the fibrinogen concentration and the third power of haematocrit (Merrill,1963; 1969).

A further property of fibrinogen may be important however. The flexibility of the red cell membrane, and therefore the ability of the red cell to deform appears to decrease with increasing fibrinogen concentration (Sirs, 1968). Since capillaries are in the order of size of the red cell itself, considerable deformation of the red cell occurs during flow through capillaries. Thus fibrinogen might be expected to have two separate and opposing influences on blood flow; an adverse effect at very low flow rate on account of yield stress and red cell aggregation, and a favourable effect on the ability of the red cell to deform to pass through the micro-circulation. At higher red cell concentrations on physical grounds it is more likely that shear dependent erythrocyte deformation is the major factor in the decreasing viscosity of blood at higher shear rates, whereas shear dependent erythrocyte aggregation assumes a greater

role at normal and low haematocrits.

Platelets

Within physiological limits platelets do not appear to have an appreciable effect on blood viscosity (Frasher, 1968). Thrombocythaemia, however, can certainly give rise to occlusive vascular disease but this may be a function of blood hypercoagulability as distinct from viscosity.

Plasma Proteins (other than fibrinogen)

Within the normal physiological range plasma proteins other than fibrinogen do not seem to influence greatly blood flow properties, although globulins in particular raise the viscosity of plasma and thus of whole blood. Chien et al (1971) studied the relative effects of fibrinogen and globulin by adding purified autologous fibrinogen to serum and Ringer suspensions of 45% erythrocytes. They confirmed that fibrinogen causes an increase in viscosity and shear dependence and deduced (by subtraction) that serum globulins have a fibrinogen-like action. The relative effectiveness of plasma proteins in interacting with erythrocytes is in the order fibrinogen, globulins and albumin and is strongly dependent on the molecular weights of the proteins.

Abnormal proteins such as occur in the unusual disorders macroglobulinaemia and cryoglobulinaemia may cause severe circulatory disturbance, including digital gangrene, because of the adverse effect on blood flow in small vessels.

Hyperlipaemia and Lipoproteins

There have been reports that hypertriglyceridaemia and

hyperbetalipoproteinaemia increase the non Newtonian properties of blood, by enhancing yield stress(Merrill,1964), and that lipaemia increases blood viscosity (Bronte-Stewart, 1965; Swank, 1953). Later work by Merrill however did not confirm these findings(Merrill,1969).

It seems unlikely that the adverse effects of hyperlipidaemia on the course of vascular disease is of rheological origin. Ballantyne studied a group of claudicating hyperlipidaemic patients whose cholesterol and triglycerides were reduced by dietary means, but without significant effect on their impaired circulation (Ballantine, 1976).

Blood Sugar

Blood sugar levels do not seem to have a detectable effect on blood viscosity, and it is unlikely that the predisposition of the diabetic to vascular disease is related to a rheological effect of hyperglycaemia (Castle, 1964). However, Skovborg (1966) claimed that diabetic subjects had whole blood viscosity levels 20% higher than a controlled population.

Temperature

The viscosity of blood rises as temperature falls. In a simple suspension one would expect that the absolute viscosity would vary with temperature in the same way as the suspending fluid so that one would be able to 'correct' from one temperature to another by reference to the established values for the viscosity of the suspending fluid at different temperatures. However, in the case of blood this would assume that the viscosity of blood relative to plasma (the relative viscosity) remains constant and that such anomalous properties as yield stress also

remain constant. There are conflicting reports on this subject. Rand (1964) found that the relative viscosity fell substantially from 37°C to 22°C while Azuma (1964) reported a 10% increase in relative viscosity from 37°C to 17°C. According to Merrill (1969) yield stress is not greatly altered. It has been suggested that changes in relative viscosity with temperature are due to alterations in the shape of the red cells and Azuma (1964) attributed this to the cells becoming more spherical and less disc like. The possibility that temperature affects blood flow properties in a non linear, or unpredictable, fashion may account for some of the discrepancies in previous reports on blood viscosity, since much of the earlier work was done at 20°C, 'room temperature' or at unstated temperature.

The type of viscometer used may be relevant to the discrepancies noted. In 1973 Barbee reported studies of the effect of temperature, 23°C and 37°C respectively, on the relative viscosity of human blood in a capillary viscometer, a GDM concentric cylinder viscometer and in a Wells-Brookfield cone and plate viscometer. He found that the relative viscosity was independent of temperature at high shear rate in all of the instruments, but depended on the temperature at low shear rates only in the capillary viscometer, a possible explanation being the different type of shear field in each instrument.

Anticoagulants

Blood clotting commences as soon as blood is withdrawn from the body. Blood rheology is profoundly altered by these changes and although attempts have been made to study this, the rheology of clotting is a separate and even more difficult area

of study than that of liquid blood.

Very few studies have been made of non anti-coagulated blood, although Dintenfass has studied freshly shed not anti-coagulated blood in a cone-in-cone viscometer (Dintenfass, 1962; and 1966). He has subsequently compared these results to those obtained from a rhombospheroid viscometer with blood anti-coagulated with EDTA (Dintenfass, 1974) and with data from other workers (Copley 1973a; Copley, 1973b;Merrill,1963a) and shown them to be very similar, except at very low shear rates (less than 0.4 sec^{-1}). Earlier however, Copley (1960) had reported studies of rabbits heart blood anti-coagulated with heparin, citrate, oxalate or EDTA showing increasing viscosity with these anticoagulants in the above order. A type of capillary viscometer was used. Mayer and Kiss (1965) compared heparin, EDTA and potassium oxalate, also in a capillary viscometer, and concluded that only the last affected blood viscosity, by decreasing it. They controlled carefully the temperature of their samples and pointed out that since blood viscosity increases by 2-3% for each degree of cooling in this temperature range some of the conflicting earlier data may have arisen from temperature differences in the samples - which may vary considerably when blood is withdrawn from the donor into a syringe. Also in Copley's studies the anticoagulants were dissolved in water and added to blood in the ratio of one part anticoagulant solution to four parts of blood, which may have rendered their results unreliable.

Cokelet, 1963, used native blood immediately after withdrawal from donor and compared it with blood anticoagulated with heparin, sodium citrate, ACD, EDTA (ethylenediaminotetraacetate sodium salt) and sodium oxalate and concluded that in reasonable amounts there was no significant effect.

The effect of heparin was studied by Frasher (1968) using direct outflow of blood from an arterio-venous shunt in a dog to a specially designed capillary viscometer. Heparin did not appear to affect viscosity. It is now generally accepted that the commonly used anticoagulants do not significantly influence blood viscosity in the concentrations normally used.

FLOW PROPERTIES OF BLOOD IN VITRO

SUMMARY

Blood flow in vitro exhibits complex properties which vary with the conditions of measurement. At rapid rates of flow (shear rate greater than 200 sec^{-1}), but not so fast that flow is turbulent, it can be regarded as Newtonian; that is, shear stress is proportional to shear rate and viscosity is constant.

As shear rate is reduced blood viscosity increases, this effect increasing with increasing haematocrit. At very low rates of shear (less than 1 sec^{-1}) the increase is very large. A yield stress is present, that is a finite force is required before blood will flow at all. The presence of this yield stress depends on fibrinogen and is related to red cell aggregation.

When blood flows through small tubes its viscosity falls. This effect becomes evident with tube diameter less than 1 mm and reaches its maximum as tube diameter approaches that of the red cell, at which point blood viscosity probably approaches that of plasma.

Under constant conditions of flow haematocrit is the largest determining factor of viscosity, there being a progressively steep increase in viscosity as haematocrit increases. Empirically, haematocrit is related in a linear fashion to the logarithm of viscosity. Of the normal plasma proteins fibrinogen has the largest effect, particularly at very low rates of shear. The white cells and platelets in normal concentrations do not seem to

have a significant effect on blood viscosity nor do blood glucose or lipid levels. The commonly used anticoagulants in normal dosage do not significantly effect blood viscosity.

CHAPTER THREE

FLOW PROPERTIES OF BLOOD IN VIVO

Shear rates in the circulation

To what extent blood's anomalous flow properties can be expected to influence blood flow in the intact circulation depends upon what order of shear rate pertains in vivo.

As might be expected in view of the complex nature of pulsatile blood flow the exact shear rates existing in different parts of the circulation are not known accurately, and estimates very widely. According to Replogle (1967) who is enthusiastic about the clinical implications of blood rheology, the shear rate in the human aorta is in the order of 100 sec^{-1} and is about 10 sec^{-1} in the arteriolar bed. He also implies that shear rates in the micro-circulation approach zero and quotes the increase in viscosity (in vitro) which occurs between one sec^{-1} and 0.01 sec^{-1} (a factor of x40) as occurring at shear rates approximately those of the micro-circulation. However, he does not state how the shear rates he assumes are calculated but illustrates them in a diagram attributed to Merrill. Curiously enough Merrill (1969) concedes that in general the flow velocity is sufficiently high that mean shear stress in the vessels corresponds to the Newtonian part of the total shear stress - shear strain rate function. However he argues that polymer solutions which are Newtonian by viscometry may interact strongly where - as in the circulation - there are rapid rates of change of shear stress around an area of turbulance at a bifurcation or

obstruction, thereby dissipating energy and reducing the driving force. He also considers that red cell interaction (related to fibrinogen concentration) in the pre-capillary region as red cells converge to enter the capillary may have considerable significance. Finally, alluding to the stop/start nature of flow in the micro-circulation (Fulton, 1957)Replogle considers that the relevance of blood rheology to physiological fluid mechanics is to make stopping of flows easier, starting of flows more difficult and slow flows more energy consuming than would be expected if blood were a simple cell less micro-molecular fluid of equal viscosity. These effects are increasingly emphasised with increase of haematocrit and fibrinogen concentration.

Whitmore (1968) quotes the values of shear rate shown below - based on calculations of flow rate and size of different vessels from several authors, assuming parabolic velocity profile.

Rates of Shear in the Circulation

	At the wall (sec^{-1})	Mean (sec^{-1})
Ascending Aorta	190	130
Descending Aorta	120	80
Large Arteries	700	470
Capillaries	800	530
Large Veins	200	130
Vena Cava	60	40

McDonald (1974) quotes figures of shear rate at the wall of 80 sec^{-1} for the ascending aorta, 28 sec^{-1} for the inferior vena cava, 400 sec^{-1} for an arteriole and 400 sec^{-1} for a capillary. Burton (1965) considers that it is justified to use as an approximation an almost constant viscosity coefficient in the physiological range of blood flow.

It is evident that there are no reliable hard data on the shear rates pertaining in the intact circulation. In any event shear rate must be constantly changing throughout the cardiac cycle, and a further complication is the presence of a large reverse flow component in the larger more peripheral arteries. The high rates of flow in large arteries - down to arteriolar level - would make it likely that shear rates must be high enough for the blood to be regarded as Newtonian throughout most of the cardiac cycle. As far as the microcirculation is concerned opinions regarding shear rate are varied, but it is probably futile to regard blood rheology in bulk flow terms as the ratio of vessel size to red cell approaches unity. What is undeniable however is that blood flow comes to a halt intermittently in the micro circulation, and that from time to time blood flow in the larger veins is static, or nearly so. Thus even in the normal circulation conditions of very low or zero shear rate must apply for some of the time. Presumably, then, in the critically ischaemic limb rheological factors must play more of a part, and it is easy to imagine a cycle of reduced flow due to arterial obstruction leading to increased blood viscosity and a further reduction in flow and so on.

In particular, from the available in vitro data, the combination of raised haematocrit or raised fibrinogen level and reduced flow due to obstructive arterial disease must be particularly disadvantageous. Thus Dormandy's concept of 'rheological claudication' (Dormandy 1973a) - where relatively minor arterial disease assumes clinical significance because of a rheological abnormality has a sound theoretical basis.

Blood Viscosity in Vivo

The stated opinions as to whether blood's theoretical and in vitro properties are applicable in vivo vary from a firm view that they are (Replogle, 1967;Merrill, 1969) to an equally firm opinion that they are likely to be irrelevant (Lancet Editorial 1977; McDonald, 1974; Burton, 1965) - because of the high shear rates thought to pertain in vivo and the special nature of flow in the microcirculation.

Attempts to measure blood viscosity in vivo are beset with difficulty, since so many different factors in addition to the intrinsic flow properties of blood govern the regulation of blood flow. There are few publications on this subject.

The classic and oft quoted study of blood viscosity in vivo is that of Whittaker and Winton (1933). They noted that early studies of blood flow in tubes (Denning and Watson, 1906; Hess, 1907) showing variation with tube size and flow rate were incompatible with Poiseuille's observations and made the prediction of the viscosity of blood in a vascular bed quite impossible. They pointed out that although text books of physiology quoted a value of about 5 for the viscosity of blood (relative to water), values of between 2 and 100 times that of

water may be obtained depending on the conditions of measurement. Whittaker and Winton constructed a heart-lung (later pump-lung) preparation in which they perfused an isolated canine hind limb with, at different times, defibrinated blood of varying haematocrit, plasma and ringer lactate. Quoting the constancy of the results with changing perfusates and flow rates they argued that the diameter of the vessels did not change significantly during the perfusions and that therefore, by comparing the perfusion pressures required to maintain the same flow rates for blood and plasma, the viscosity of blood relative to plasma - the relative viscosity - could be calculated. These values were compared with data from a high velocity Ostwald type of viscometer. At 90 mm Hg pressure Whittaker and Winton found the apparent viscosity of normal defibrinated blood to be 2.2 ± 0.2, about one half the value given by the glass viscometer. They attributed the low value of the apparent viscosity to the small diameter of the blood vessels in which most of the arterio-venous pressure fall occurs, invoking the Fahraeus-Lindqvist effect (the increasing relative importance of the cell free outer zone as tube diameter decreases) as explanation.(It has been pointed out that a reduction in viscosity of this magnitude occurs in capillary tubes of about 30-40 μ which is approximately the size of the terminal arterioles and venules in which most of the pressure drop in the human limb occurs (Strandness, 1975)).

Determination of relative viscosity in a vascular bed was the main object of what must have been a highly temperamental experimental arrangement (3 pump-lung preparations were used in

parallel, involving 260 glass-rubber connections), and low flow states were not particularly studied. However, Whittaker and Winton did note that if the arterial pressure was less than 50 mm Hg, viscosity increased slightly and that at higher corpuscular concentration the increase of viscosity with reduction of arterial pressure was greater.

With regard to the relationship between 'hind limb' viscosity and haematocrit they concluded that although the actual values of the apparent viscosity of blood are widely different in the limb and in a viscometer, the ratio of the viscosity of an abnormally concentrated blood to that of normal blood is not very different in the two, and viscometry readings yielded a fair indication of the increased resistance to circulation in polycythaemia. They did not feel that viscometry would be helpful in low haematocrit conditions because of the wide variations between hind limb and viscometer data.

Interestingly the findings of Whittaker and Winton with regard to the discrepancy between blood viscosity in vitro and vivo were confirmed more recently (Benis, 1973) but this newer work demonstrated that the cause of the phenomenon could not be due to an alteration in cell distribution (as in the Fahraeus-Lindqvist effect) since a similar effect was observed when cell free perfusates of different viscosity were used.

Benis considered the likely explanation to be the occurence of non linear inertial pressure losses in the larger vessels of the hind limb, the effect of the pressure losses disproportionately affecting the fluid of lower viscosity and thereby reducing the relative viscosity of blood and plasma. This more recent work

however, confirmed the basic relationship between resistance to flow in the hind limb and haematocrit. This is not dissimilar to the published results of in vitro viscometry and illustrates the progressive rise in resistance to flow as haematocrit increases.

Again however, Benis et al did not study blood under low flow conditions and the 'blood' they used was in fact a suspension of washed red cells in ringer albumin solution, and therefore did not contain fibrinogen (or globulins). It was perfused in a non-pulsatile fashion and was not oxygenated. Previously the same authors (Benis, 1970) had conducted similar experiments in which the effect of haematocrit on pressure-flow relations in the perfused (steady flow) isolated hind paw of the dog had been studied. Again they found that the pressure flow relationship was non linear and this they attributed to non Newtonian viscosity and vessel distensibility as well as inertial pressure losses. At physiological perfusion pressures, inertial losses accounted for about 40% of the total pressure drop for cell free albumin ringer solution, and decreased to about 5% for blood of haematocrit 50.

Levy and Share (1953) also studied the influence of erythrocyte concentration on the pressure-flow relationship in the dog hind limb in a preparation in which the sciatic and femoral nerves were divided, cannulae inserted into the femoral artery and vein and the collateral circulation prevented by applying two wire tourniquets about the remaining tissues of the upper thigh. Perfusion of blood was performed directly from the dog's own femoral artery and haematocrit varied by replacing an

arbitrary quantity of the dog's blood by either packed cells or plasma. The authors considered that this preparation represented 'moderate dilatation' and found that the pressure flow curves were parabolic, convex to the pressure axis. The logarithmic slopes were equal for all haematocrit ratios in the given experiments and therefore the relevant apparent viscosities were independent of variations in pressure. Their results also indicate that the degree of non linearity of the pressure flow curve is independent of haematocrit which is at variance with the results of Whittaker and Winton (1933) and Benis et al (1973). Levy and Share also studied pressure flow curves under conditions which they considered to represent maximal vasodilatation by perfusing the preparation with de-oxygenated blood after 10 minutes or more of ischaemia. In some of these experiments they also added sodium nitrite to the blood to ensure maximal vasodilatation. The blood was perfused in a non pulsatile fashion from a reservoir. For the 'maximal vasodilatation' preparation the curves relating pressure and flow were virtually linear, and the relative apparent viscosity 'intimately dependent upon pressure, becoming significantly greater when progressively lower reference pressures were chosen". They explain this paradox on the basis that in the non maximally dilated preparation there is a passive increase in the diameter of arterioles or an opening of previously closed capillary beds with increasing perfusion pressure. It appears however, that there were technical problems with their experimental model since only six out of fifteen denervated preparations were considered to be sufficiently free from fortuitous alterations of vasomoter

tone. Also the lowest haematocrit they used was 22%, the apparent viscosity of plasma being deduced by extrapolation.

Studies of a similar nature by Pappenheimer and Maes (1942) suggested that the pressure flow relationship was linear over a wide range but, rather like Whittaker and Winton they noted a deviation from linearity below critical values of flow. Conversely, Green (1944) found flow to be an exponential function of pressure in a series of experiments in which pressure flow relations were studied by recording either the arterial inflow or the venous outflow at a series of perfusion pressures in various vascular beds in the hind limbs of anaesthetised dogs.

It is evident from the foregoing that there is no clear consensus of evidence from isolated limb perfusion experiments as to the nature of in vivo blood behaviour at low flow rates, although there is agreement that resistance in vivo increases considerably with increasing haematocrit.

There appears to be only one study in the literature aimed specifically at determining blood viscosity in vivo at low rates of flow (Djojosugito, 1970). Djojosugito and his colleagues were concerned that the linear - or almost linear - pressure flow relationships obtained in the earlier studies quoted above were influenced by smooth muscle activity and suggested that active vascular smooth muscle adjustments were taking place to counteract the physical distention of the resistance vessels at higher pressures and flows (Folkow and Lofving, 1956). They felt that if the vascular bed is to be used as a viscometer all smooth muscle activity should be abolished and the passive-elastic behaviour of the vascular bed fully displayed. In their

experiments Djojosugito's group perfused the isolated calf muscles of the cat with blood from the contra-lateral femoral artery and controlled the rate of flow with a screw clamp on the polyethylene catheter connecting the donor femoral artery to the test preparation. Pressure flow curves were then obtained down to perfusion pressures of 10 mm Hg or less. In order to minimise changes in smooth muscle tone they obtained maximal dilatation by exercising the calf muscles with electrical stimulation of the sciatic nerve, and also infused what they describe as 'huge doses' of isoprenaline and acetyl choline (25-50 micrograms). Values for in vivo viscosity were obtained by comparing the pressure flow curve of blood with that of a Newtonian fluid of known viscosity (Dextran-Tyrode solution). They concluded, in agreement with previous work, (Whittaker and Winton, 1933; Benis, 1973) that apparent viscosity in vivo was approximately 50% lower than in vitro values, and that although in vivo viscosity increased at very low rates of flow it was as a maximum only doubled. This maximum viscosity occurred at a perfusion pressure of approximately 10 mm Hg,below which viscosity decreased again. They concluded that the steep rise of viscosity in vitro at low shear rates had no counterpart in vivo. These conclusions were however based on only six experiments and changes in haematocrit were not studied. An odd feature of their results is that the in vitro value of blood viscosity at high shear rate (230 sec^{-1}) varied remarkably with what would be expected. One animal haematocrit 37, had a viscosity of 5.7 cps while another, haematocrit 42, had a viscosity of 3.5 cps - a very wide

variation for which the explanation is not clear.

A rather different approach to the determination of in vivo blood viscosity was adopted by Frasher and colleagues at the University of Southern California. In a series of publications (Frasher, 1967a, b, 1968, 1971; Meiselman, 1971, 1972) they described a variable shear rate, non pulsatile capillary viscometer which measures the viscosity of blood as it flows directly from a chronic arterio-venous shunt between the carotid artery and jugular vein of the dog. The tubes used were of diameters 85 and 200 microns respectively and the shear rates obtained varied between 120 sec^{-1} and 1,000 sec^{-1}. Haematocrits ranged from 30 - 45. In particular, they studied the effect of heparinisation, platelet withdrawal and defibrination (by infusion of thrombin). They stated that neither heparin injection nor platelet removal affected the measured viscosity at either tube size, which is in agreement with previous studies. They analysed the effect of shear rate and haematocrit on apparent viscosity by regressing the measured apparent viscosity against shear rate and haematocrit according to two equations; one assuming a linear relationship, the other a power law. The power law relationship showed a distinctly better correlation again consistent with previous studies (Merrill,1969). More surprisingly they were unable to demonstrate a difference in measured viscosity between the 200 micron and 85 micron tubes, whose sizes are well within the range where the Fahraeus-Lindqvist effect would be expected to lead to reduction in haematocrit, and hence viscosity, of the blood issuing from the smaller tube. However, a difference was

detected between the effect of shear rate on viscosity in the two tubes, the regression coefficients being significantly higher in the 85 micron tube. This they assumed to be due to alterations in the flow patterns at the entrance and along the length of the measurement tubes.

In the only study, other than that reported later in this thesis, to investigate directly the effect of fibrinogen on in vivo blood rheology, Meiselman et al (1972) used their A-V shunt capillary viscometer to examine changes in viscosity during defibrination by infusion of thromboplastin, (later bovine thrombin), into the vena cava of the experimental dog. They assumed that any micro aggregates or thrombi produced would be retained by the lungs. Fibrinogen levels fell significantly and were reduced to less than 10% of the initial value for two hours or more. Measurements were made at three shear rates approximating to 560 sec^{-1}, 140 sec^{-1} and 28 sec^{-1}. They observed that viscosity decreased as shear rate increased, that apparent viscosity of dog blood is a direct function of haematocrit in the range 25-45%, and that the effect of fibrinogen on the rheological properties of dogs blood does not appear to be significant. Although not mentioned in the publication quoted above, this seems to be in contradiction to earlier accounts of the same, or similar, experiments contained in a review article by Wayland (1967) which describes a decrease in apparent viscosity with declining fibrinogen level and suggests also a role for products of fibrinolysis in determining the ability of fibrinogen to act as a cement in forming red cell aggregates - which are thought to be important in determining the rheological properties

of blood.

There are obviously limitations as to how far one can extend these observations, made under conditions of steady flow in a rigid capillary tube, and at a shear rate not sufficiently low for fibrinogen to be expected to exert a great effect on blood's anomalous viscosity. If the effect of fibrinogen on red cell interaction is important in the circulation one would expect it to be so in conditions where blood flow is either very slow or of a stop/start nature, or flowing through a series of complex junctions. None of these conditions is reproduced in Frasher and Meiselman's model.

There are obvious difficulties in the construction of pressure-flow curves for blood in humans, but in an ingenious experimental procedure involving venous occlusion plethysmography in human volunteers, Collins (1967) constructed pressure flow curves following sudden reduction in perfusion pressure. Pressure flow curves for the hand were parabolic, convex to the pressure axis (in line with the animal work described above), but when the skin was rendered ischaemic with adrenaline and pressure flow curves constructed for what seemed to be forearm muscle, the curves were concave to the pressure axis. This they attributed to auto regulation.

The underlying theme to this thesis is that it may be possible to treat inoperable arterial insufficiency by modifying blood flow properties. Encouragement for this is provided by reports of blood's anomalous low flow properties in vitro which, if reflected in vivo, would place great importance on the

increase in viscosity which occurs at low flow rates, this increase being related to fibrinogen concentration and haematocrit. However the effect of reduced flow and elevated haematocrit,and the effect of fibrinogen concentration at very low flow rates do not seem to have been adequately investigated in vivo.

CHAPTER FOUR

EFFECT OF DEFIBRINATION ON BLOOD FLOW THROUGH A CRITICAL ARTERIAL STENOSIS

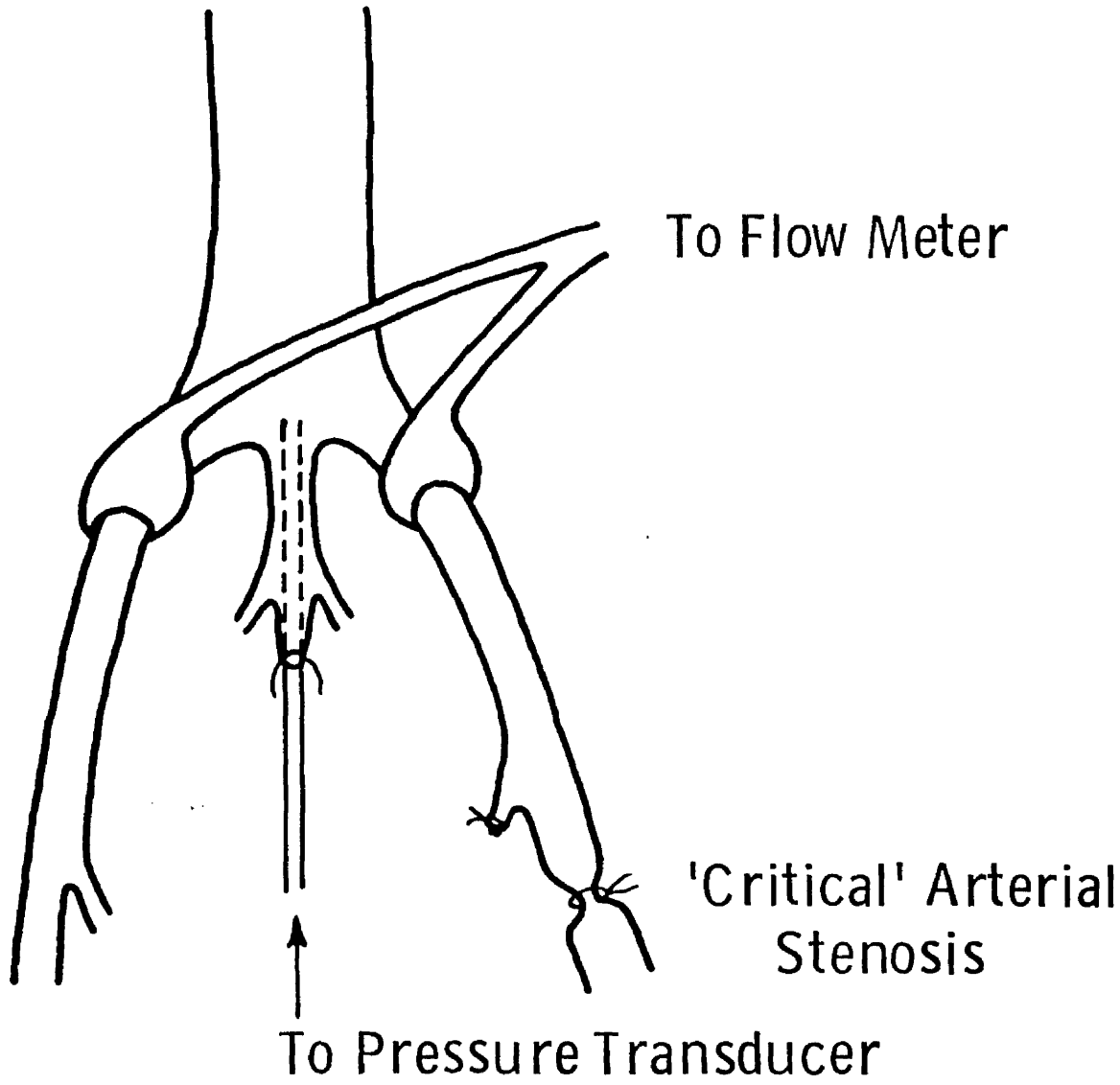
The difficulties of establishing the rheological properties of blood in the intact circulation are very considerable, and the bulk of this thesis is devoted to such work. However it seemed reasonable to determine in a simple canine model if the removal of fibrinogen from the circulating blood would increase flow in an ischaemic limb (Barrie, 1976).

Ancrod, a purified enzymatic derivative of the venom of the Malayan Pit Viper, breaks down fibrinogen to degradation products which are removed from the circulation by the reticulo-endothelial system, the resultant effect being complete defibrinogenation (Reid, 1968). Unlike the syndrome of disseminated intra vascular coagulation, other clotting factors are not affected, although the split products formed by the degradation of fibrinogen may exert transient anti-coagulant and antiplatelet actions. (Kowalski, 1964; Larrieu, 1966; Prentice, 1969).

Clinical reports of ancrod's effect in arterial disease have been conflicting: Ehringer et al (1972) claimed to show a beneficial effect with ancrod treatment in the therapy of peripheral ischaemia as did Ehrly (1973), but subsequent experience has been disappointing. Charlesworth's group used ancrod to treat patients with advanced ischaemia but were unable to show convincing improvement even in this group (Humphreys, 1977).

Materials and Methods (Fig 7)

Eleven mongrel dogs, weighing 18-22kg, were anaesthetised with sodium pentobarbitone, 30 mg/kg, and ventilated with air via a mechanical respirator. Thoracotomy was performed through the left fifth interspace and a non-cannulating electromagnetic flow probe placed around the ascending aorta. A lower mid line abdominal incision was made and the aortic trifurcation exposed. The incision was extended into the left groin to allow exposure of the left iliac and femoral arteries. A polyethylene catheter (internal diameter 0.85 mm)was inserted into the abdominal aorta via the median sacral artery and the common internal iliac artery ligated. The catheter was connected to an inductance type pressure transducer (Hewlett Packard 1280) to allow continuous monitoring of aortic pressure. Electromagnetic flow probes were placed around both iliac arteries just distal to the aortic trifurcation. On the left side, all of the iliac and femoral arterial branches (including the deep femoral artery) between the probe and the superficial femoral artery were ligated. The right ilio-femoral arterial supply was not disturbed. After an interval to allow stabilisation of the preparation, the values of cardiac output, arterial pressure and each iliac arterial flow were recorded. A "critical" arterial stenosis was produced by tying a ligature around the left superficial femoral artery and reducing the flow to the minimum that could be obtained without occluding the vessel completely. The preparation was then allowed to stabilise for approximately 45 minutes. Animals comprising the treated group, 7 in all, were infused intravenously with ancrod 1 unit/kg in



Experimental Model for Ancrod /Critical Arterial Stenosis Experiments

FIG 7

50 cc normal saline over 30 minutes. Four control animals were given a similar volume of saline only. The haemodynamic variables of the animals were recorded on a direct writing recorder for the remaining three hours of the experiment. Zero flow references were obtained periodically for the iliac probes by occlusion of the arteries just distal to the probes. On the left side the absence of collateral supply was confirmed by checking that zero flow was obtained by occlusion of the superficial femoral artery immediately proximal to the stenosis. The diastolic quiet interval was taken as zero flow for the ascending aortic probe.

Results

Table 1 shows the mean changes, with standard deviations, in cardiac output and iliac arterial flow in the four control and seven treated animals. In both groups, cardiac output fell during the experiment, the decrease being greater, but not significantly so, in the treated group (Figure 8).

Although there was considerable individual variation in both treated and untreated animals the mean iliac arterial flow in the non ischaemic limb decreased by a greater amount than cardiac output, there being no significant difference between the treated and untreated animals (Table 1, Column 1).

By contrast, there was a distinct difference between treated and untreated animals with regard to flows through the ischaemic limb (Figure 9; Table 1, Column 2). During the three hours after ancrod infusion the mean iliac flow of the control group decreased by 34% while the mean iliac flow of the treated group increased by 20%. Individually blood flow

TABLE 1

Comparative Effects of Ancrod on Blood Flow through Ischaemic vs Non-Ischaemic Limb

Time period after Ancrod Infusion Min.	1 Iliac Flow non-ischaemic Limb, ml/min		2 Iliac Flow ischaemic limb ml/min		3 Cardiac Output ml/min		4 Haematocrit, %	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Pre-treatment	218 (108)	204 (79)	74 (13)	49 (22)	3520 (920)	2860 (750)	44.8 (8.2)	43.6 (5.3)
30	221 (94)	185 (66)	70 (14)	51 (18)	3430 (710)	2800 (800)	44.8 (8.2)	44.2 (5.1)
60	202 (82)	170 (61)	62 (12)	53 (22)	3280 (670)	2780 (790)	44.8 (8.2)	44.3 (5.2)
90	184 (75)	156 (48)	60 (13)	55 (25)	3230 (740)	2450 (620)	45.1 (8.0)	44.0 (5.8)
120	176 (77)	161 (43)	57 (15)	60 (29)	3050 (780)	2560 (770)	46.8 (7.9)	43.8 (5.0)
150	173 (70)	153 (53)	53 (10)	60 (28)	3000 (670)	2129 (660)	46.0 (6.0)	44.8 (5.0)
180	174 (56)	148 (68)	50 (9)	66 (37)	3110 (880)	2000 (570)	45.5 (6.0)	45.0 (5.0)

Numbers in parenthesis indicate 1 SD.

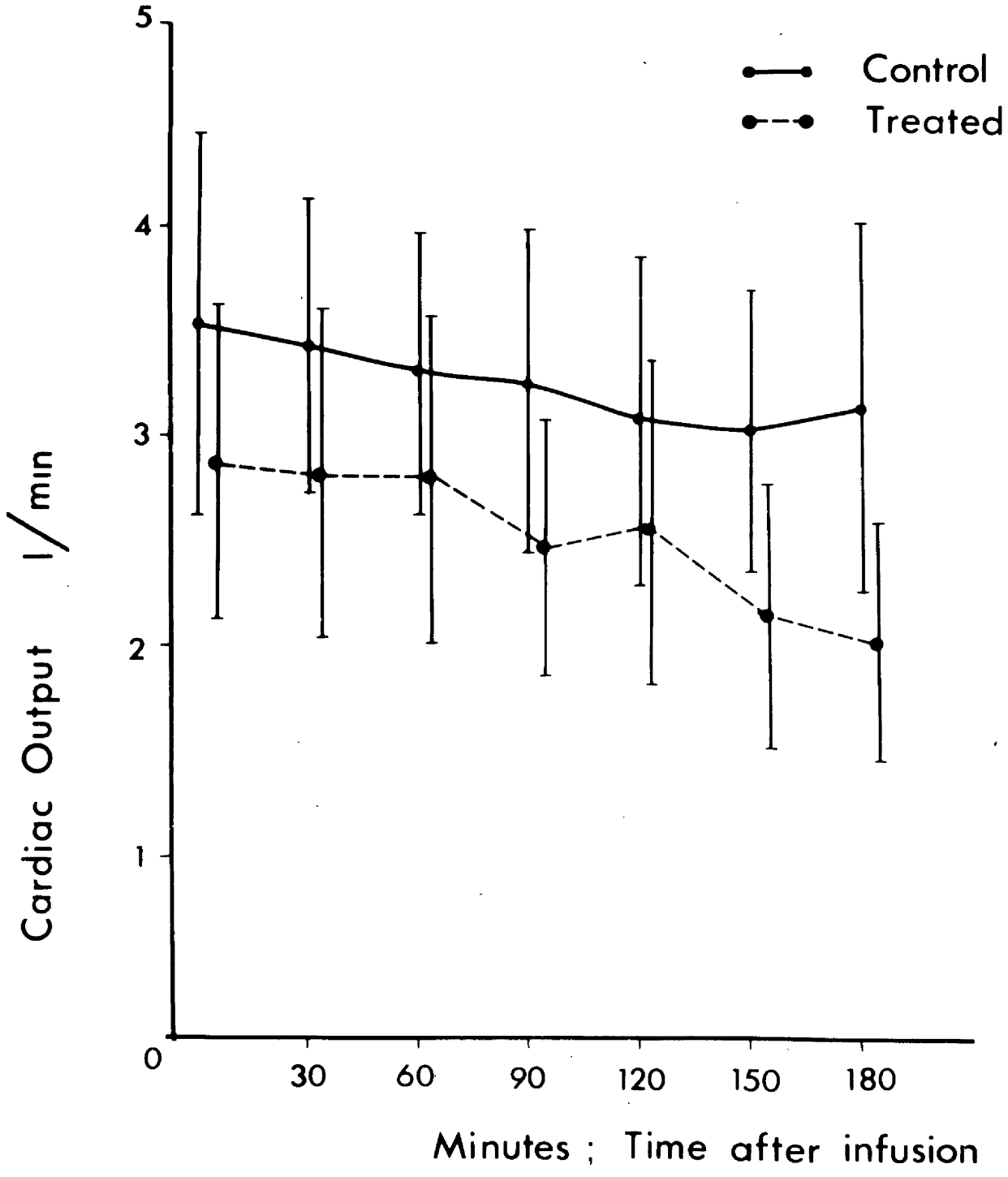

5
4
3
2
1
0
Control
Treated
Cardiac Output l/min
30
60
90
120
150
180
Minutes ; Time after infusion

FIG 8

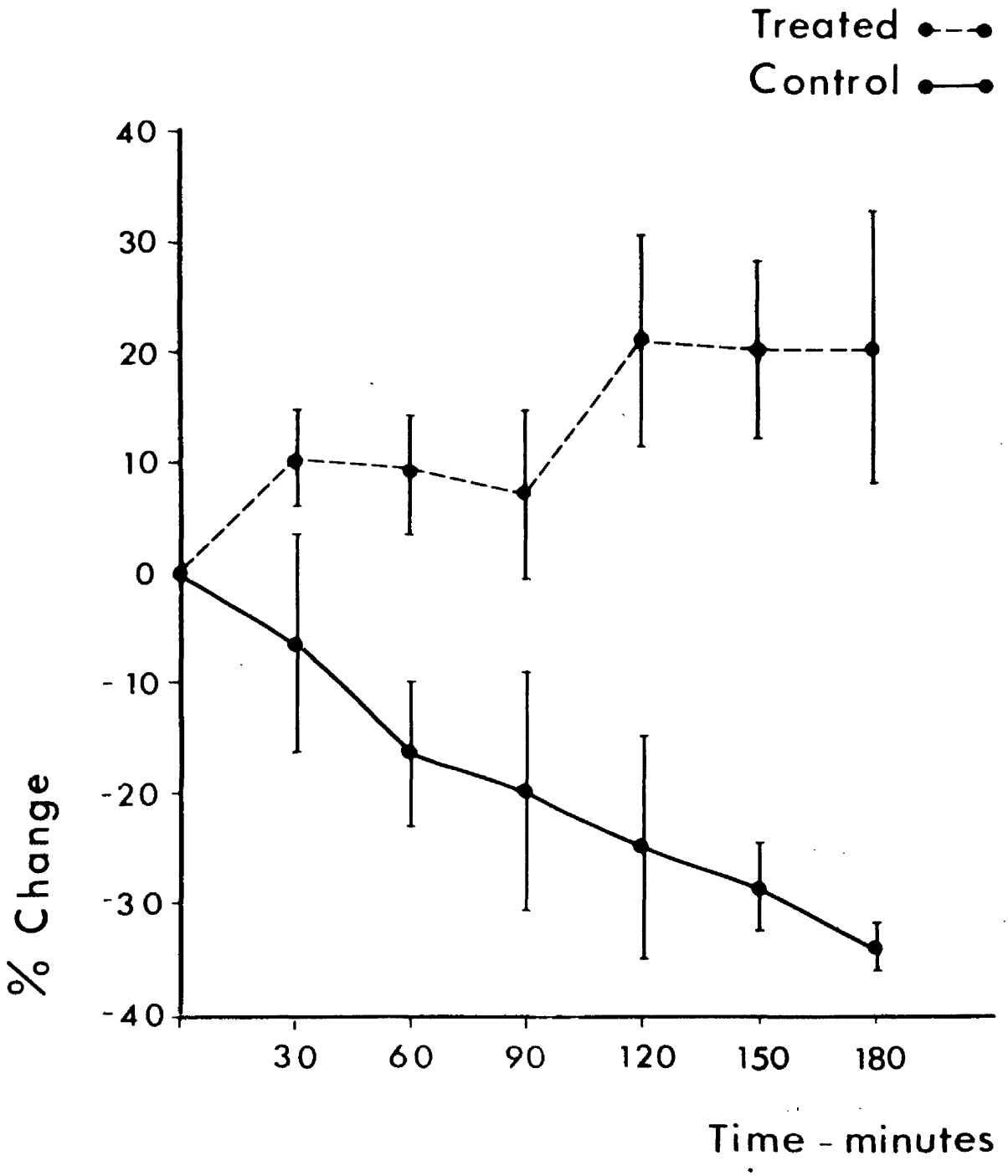

Treated
Control
40
30
20
10
0
-10
-20
-30
-40
% Change
30
60
90
120
150
180
Time - minutes

FIG 9

decreased in all the ischaemic limbs in the control group and increased in all of the treated group. After 60 minutes the difference between the two groups is statistically significant ($P < 0.001$). Since cardiac output decreased by a greater amount in the treated group this increase is all the more striking. During the three hours of the experiment the haematocrit remained constant in each group (Table 1, Column 4).

Discussion

The dose of ancrod administered in this study, 1 unit/kg is sufficient to reduce the plasma fibrinogen concentration in dogs to very low levels (unmeasurable by routine methods) within two hours (Reid, 1968). Although viscosity measurements of blood differ with the type of viscometer used and the rate of shear at which they are measured, there is general agreement that defibrinogenation reduces blood viscosity. At the shear rates encountered in the normal limb (thought to vary from 100 sec^{-1} to 10 sec^{-1}) the reduction in whole blood viscosity achieved by defibrination is in the order of 20% (Ehrly, 1973). However, as shear rate is reduced, the viscosity of normal blood increases and there is good evidence that a yield stress exists (Merrill,1969). It has been claimed that these effects are largely dependent on the presence of fibrinogen, since suspensions of red blood cells in saline have been shown to behave in an almost Newtonian fashion (Merrill,1969). Thus, at extremely low shear rates, the concentration of fibrinogen may

have a profound effect on the flow properties of blood. This may explain why, in our experiments, defibrination with ancrod increased blood flow through the ischaemic, but not the normal limb of the treated animals.

These experiments involved a comparatively small number of animals, however, and a number of objections can be made to the above hypothesis.

Firstly, it is difficult to obtain a stable preparation with a stenosis of the type used and there is a considerable tendency for the flow rates to vary. The stability and accuracy of electromagnetic flow meters, particularly at low flow rates is a further difficulty and it may be relevant that some of the greatest percentage increases in flow are recorded where flow rates are lowest initially and thus errors of measurement potentially greater. The control group by chance did not appear to have as tight a stenosis as the treated group, who therefore had lower initial flow rates. The cardiac output and limb flows in the control animals, and non ischaemic limbs in the treated animals, fell considerably as did cardiac output. The decreased cardiac output is probably not related to defibrination since almost as large a decrease occurred in the control animals as in the ancrod treated group, but may be attributable to the pentobarbitone anaesthesia. Indeed, Nash et al (1956) found a steady decline in cardiac output to 50% of the original value after three hours of pentobarbitone anaesthesia. Also, in these experiments, to prevent possible haemodilution and resultant decrease in blood viscosity, virtually no supportive intravenous fluids were given.

If ancrod effectively reduces blood viscosity in vivo it would have been expected that cardiac output would increase compared to controls, but the opposite effect was observed, implying that in vivo viscosity was not reduced.

An alternative explanation of these results could be that the highly efficient anticoagulant effect of ancrod prevented the decrease in flow which occurred in the control ischaemic limbs - where clot formation could have occurred - and allowed some form of autoregulation to take place in the anticoagulated ischaemic limb.

Although these results, in a small number of experimental animals, are of interest, it may be that this particular model does not sufficiently separate changes due to rheological factors from those due to, for example, alterations in vascular tone or blood coagulability.

CHAPTER FIVE

EXPERIMENTS USING ISOLATED HIND LIMBS

In a vascular bed peripheral resistance, that is the ratio of perfusion pressure to flow, has two components, the intrinsic resistance of blood to flow - its viscosity - and a component related to the dimensions and geometry of the vascular bed. Unfortunately for the investigator vascular dimensions are subject to very large variations and are influenced by both local and systemic factors. It follows that to measure viscosity in vivo one requires to know simultaneously the pressure and flow rate through the particular vascular bed, and either to keep the geometric component constant or know enough about the changes to apply a correcting factor. In practice, this is unlikely to be achieved.

The control of vasomotor tone is an enormously complex subject which can only be summarised briefly here. The nervous system constantly alters vascular tone and cardiac output to meet the varying demands of tissues, maintain systemic arterial pressure and still preserve the essential functions of heart, brain and other vital organs. This control is largely exercised via the sympathetic nervous system, which appears capable of producing a vasodilator effect, in addition to its more commonly recognised vasoconstrictor action. The parasympathetic nervous system also has a vasodilator action, which may be mediated by a bradykinin like substance. The wide variety of vascular changes mediated through the nervous system are not predictable enough to be allowed for in calculating the

role of blood viscosity from a pressure/flow relationship and thus a suitable experimental model would require to be denervated.

However even in a denervated preparation wide changes in vascular dimensions, and therefore peripheral resistance may occur. The alpha and beta receptors of the arterioles are still sensitive - probably even more sensitive - to the effect of circulating catecholamines, bradykinins, histamine and other unidentified vasoactive substances. The vascular tonus of skeletal muscle is particularly liable to wide changes, probably due to the preponderance of beta receptors which are considered to be not innervated but activated by circulating l-ephinephrine or injected beta stimulators such as isoprenaline. Similarly skin has a most complex variety of available control mechanisms. Rushner (1970) remarks in a review of peripheral vascular control that the overwhelming mass of literature on the subject defies precise description and concludes that the vascular reactivity of skeletal muscles and skin are influenced by a bewildering number of factors.

Since the object of this thesis is to compare change in vascular resistance attributable to the viscosity of blood, the tone of the resistance vessels must remain reasonably constant or at least not change independently of the variable under examination.

Initially an attempt was made to examine changes in blood flow measured with the electromagnetic flow meter in the femoral artery of the intact dog with changes in the

composition of blood but it proved impossible to obtain a sufficiently stable preparation, even when the limb was sympathectomised. In addition, without isolating the entire hind limb it proved impossible to secure low enough rates of flow, since even when the femoral artery was ligated a substantial arterial input still occurred via collateral vessels.

For these reasons it was felt that isolated organ perfusion offered the only prospect of fulfilling the object of the study, to compare changes in viscosity with flow rate, haematocrit and fibrinogen concentration. A volume of literature exists on models using the canine hind limb and this appeared a suitable starting point (Table 2). To resemble in vivo conditions as closely as possible such a model should provide pulsatile flow with fresh oxygenated whole blood, and perfusion pressure and flow should be accurately measured over a wide range of pressure and flows, the procedure to be carried out at body temperature. It is essential to study the effect of haematocrit, in particular high haematocrits. None of the studies reviewed previously had fulfilled all of these conditions. With this in mind the model described here was developed (Barrie, 1977).

TABLE 2

Author	Preparation	Year	Temp	Oxygenated Blood	Pulsatile Flow	Composition of Blood	Range of Perfusion Pressure (mm Hg)	Range of Haematocrit %
Whittaker and Winton	Canine hind limb	1933	37°C	Yes	No	Defibrinated	over 40-150	0 - 80
Levy and Share	Partially isolated canine hind limb	1953	37°C	In some of the experiments	In some of the experiments	Normal		22 - 45
Benis et al	Canine hind paw	1970		No	No			
Benis et al	Canine hind limb	1973		No	No	Washed red cells in ringer albumen		
Pappenheimer & Maes	Canine hind limb	1942	37°C	Yes	No	defibrinated	approx.50-120	not studied
Green	Canine hind limb - various segments	1944	37°C or less	Yes	No	Normal	0 - 140	not studied
Smith and Shepherd	Altitude aclimatised canine hind limb	1974	37°C	No	No	Normal	40 - 200	20-40 and 60%
Djojosugito	Partially isolated gastrocnemius muscle of cat	1970	37°C	Yes	Variable-since flow controlled with gate clamp	Normal	10 mm Hg to normal	35 - 45
Wayland (1967) Frasher (1967a,b) Meiselman	Variable shear rate, capillary viscometer attached to canine arterio venous shunt		37°C	Yes	No	Normal, defibrinated, thrombocytopaenic	shear rates of 560 sec^{-1}, 140 sec^{-1}, and 28 sec^{-1}	25 - 45%

The Model

Mongrel dogs, weighing 18-25 kg were used for the experiments. Each animal was anaesthetised with pentobarbitone, 30 mg/kg intravenously, and ventilated with air via a piston ventilator. Small incremental doses of pentobarbitone were given as necessary to maintain anaesthesia for the duration of the experiment, some four to six hours usually.

Using electro-cautery one hind limb was completely isolated at the hip joint except for the femoral artery and vein, care being taken to avoid obstructing either of the femoral vessels. All collateral vessels were ligated or cauterized. When the limb was attached only by the femoral artery and vein 5,000 units of heparin were given intravenously to the donor dog and the vessels clamped. T shaped polyethylene cannulae (internal diameter 3.1 mm) were inserted into the divided ends of the femoral artery and vein and the clamps released. With the side limbs of the cannulae clamped the circulation was re-established via the donor dog, having been interrupted for less than one minute.

When the femoral artery and vein proximal to the T shaped cannulae were clamped and the side limbs of the cannulae opened, blood could be circulated via the 'test circuit' illustrated (Figure 10). This simple circuit consisted of a reservoir from which oxygenated blood was pumped in a pulsatile fashion, through connecting tubing to the femoral artery of the dog. The venous return was routed back to the reservoir, the donor dog being completely isolated from the hind limb circulation.

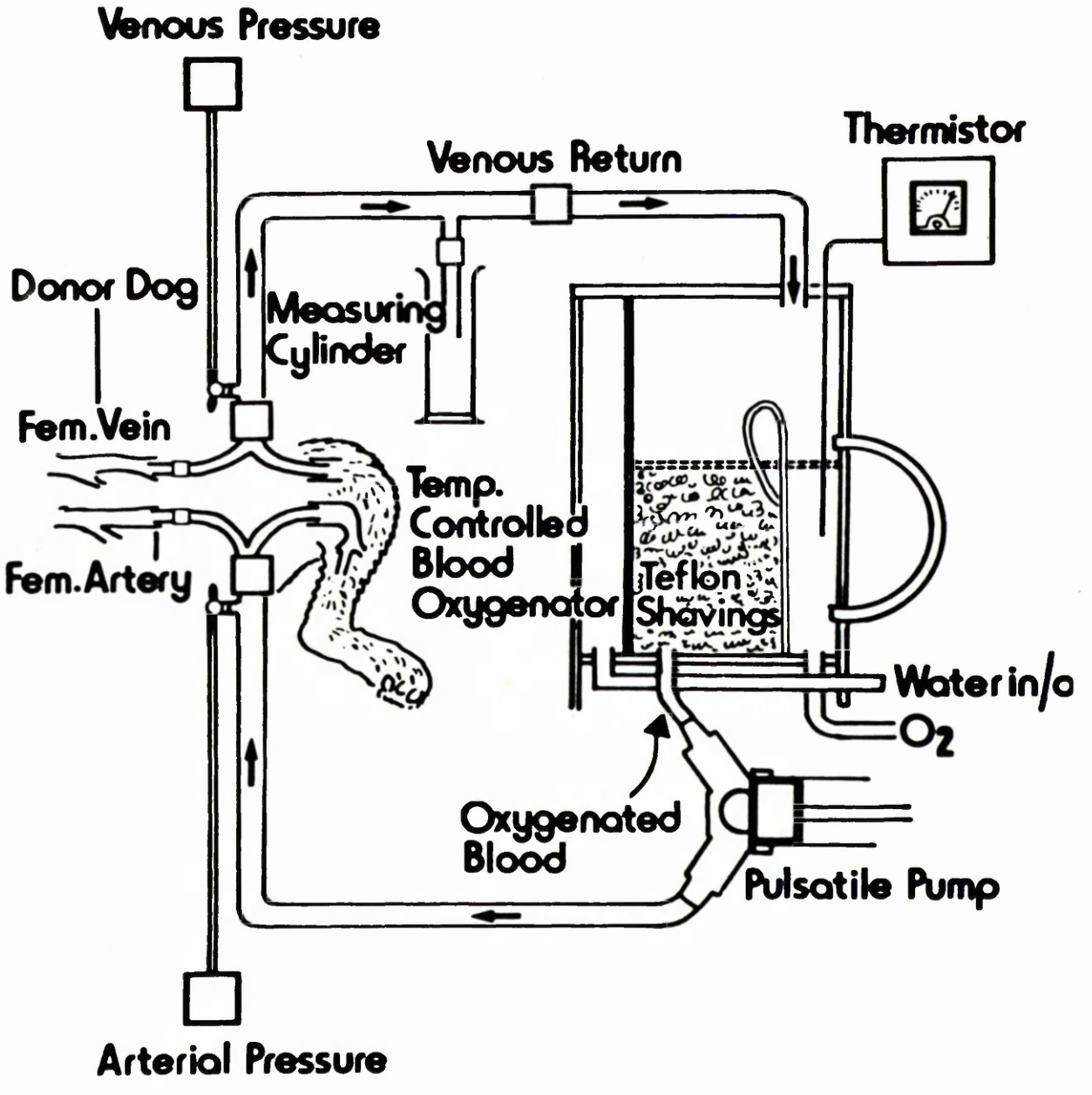

Venous Pressure
Thermistor
Venous Return
Donor Dog
Measuring Cylinder
Fem.Vein
Temp. Controlled Blood Oxygenator
Fem.Artery
Teflon Shavings
Water in/o
O2
Oxygenated Blood
Pulsatile Pump
Arterial Pressure

FIG 10

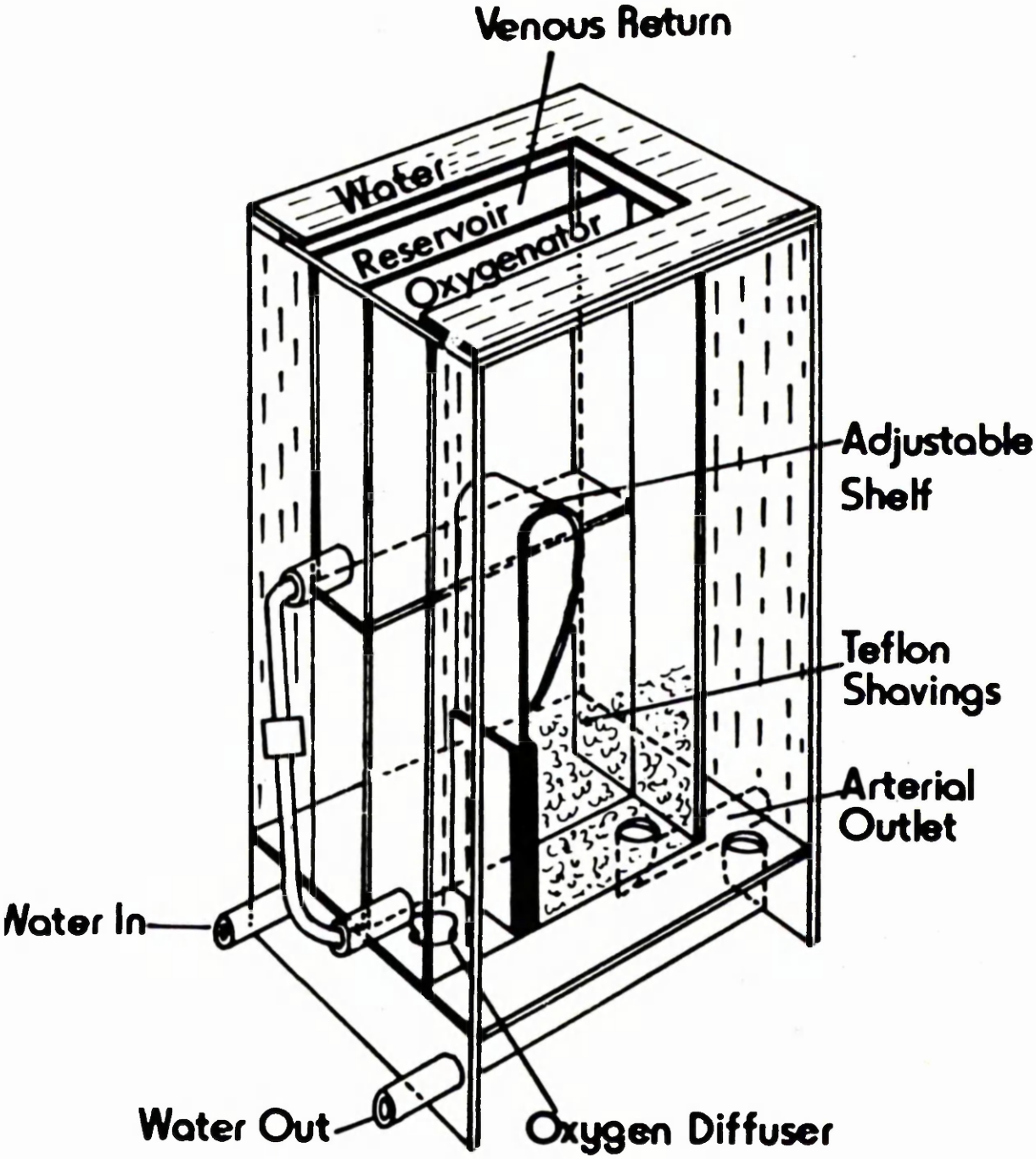
By means of a side arm the venous flow could be readily diverted to a measuring cylinder where the flow rate was measured with a stop watch. The arterial and venous pressures were accurately monitored by pressure transducers (Hewlett Packard 1280c) connected to ports immediately proximal to the limb, and recorded on a multi-channel recorder (Hewlett Packard 7700).

The Oxygenator

For the circuit a re-usable, low priming volume, temperature stable reservoir/blood oxygenator was required but no such device appeared to be available commercially. The apparatus described here was therefore constructed of readily available materials (Figure 11).

The unit, made of Plexiglass, consisted of a blood reservoir of approximately 800 millimeters volume, an oxygenation chamber and a de-foaming chamber, surrounded on three sides by a water jacket.

Blood passed from the reservoir to the oxygenation chamber where it was oxygenated by 97% oxygen/3% carbon dioxide mixture (from a commercially available cylinder) flowing through an oxygen diffusor obtained from a Travenol 2LF bubble oxygenator (normally used for human cardio pulmonary bypass procedures). The oxygenation chamber contained a movable partition, adjustment of which varied the height of the column of blood being oxygenated and thus provided a method of altering oxygenation in addition to alteration of the gas flow rate. The oxygenated blood bubbled over the partition into the defoaming chamber, where it was defoamed by contact



Schematic illustration of the oxygenator ;ed in these studies.

FIG 11

with Teflon shavings.

In the construction of the device $\frac{1}{8}$" (3.2 mm) Plexiglass was used for blood/water interfaces and 3/16" (4.8 mm) for air/water or air/blood interfaces. Water was circulated from a temperature bath by a submersible pump at a rate of 4 litres per minute. A water bath temperature of 40°C was normally required to maintain the blood in the oxygenator at 37°C.

At the blood flow rates required in our experiments, 5-300 mls/min, 95-100% haemoglobin oxygen saturation was obtained with O_2/CO_2 flow rates of 0.2 - 0.4 litres per minute. By adjustment of the gas flow rate the arterial $P^{O}2$ and $P^{CO}2$ could be maintained within reasonably normal physiological limits.

The Pump

For these experiments a pump was required which would have both a pulsatile action and be capable of providing flow rates of from virtually zero to 400 mls/min. Such a pump, designed for isolated organ perfusion, or other situations where human cardiac output is to be mimicked, is marketed by Harvard Pumps Limited, Model 1405. Flow could be varied with this pump in two ways; the stroke rate could be varied from 0-200 strokes per minute and the stroke volume from 0-10 c.c. Each of these could be varied independently giving a flow range of from 0-2,000 mls/min. Since the object of these experiments was to mimic the ischaemic limb it was decided to fix the stroke rate at 100 beats per minute and achieve changes in flow rate by varying the stroke volume. A screw adjustment provided fine and reproducible control over this. When the screw was turned

clockwise the piston was held shut and the stroke volume became effectively zero. By rotating the screw anti-clockwise a progressively greater stroke volume could be achieved. This adjustment could be made while the pump was running and thus during an experiment, by altering the stroke in increments from 0-4 cc,the flow rate could be altered from 0-400 cc per minute.

The connecting tubing used in the perfusion apparatus was Tygon (Medical Grade) tubing of internal diameter 3/16" (4.8 mm). Y shaped connecting pieces were used to provide ports for the pressure transducers while introducing minimal disturbance to flow. Similar connecters were used for the femoral vessels, and to provide a means of diverting the venous flow to the measuring cylinder. By appropriate positioning of clamps blood flow could be diverted as required from donor dog to perfusion apparatus and venous return to the measuring cylinder instead of the reservoir.

Although the apparatus was dismantled and thoroughly cleaned with antiseptic solution (5% Hexachlorophane) before and after use, in view of the relatively short duration of the experiments no attempt was made to render it completely sterile. Prior to use any residual antiseptic was flushed out by circulating sterile saline through the apparatus for 15 minutes.

Experimental Procedure

The aim of the experiments was to obtain a series of measurements of arterial pressure, venous pressure and flow rate at mean arterial pressures ranging in increments from normal to about 5 mm Hg and increasing again in similar

increments. To try and exclude the influence of changes in vessel size, on occasions the reverse order was employed, the starting point was in the middle of the perfusion range, or the order was randomly varied.

The test blood was placed in the oxygenator and the clamps arranged so that blood was pumped via the test circuit. The first 200 cc or so of blood issuing from the limb was diverted to a measuring cylinder and discarded to try and keep mixing of the test blood with the limb donor dog blood to a minimum. The venous blood was then returned to the reservoir to complete the circuit. At each change in the flow rate the pressure tracings (venous and arterial) were allowed to stabilize - which occurred in a few seconds - and the flow recorded. The flow rate was then changed by increasing or decreasing the stroke volume of the pump until a series of measurement had been made with each point having been approached from below and above.

Samples of blood were taken at the start and finish of each experiment for measurement of haematocrit, plasma protein, fibrinogen, and in vitro viscosity.

On completion of perfusion of test blood the clamps were rearranged and the limb perfused by the limb donor dog. The composition of the test blood could then be altered or replaced as required. In practice it was possible to change the composition of the test blood up to 4 times during one experimental session.

At least once during each experiment a series of pressure/flow measurements were made with Dextran 40/10% normal saline at 37°C in order to provide a standard with which to compare the results from different experimental preparations. Dextran 40 seemed to lend itself to this purpose since it is commonly used in human intravenous infusions as a plasma volume expander and is not considered to be vasoactive. Its effect on blood viscosity is now agreed to be limited to that obtained by dilution (Kilman, 1967; Dormandy, 1971), despite early reports to the contrary. By coincidence its viscosity (approximately 4 centipoise) approximates to the of "normal" blood, that is blood of haematocrit 40 measured at shear rates over 200 sec^{-1}, the asymptotic value.

Alteration in Haematocrit

In the experiments in which haematocrit was to be varied blood was drawn from donor dogs into citrate phosphate dextrose collection bags, of the type used for collecting human donations for transfusion purposes. Still in the collection bags this blood was then centrifuged at 3500 r.p.m. for 5 minutes and stored for the duration of the experiment in a water bath at 37°C until ready for use, a period which was not greater than 3 hours. The bags were kept vertical so that the supernatant plasma could be decanted, and the plasma and concentrated red cells thereby obtained remixed in suitable proportion to provide test blood of haematocrit less than 10 to greater than 70. As far as possible direct exposure of blood to air was avoided.

Alteration in Fibrinogen Concentration

The fibrinogen level in the test sample was varied in one of two ways:- reducing the fibrinogen concentration by defibrinating the donor dogs' blood, or raising the fibrinogen concentration by adding canine fibrinogen to the test blood.

Defibrinated blood was obtained by treating blood donor dogs with ancrod. Ancrod is a proteolythic enzyme derived from the venom of the Malayan Pit Viper (Reid, 1968). It has a thrombin like action on fibrinogen but whereas thrombin splits off both fibrinopeptide A and B from the fibrinogen molecule, with the formation of a stable polymer, fibrin, ancrod splits off only fibrinopeptide A and the resulting products are cleared from the circulation by the reticulo endothelial system. This results in the effective removal of fibrinogen - and only fibrinogen - from the blood. Initially however, a high circulating level of fibrinogen breakdown products is produced, but their level falls to only slightly greater than normal after the initial reduction in fibrinogen level. For this reason the donor dogs in these experiments were treated with ancrod for 72 hours prior to the collection of blood. The dosage used, one unit per kilogram per day by intravenous injection, was sufficient to produce complete defibrination but did not have any obvious toxicity, other than a pronounced bleeding tendency. Dogs treated in this fashion thus provided a supply of blood, of very low fibrinogen concentration, but normal in other respects.

Ancrod added to blood in vitro will produce a form of clotting, since its effect in vivo depends on the reticulo endothelial system to clear the breakdown products of fibrinogen. Since a slight amount of mixing occurs with the blood of the limb donor dog when the blood in the perfusion circuit is changed, any ancrod remaining in the blood donor dogs' circulation was inactivated by the addition of anti-ancrod, a specific antidote prepared from goat gamma globulin (Provided by Abbott Laboratories, Chicago, USA).

The second method of altering fibrinogen concentration was to utilise a supply of autologous canine fibrinogen from a separate pool of donor dogs. Blood was drawn from these dogs into citrate phosphate dextrose collection bags and centrifuged at 3250 r.p.m. for 5 minutes. Purified fibrinogen was separated from this plasma by the method of Atencis (1965). The plasma was diluted with an equal volume of 0.09 M sodium citrate and centrifuged for 10 minutes to remove any remaining red blood cells or platelets. Two thirds of that volume of 4 M ammonium sulphate was added slowly and with stirring to the plasma-citrate mixture to precipitate the fibrinogen. After centrifugation at $4^{o}C$, the clear supernatant was discarded and the precipitate washed three times with 1 M ammonium sulphate solution. The precipitate was then redissolved in a volume of 0.005 M sodium citrate equal to the original volume of plasma and the fibrinogen again precipitated by the addition of one third that volume of 4 M ammonium sulphate. This precipitate was washed twice with 1 M ammonium sulphate, and, after the final centrifugation, was stored at $4^{o}C$ for a period of from

3-12 hours and then centrifuged to remove any cold - insoluble material.

Up to 1 gram of this purified fibrinogen was added to normal blood in each of four perfusion experiments.

Alteration in Temperature

The temperature of the test blood could be altered simply by appropriate adjustment of the temperature of the water bath used for storing the blood. The water from this bath also circulated through the water jacket of the reservoir/ oxygenator, and the heated pad on which the isolated limb lay.

Plasma Substitutes

Suspensions of red cells in saline or Dextran were prepared by simply resuspending the packed cells in the appropriate solution. Obviously a certain amount of 'trapped' plasma remains but this volume is small, and attempts to remove it by washing the red cells were considered undesirable in view of possible damage to red cells.

IN VITRO MEASUREMENTS

Measurement of Plasma Proteins

Plasma proteins were measured by the biuret method, a simple, quantitative colourimetric procedure which has been in use for a number of years. (Kingsley, 1942). Copper in the biuret reagent reacts with the peptide bonds of serum proteins to form a purple colour with an absorption maximum at 545 nm. The colour is proportional to the total protein concentration in plasma.

Measurement of Plasma Fibrinogen

Plasma fibrinogen was measured by an immunodiffusion technique using commercially prepared agar plates in which a specific fibrinogen precipitating antiserum is present in uniform concentration (M-Partigen, immunodiffusion plates, Hoechst Pharmaceuticals, Salisbury Road, Hounslow, Middlesex, UK). When a protein antigen in solution (i.e. the fibrinogen in the sample to be measured) is applied to a cylindrical application well, it diffuses radially into the thin film of agargel and combines with the antiserum forming a visible ring of precipitin. The fibrinogen concentration is proportional to the volume of the precipitin cylinder when diffusion ceases, i.e. when all the available fibrinogen has combined with antibody. Since the thickness of the gel is constant the area of the diffusion ring is proportional to fibrinogen concentration. In practice the plates were calibrated with standard fibrinogen solution, and each sample was measured twice, using three different dilutions each time. The mean of the values was taken as the fibrinogen concentration.

Haematocrit

Haematocrit was measured by the microhaematocrit technique, no correction being applied for plasma trapping.

Measurement of Blood Viscosity in Vitro

Over the years very many types of viscometer have been used, but only a few are suitable for the measurement of whole blood viscosity. Since blood is non Newtonian it is essential that viscosity is measured at several different, known, shear rates, and that measurements can be made on reasonably small samples. It is also desirable that the temperature of the sample be

maintained at 37°C.

Of the instruments in use, the simplest and least expensive is the tube or capillary viscometer, in which the rate of flow through a tube of known dimensions, at a given pressure difference, is measured. Until recently by far the most widely used instrument for the measurement of blood or plasma viscosity has been the Ostwald viscometer or variations of it (Figure 12). This is a type of capillary viscometer in which the time taken for a meniscus of test fluid to flow from a reservoir into a capillary tube is noted. This can either be done with the effect of atmospheric pressure only, or the device can be "pressurised" by applying a known pressure as a driving force. If the dimensions of the tubing are known precisely the viscosity of a Newtonian fluid can be calculated from Poiseuille's equation

$$\eta = \frac{\Delta P \pi R_1^4}{8 l Q}$$

This ignores the effect of pressure losses due to inlet and outlet changes for which corrections would need to be applied to obtain strict accuracy. In practice such viscometers are calibrated by measuring, at a particular pressure difference, the flow rate of a Newtonian fluid of known viscosity such as water, and comparing to it the flow rate of the test substance at the same pressure difference. Entrance and exit effects are assumed to be the same.

Capillary viscometers are very suitable for use with Newtonian fluids, where viscosity is independent of shear rate and thus the rate of shear at which the measurement is made is unimportant. They have the additional advantages of being

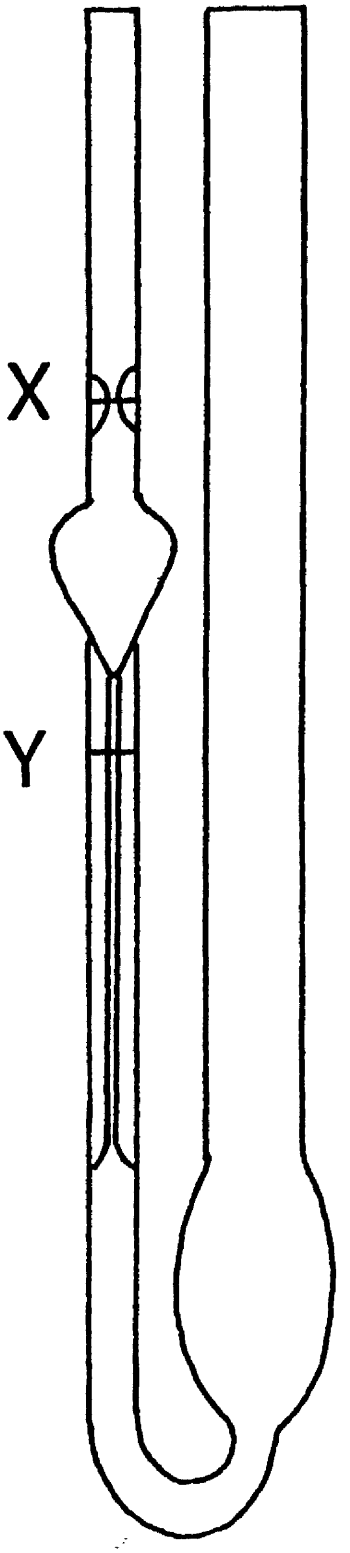


FIG 12

inexpensive, easy to operate, use small samples of test fluid, and give reproducible results. However, they are not suitable for use at low shear rates and they subject the samples being measured to a varying shear rate across the diameter of the tube (since shear rate is maximal at the centre of the stream but zero at the wall). Also, very fine capillaries have often been used since with a small tube radius streamline flow is ensured and a substantial pressure drop occurs. If the diameter of the capillary however is less than about 300 microns the viscosity of blood is artificially reduced (the Fahraeus Lindqvist effect) and diminishes with decreasing tube size. Thus capillary viscometers are not well suited to the measurement of whole blood viscosity but are reasonably suitable for Newtonian fluids such as plasma.

The second main type of viscometer in use depends on measuring the resistance of the fluid to a body moving through it. The best known of these are the various types of falling ball viscometer, but numerous other ingenious devices have been described using, for instance, a vibrating reed. The disadvantage which disqualifies these for use in measuring blood viscosity is the difficulty in knowing, and controlling, the shear rate being applied.

For the past few years it has been accepted that the rotational viscometers are the most suited to the measurement of blood viscosity. The principle is that the test fluid is sheared between two concentric, or more precisely co-axial cylinders, one of which rotates; or between a shallow cone rotating in a fixed cup, or slightly shallower cone. The

viscous properties of the fluid are calculated from the torque developed between the two components. The co-axial cylinder type of viscometer, sometimes referred to as a Couette viscometer, after its inventor, allows a reasonable approximation to constant shear rates, if the gap between the cylinders is small. For a Newtonian fluid viscosity,

$$n = \frac{K \cdot M}{V}$$

where M is the measured torque, and V the angular velocity of the rotating outer cylinder. The constant K is related to the radius of the cylinders and the depth to which the inner cylinder is immersed in the liquid.

The cone in plate viscometer is a modification of this principle. The cylinders are replaced by a plate - or flat bottomed cup - in which the sample is placed and suspended in it is a wide angled cone. The cone is usually truncated (i.e. the tip is cut off), and the bottom of the cone almost touches the plate. Provided an almost flat cone is used the rate of shear across the sample is virtually constant because, in moving further away from the centre of the cone, the increasing angular velocity is compensated for by the increasing depth of the sample being sheared.

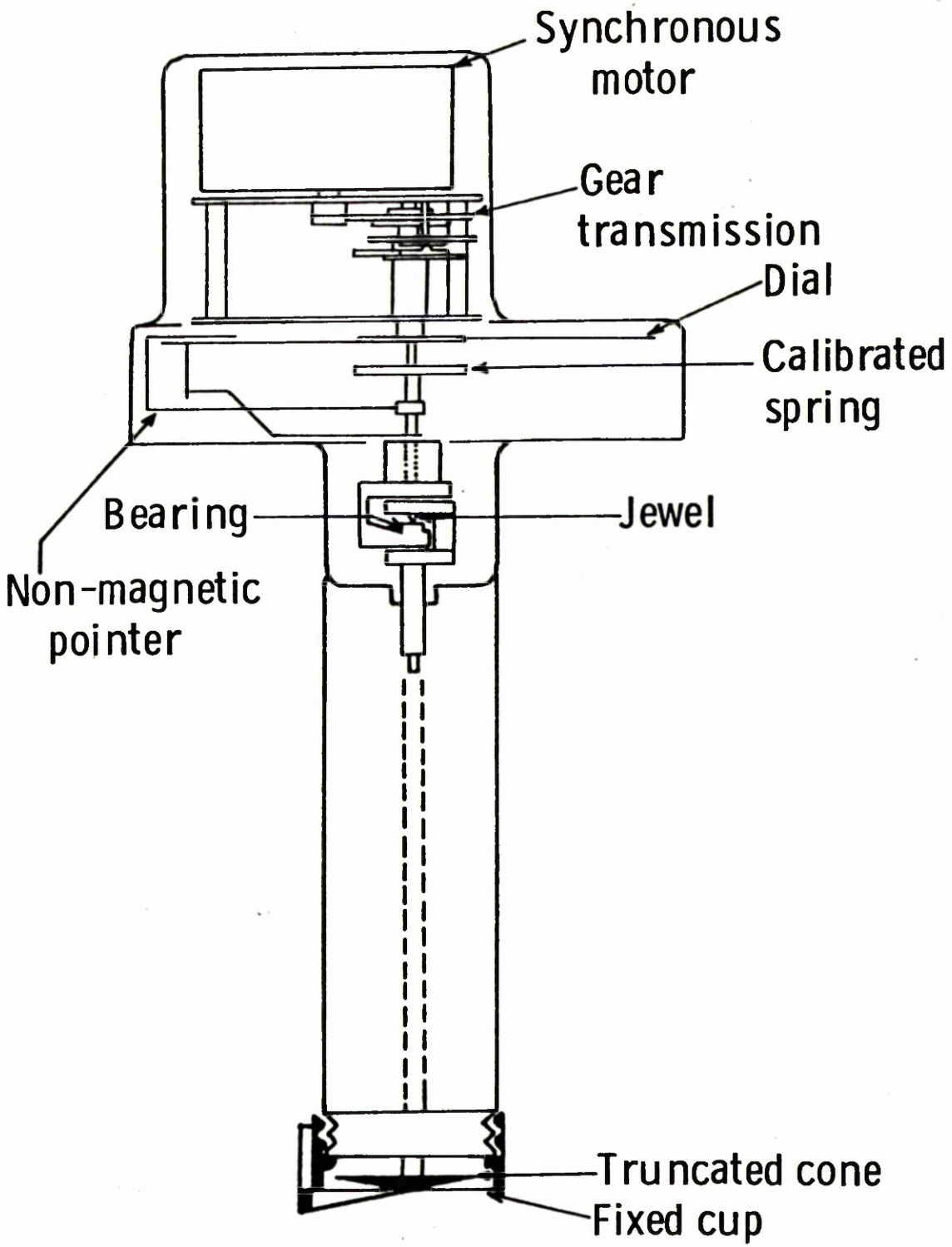
There are many more complex - and expensive - instruments designed to test the behaviour of visco-elastic substances, in which the lower cylinder, cone or plate oscillates and the curve registering the torque in the other component is analysed.

These are outwith the scope of this thesis.

The instrument used in the experiments described here is the Wells-Brookfield microviscometer. This is a modification of an industrial viscometer (Brookfield Synchro-electric Viscometer, Brookfield Engineering Laboratories, Stoughton, Mass. USA) and is of the cone-plate type.

The flat cone can be rotated at different speeds upon the fixed plate, thereby shearing the test blood at different pre-selected rates of shear. The resultant torque is a function of the shear stress in the blood. The shear rate can be calculated since the dimensions of the cone and the speed of rotation are known. It is therefore possible to obtain a series of measurements of shear stress at different shear rates and since viscosity is the ratio of shear stress to shear rate, viscosity at different shear rates.

In this particular instrument (Fig 13) torque is measured by a calibrated beryllium copper spring mounted in the drive shaft. Attached to the spring is a pointer which is shaped to indicate on a dial, the dial rotating in phase with the drive shaft,so that the deflection on the dial increases linearly with torque applied to the spring. The speed of rotation, and hence the shear rate, is selected by a simple clutch and reduction gear attached to the synchronous motor which powers the drive shaft. The speeds of rotation used in our experiments were:



WELLS-BROOKFIELD VISCOMETER

FIG 13

Revolutions per minute	Shear Rate (Sec^{-1})	Correction Factor to dial reading
60	230	x 0.1
30	115	x 0.2
12	46	x 0.5
6	23	x 1
3	11.5	x 2
1.5	5.75	x 4

The instrument is so calibrated that the dial, which is graduated from 0-100, reads directly in centipoise at a spindle speed of 6 r.p.m. corresponding to a shear rate of 23 sec^{-1}. For the other speeds an arithmetic conversion factor is required. It follows that at rapid shear rates the percentage error is less. The manufacturer claims an accuracy at any rotational speed of within 1% of full scale deflection.

The stationary plate is a metal cup, surrounded by a water jacket through which water is pumped from a water bath to maintain the sample at a chosen temperature. This unit is attached to a heavy aluminium cylinder projecting down from the motor housing, surrounding the drive shaft to the level of the cone. The cup device is held in place by a spring clip. A threaded adjusting ring provides the means of raising or lowering the cup relative to the cone, the optimum adjustment being when the cone just clears the plate.

The volume of blood sample required for the instrument is 1 cc but a slight excess does not introduce an error, since it flows on top of the cone and does not affect the transmitted torque.

The instrument is relatively inexpensive and simple to use. It overcomes the disadvantages of the capillary viscometer in that it allows estimation of blood viscosity at a range of shear rates, while avoiding the expense and complexity of the more specialised instruments. It is not designed to estimate blood viscosity at very low shear rates but is reasonably accurate down to a shear rate of 5.75 sec^{-1}.

CHAPTER SIX

RESULTS

Data from 30 separate limb perfusion models are summarised in this section. The pressure flow data from the experiments are shown in the appendix.

The results of two experiments, Nos. 6 and 7 are shown individually (Figures 14 & 15) as illustrative of the series. At each increment on the pressure/flow curve, the perfusion pressure (mm Hg) Δ P, (mean arterial pressure - mean venous pressure) is divided by the flow rate (cc per minute), Q, to give an arbitrary value for resistance, R.

$$R = \frac{\Delta P}{Q}$$

This is done for each of the bloods perfused and for Dextran. This value, R, is then plotted against perfusion pressure to show the relationship between resistance and perfusion pressure. It appears that the resistance (R) is virtually independent of pressure for blood of low haematocrit but as haematocrit increases there is a progressive deviation from linearity at low perfusion pressures. Inevitably in a preparation of this kind there is variation from experiment to experiment but in each model the same pattern was observed.

Method of Calculation

Although the limbs used in the experiment were approximately the same size, in order to compare data from one experiment with another some form of normalisation is required. Since each limb was perfused under the same conditions with Dextran 40/10% N Saline, by dividing each value of resistance for test blood by

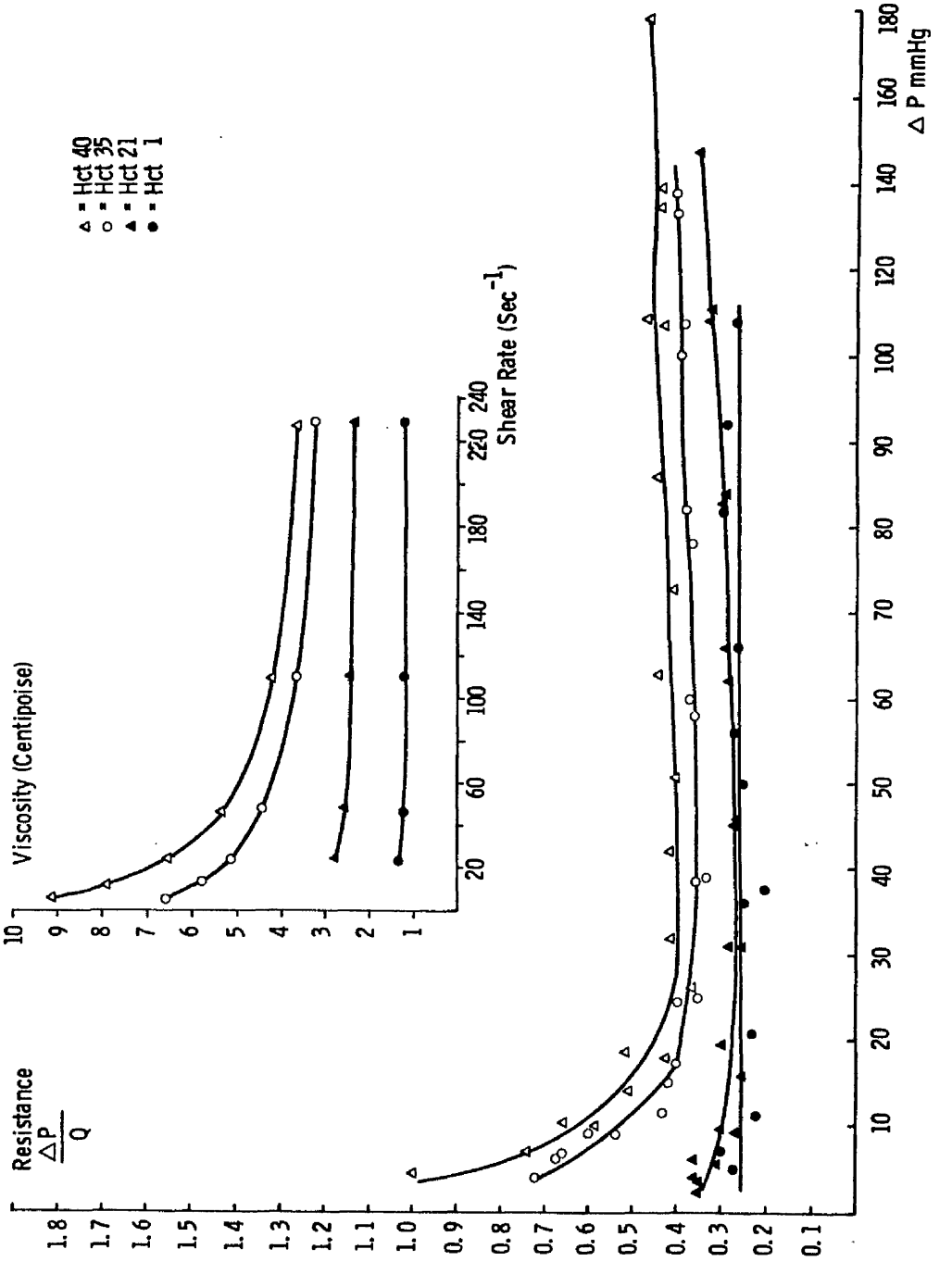


FIG 14 - Experiment 6

The inset graph is from the Wells-Brookfield Viscometer

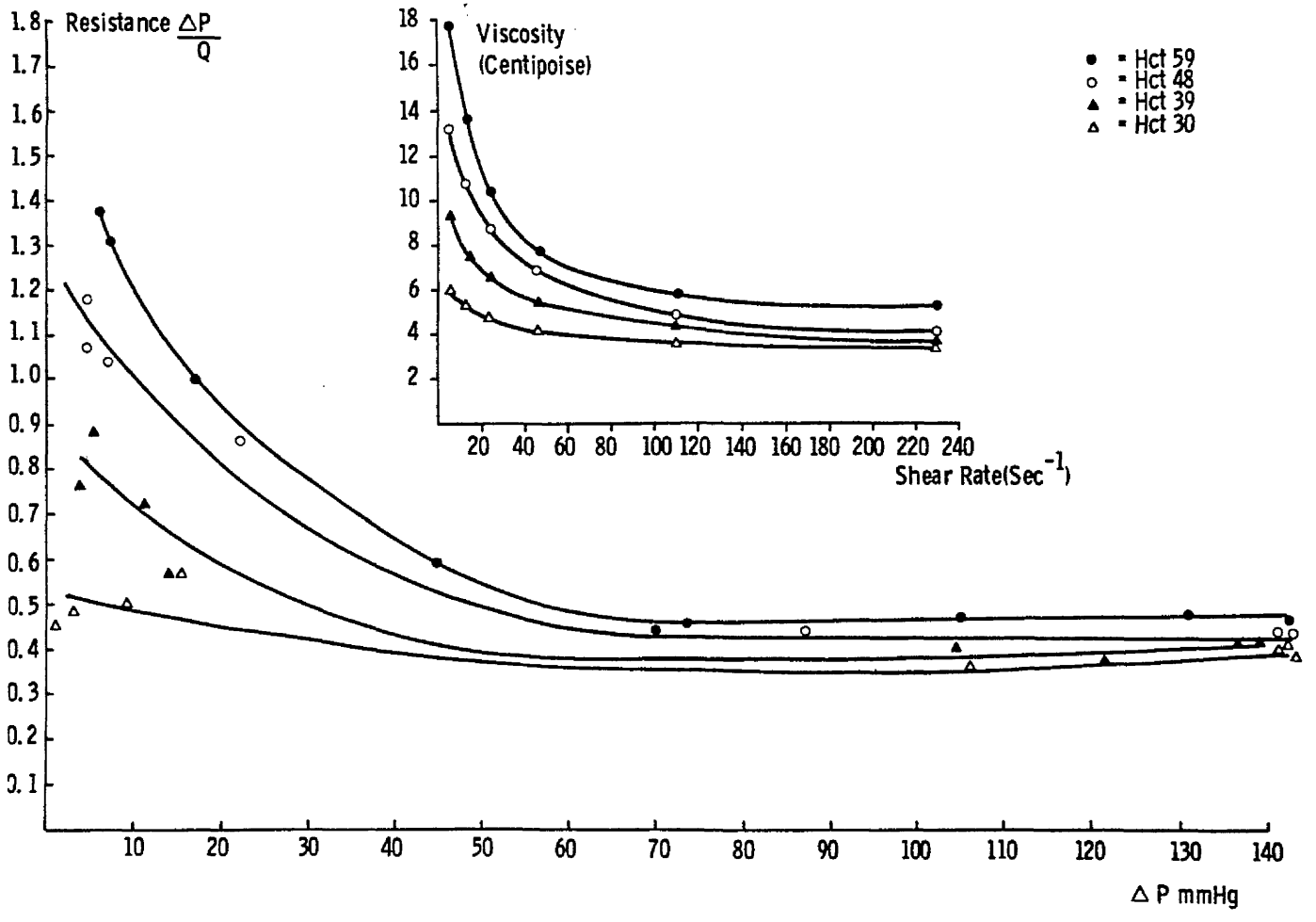


FIG 15 - Experiment 7

The inset graph is from the Wells-Brookfield Viscometer

the corresponding value of resistance for Dextran, a "normalised" resistance is obtained. In practice the Dextran perfusions were done fairly rapidly to minimise the length of time the limb was ischaemic, and perhaps because of this the resistance values obtained for Dextran were more scattered at the lowest perfusion pressures. To allow for this a 'Dextran' value for each experiment was obtained by taking the mean of the resistance values for Dextran at perfusion pressure greater than 30 mm Hg. This value ranged from 0.4 to 0.5. For each experiment each resistance value for blood was expressed as a percentage of this Dextran value thus making the results comparable from experiment to experiment. This method, however, assumes that the resistance of Dextran is constant. Although Dextran is effectively Newtonian in vitro (that is, its viscosity is independent of shear rate in a viscometer), the resistance as measured in the limb perfusion model tended to rise slightly at both the lowest and highest perfusion pressures. This trend was observed in almost all of the experiments, and presumably represents a characteristic of the limb itself - such as distensibility of the arteries - rather than a property of Dextran. Support for this view is provided by the fact that similar behaviour is shown by blood of haematocrit less than 20.

The magnitude of this variation was assessed by expressing each of the Dextran values as a percentage of the mean for Δ P greater than 30 mm Hg for that experiment. These values for all the experiments were then plotted against perfusion pressure. When the results are grouped according to perfusion pressure (Figure 16) it is seem that the values at the lowest (A) and the

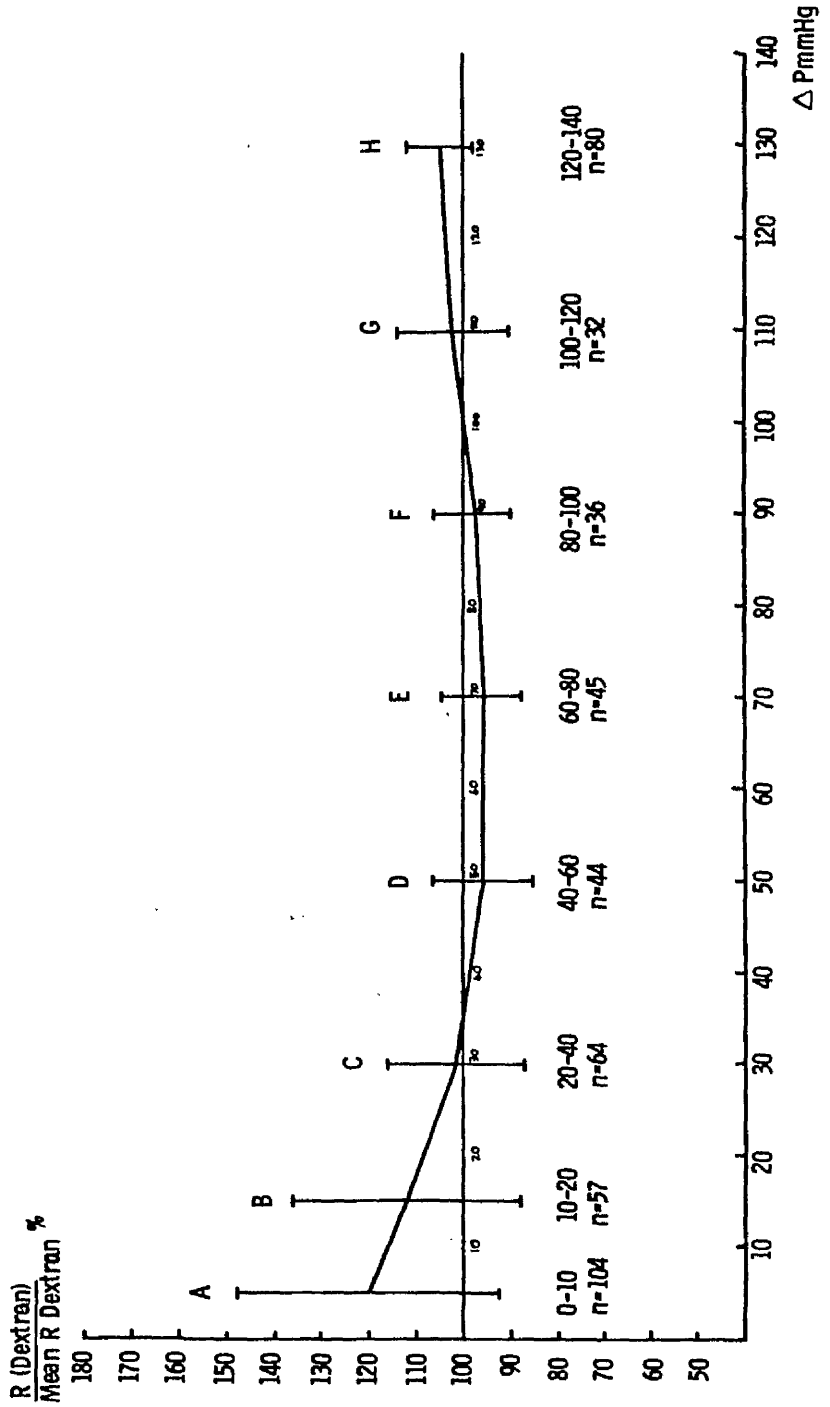


FIG 16

The variation of resistance for dextran with perfusion pressure

Comparison of means - Unpaired t test A v D, $p < 0.001$
H v D, $p < 0.001$
A v H, $p < 0.001$

highest (H) end of the range of perfusion pressure are higher than the middle (D,E,F) and that the lowest perfusion pressure yields a higher resistance than the highest. Although the variation of resistance with perfusion pressure for Dextran is small when compared to that of blood of normal haematocrit, the differences between the resistance at both the lowest perfusion pressure and the highest perfusion pressure are statistically different from the mid range of perfusion pressures (Figure 16). Because of this the reciprocal values of the mean at each perfusion range were used as correction factors and applied to the normalised resistance values. Thus in each experiment the value of resistance for blood was expressed as a percentage of the mean of the resistance values for Dextran at perfusion pressures greater than 30 mm of Mercury for that individual experiment. These percentage values were then corrected by the correction factors derived above. The correction values are:

Perfusion Pressure (P) mm Hg			Correction Factor
0	-	10	.83
11	-	20	.89
21	-	40	.99
41	-	60	1.04
61	-	80	1.04
81	-	100	1.02
10.1	-	120	.98
		120	.96

Finally for each blood perfusion in each experiment the corrected normalised values for resistance were plotted against Δ P and this graph used to obtain values at standard increments of Δ P to allow comparison of resistance at the same Δ P for different experiments.

An alternative, and very much simpler method of calculation would be to plot in each experiment the value of R for the test blood and for the Dextran against Δ P and take a ratio of one to the other, expressed as a percentage. This would have yielded very similar results in the experiments where a Dextran curve could easily be drawn, but in several of the experiments at the lowest perfusion pressures the Dextran results were rather scattered making the drawing of such a line unsatisfactory, hence the adoption of the somewhat cumbersome method described above.

Reproducibility

The duration of these experiments after isolation of the limb was between two and three hours. During this time the limb was perfused via the cannulae either by the limb donor dog, or by the perfusion circuit. During the development of the perfusion apparatus it was established that continuous perfusion of the limb yielded a steady value for resistance, at a constant flow rate, for over three hours. However, since it was the intention to alternate the limb's circulation between the perfusion apparatus and the limb donor dog so that the test blood might be changed, two preliminary experiments were performed in each of which the same blood, haematocrit 48 and 38 respectively, was perfused four times in succession, the limb donor dog being connected to the limb for 10 minutes between each perfusion.

As Dextran was not used the values obtained are not included in the final results, but serve to illustrate the reproducibility of the method of obtaining the pressure/flow curve (Figures 17 and 18). On the linear part of the graph the results are virtually identical, with as expected, more variation at the lowest perfusion pressures. However, even at the low flow rates the curves obtained are acceptably close.

EFFECT OF HAEMATOCRIT ON VISCOSITY AND RESISTANCE

Viscosity in Vitro (Wells-Brookfield Viscometer)

Other than at very low shear rates viscosity in vitro is determined largely by haematocrit. The relationship at 230 sec^{-1} is shown below (Figure 19). This is clearly non linear, but when log viscosity is substituted a reasonable linear relationship appears (Figure 20), the coefficient of correlation being 0.97. As shear rate is reduced viscosity increases, this effect increasing with increasing haematocrit (inset Figures 14 and 15). Since plasma and blood of low haematocrit are little affected by shear rate, as shear rate is reduced the slope of the log viscosity/haematocrit regression line increases but the intercept remains approximately constant. Thus at 11.5 sec^{-1} the slope is 0.0178 (Figure 21) compared with 0.0113 at 230 sec^{-1} and 0.0201 at 5.75 sec^{-1} (Figure 22). The linear relationship between log viscosity and haematocrit is maintained at 11.5 sec^{-1} and 5.75 sec^{-1} the coefficients of correlation being 0.96 and 0.95 respectively.

Vascular Resistance (Limb perfusion model)

With any particular blood sample resistance tended to be constant with perfusion pressures greater than about 80 mm Hg, resistance increasing as perfusion pressure fell below this level.

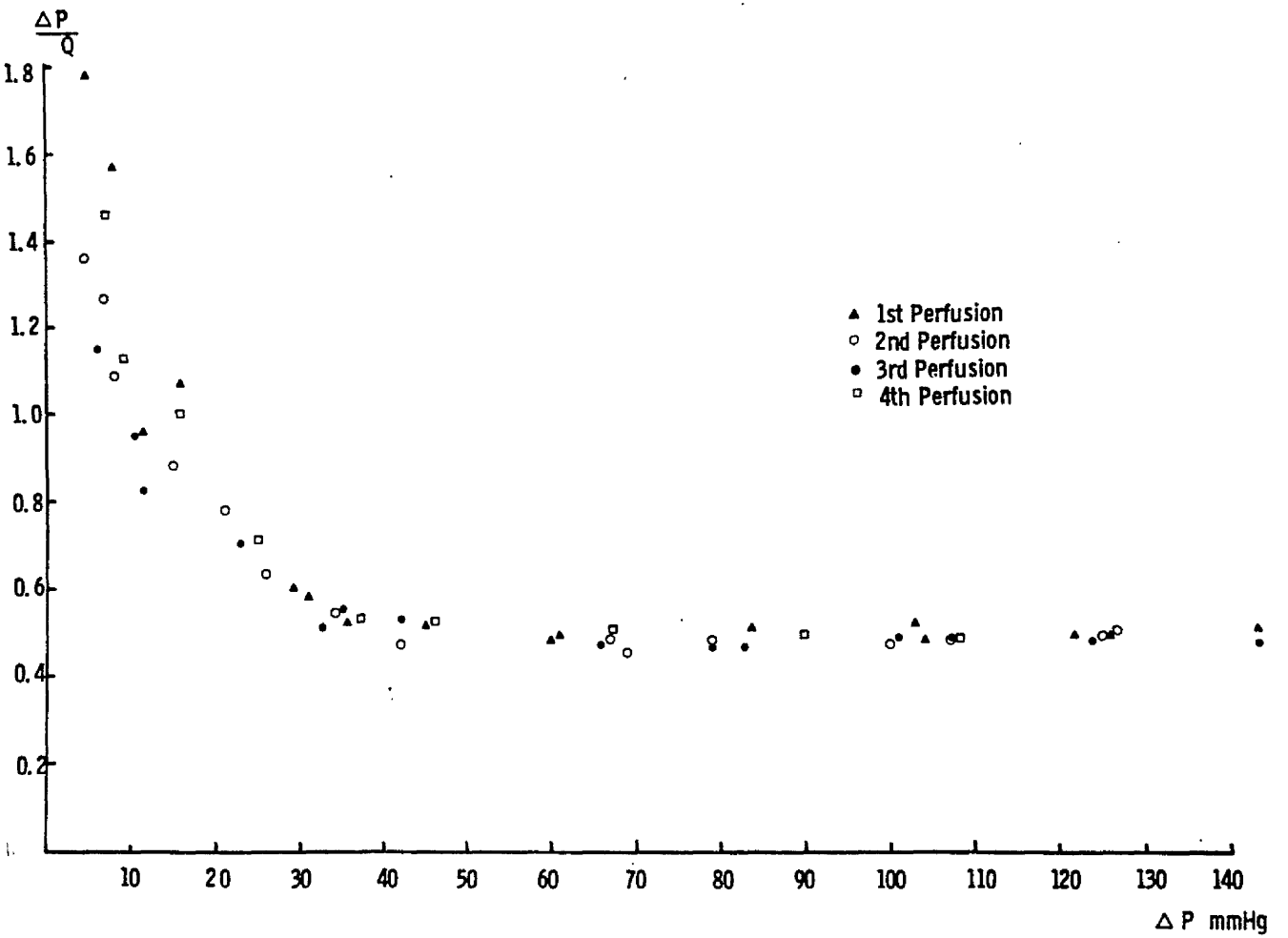


FIG 17

Preliminary experiment 1, Haematocrit = 48

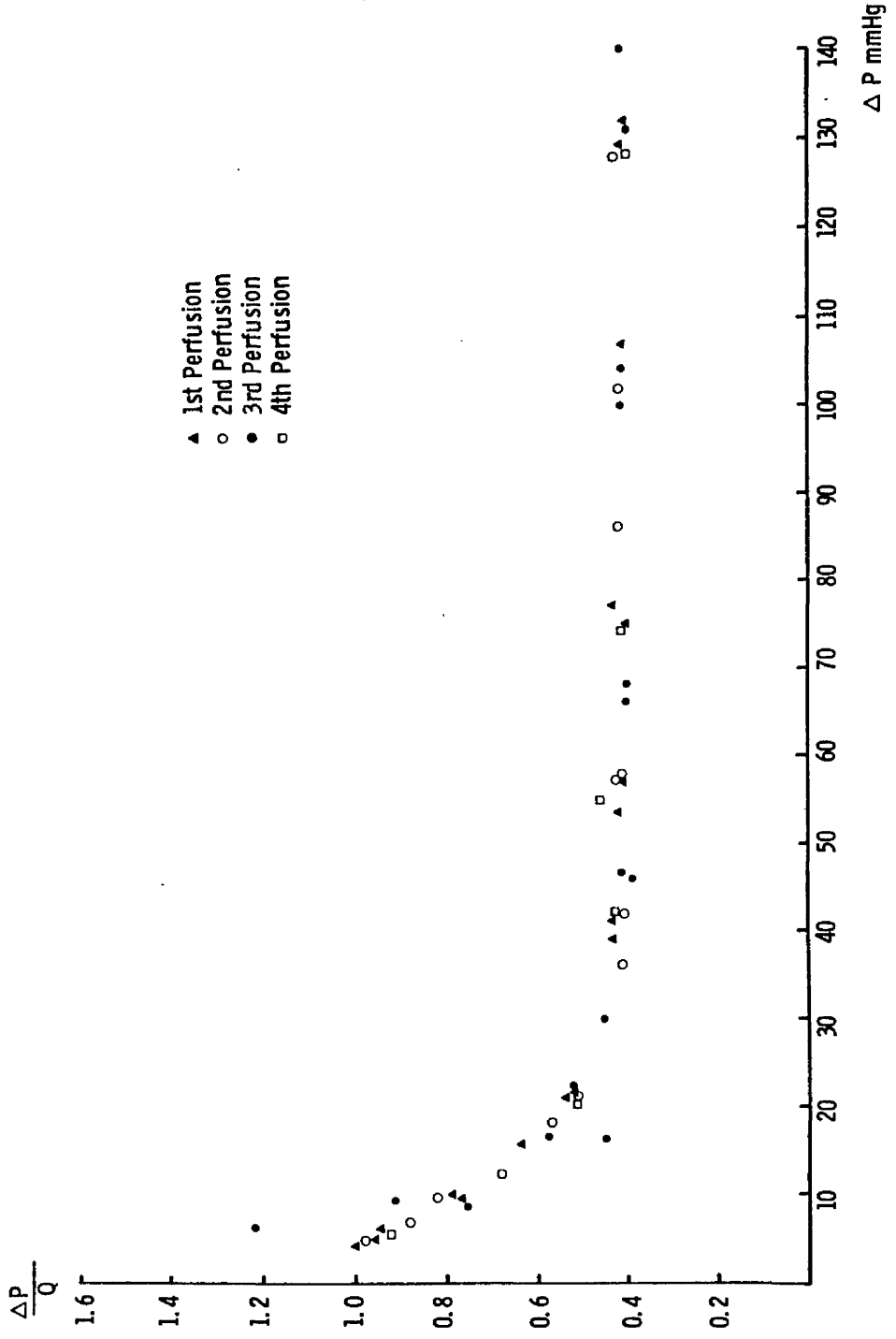


FIG 18

Preliminary experiment 2, Haematocrit = 38

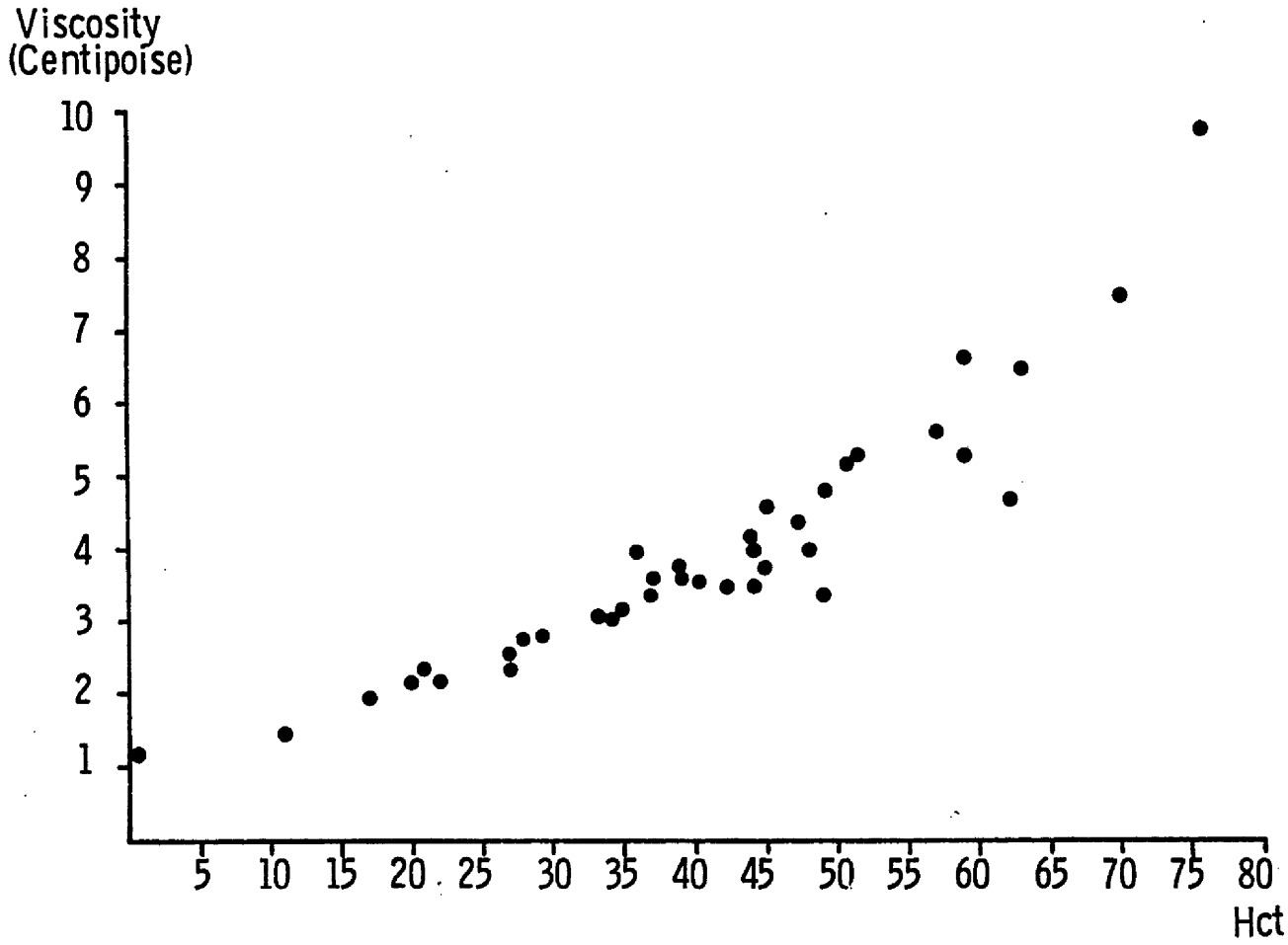


FIG 19

The relationship between viscosity and haematocrit for normal blood (experiments 1-13). Wells-Brookfield viscometer at 230 sec $^{-1}$

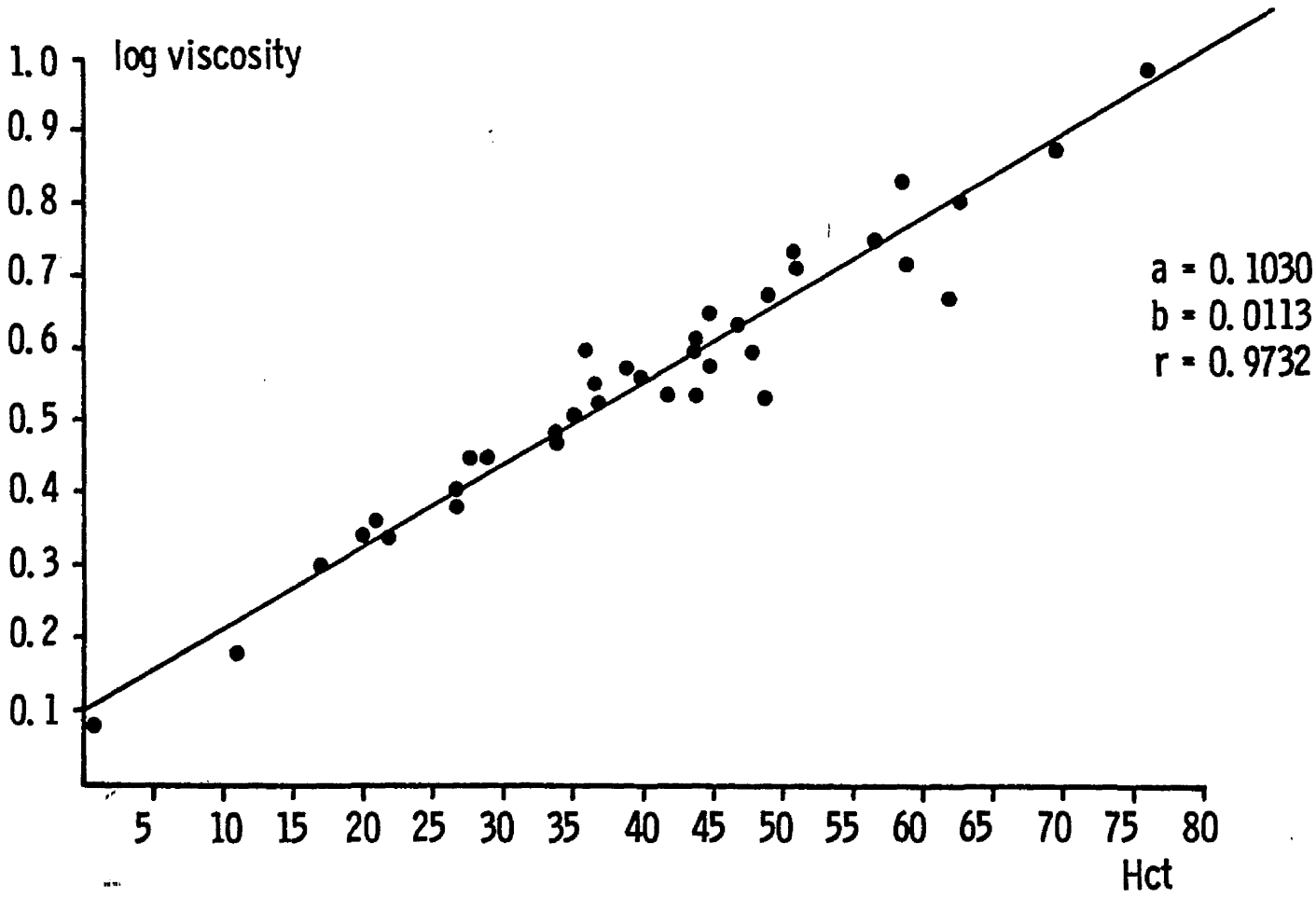


FIG 20

The relationship between log viscosity and haematocrit for normal blood (experiments 1-13) Wells-Brookfield viscometer at 230 sec $^{-1}$

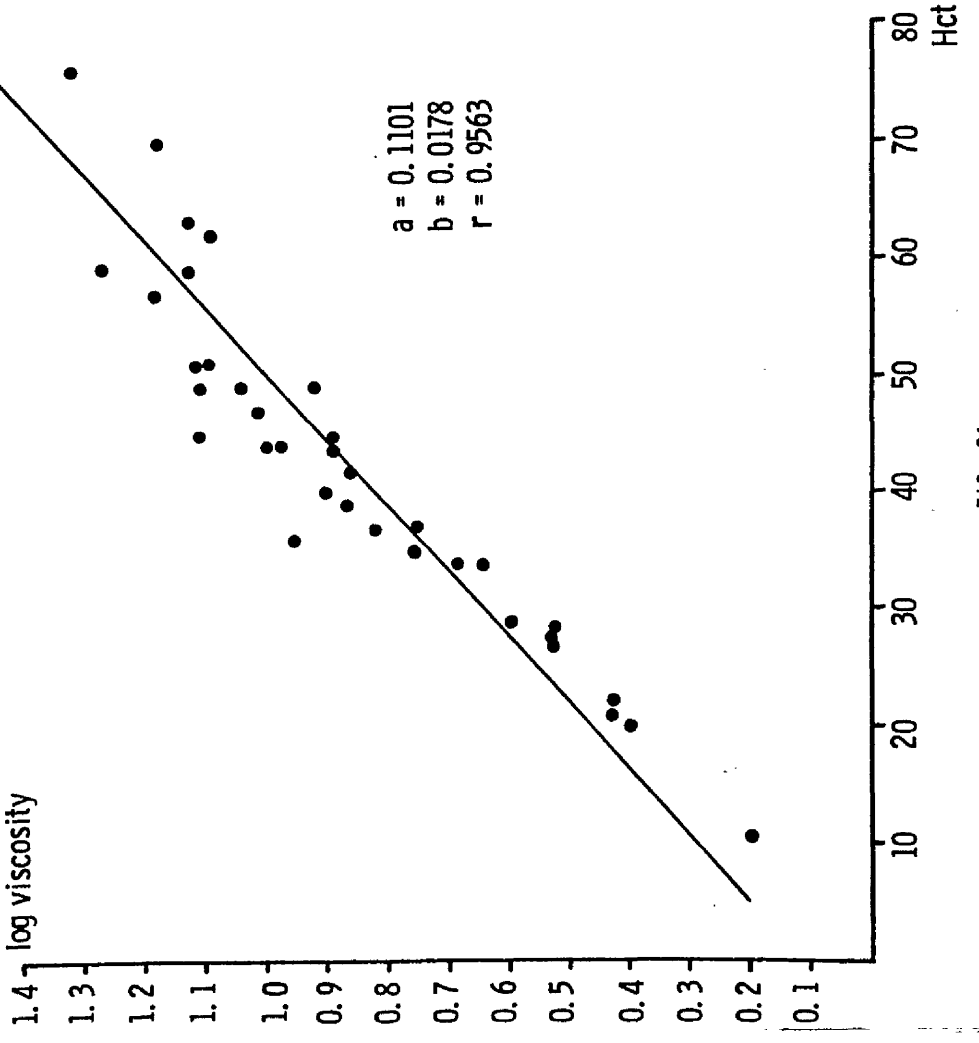


FIG 21

The relationship between log viscosity and haematocrit for normal blood (experiments 1-13) Wells-Brookfield Viscometer at 11.5 sec^{-1}

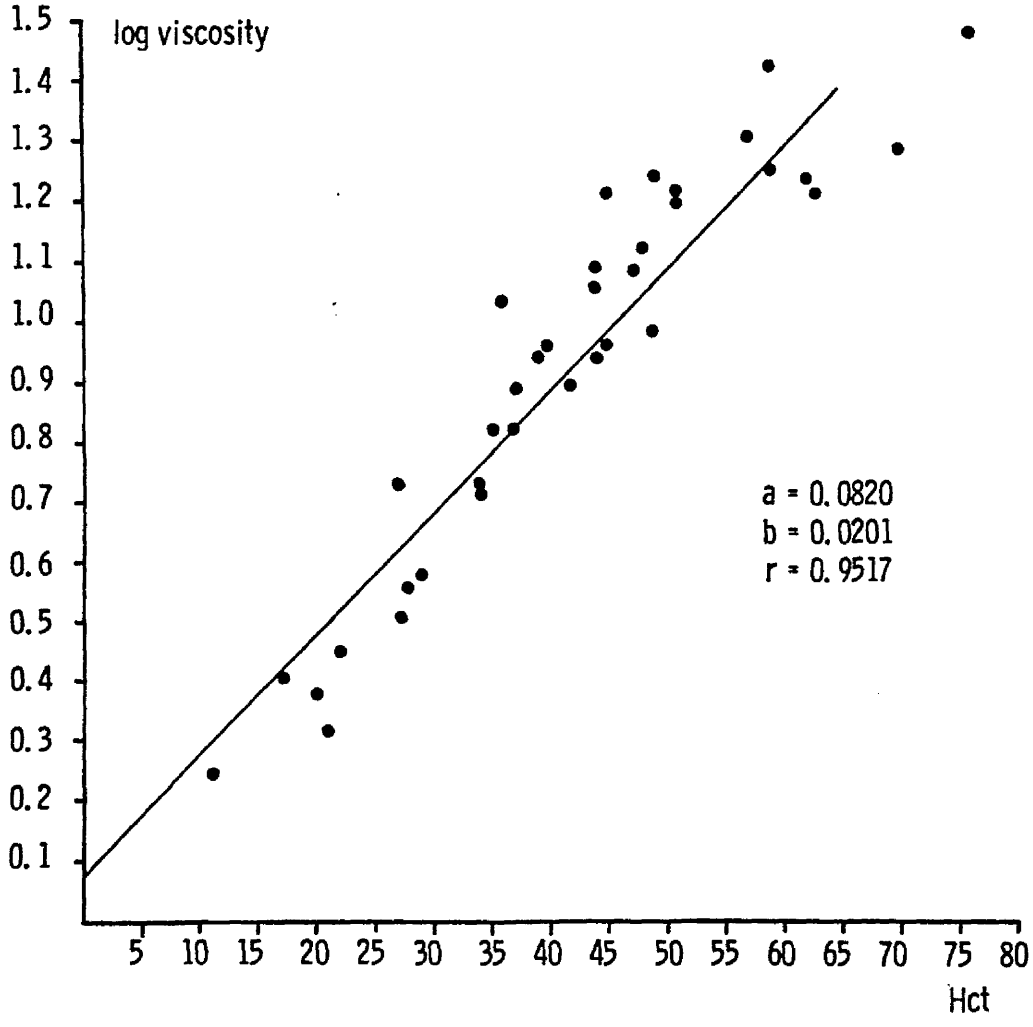


FIG 22

The relationship between log viscosity and haematocrit for normal blood (experiments 1-13) Wells-Brookfield Viscometer at 5.75 sec^{-1}

As with the relationship between viscosity and shear rate this effect was minimal when haematocrit was less than 20 or so but increased considerably with increasing haematocrit thereafter. At $\Delta P > 80$ mm Hg resistance increased with increasing haematocrit (Figure 23). Between haematocrit 30 and haematocrit 70 this relationship appears linear but the increase of resistance with haematocrit is considerably less than that of viscosity with haematocrit.* (Figure 19). From haematocrit 30 to haematocrit 70 resistance in arbitrary units increases from 83 to 128 (Figure 23), just over 50%, while the corresponding figures, in centipoise, for viscosity at 230 sec^{-1} (Figure 19) are 2.44 and 7.18, an increase of nearly 200%. At the lowest perfusion pressures however, the slope is considerably steeper (Figures 24 and 25). The corresponding increases in resistance from haematocrit 30 to haematocrit 70 at P=10 mm Hg and $\Delta P = 5$ mm Hg being from 121 to 339 and from 138 to 417, increases of 180% and 200% respectively. In making these calculations the linear regression at each perfusion pressure of log resistance against haematocrit was drawn. The value of the equations are shown in Figure 26, each coefficient of correlation exceeding 0.8. Values derived from similar regression lines of log viscosity versus haematocrit (Figures 20, 21 and 22) for values of haematocrit 30 - 70 and shear rate 230, 11.5 and 5.75 are shown in Table 3. It is seen that both resistance and viscosity rise considerably as shear rate or perfusion pressure (or flow rate) approaches zero, and as haematocrit rises. The

* Note that the units for vascular resistance are in arbitrary 'percentage' values whereas the in vitro viscosity graphs are in centipoise.

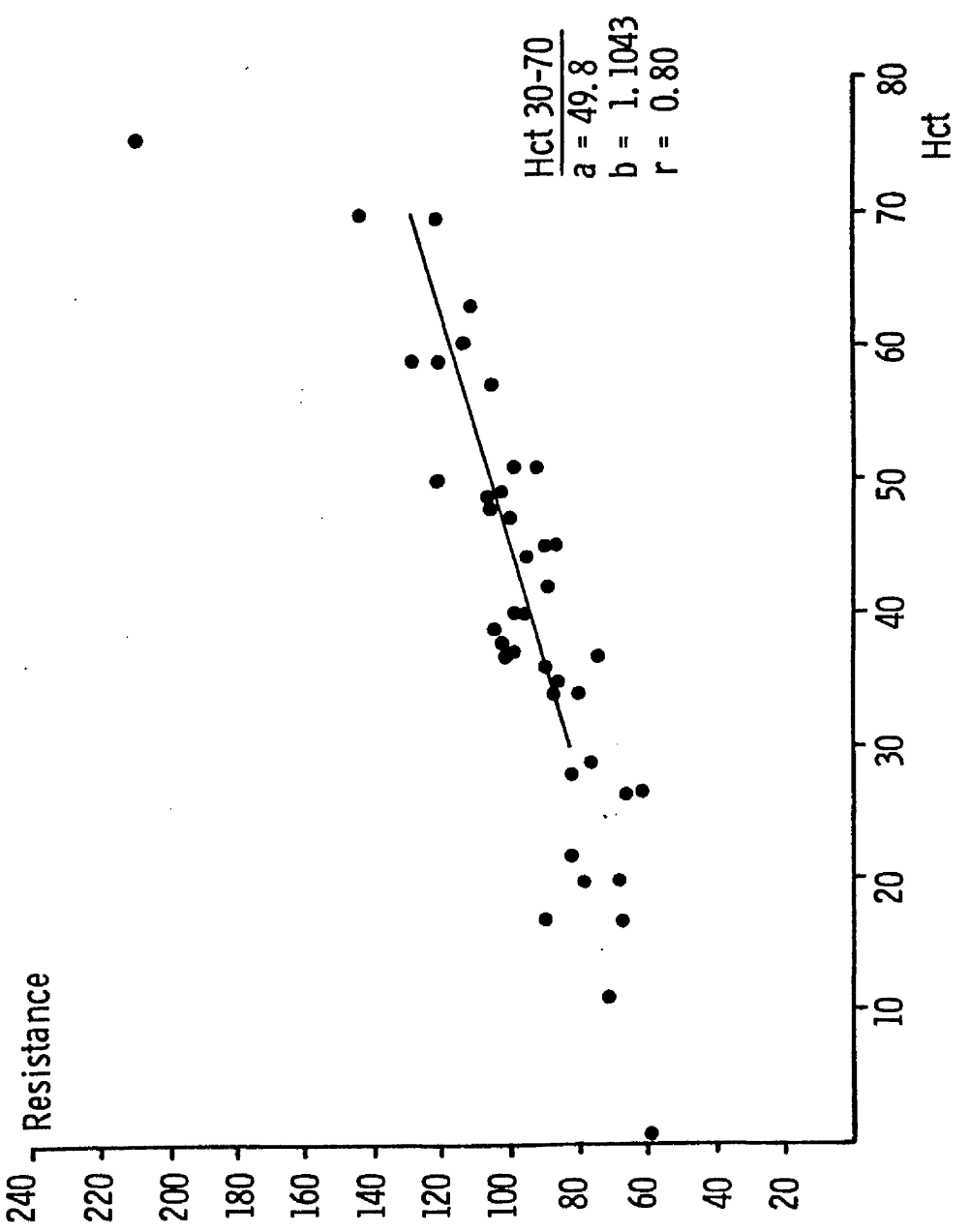


FIG 23

The relationship between resistance (arbitrary units) and haematocrit, $\Delta P > 80$ mmHg. Experiments 1-13

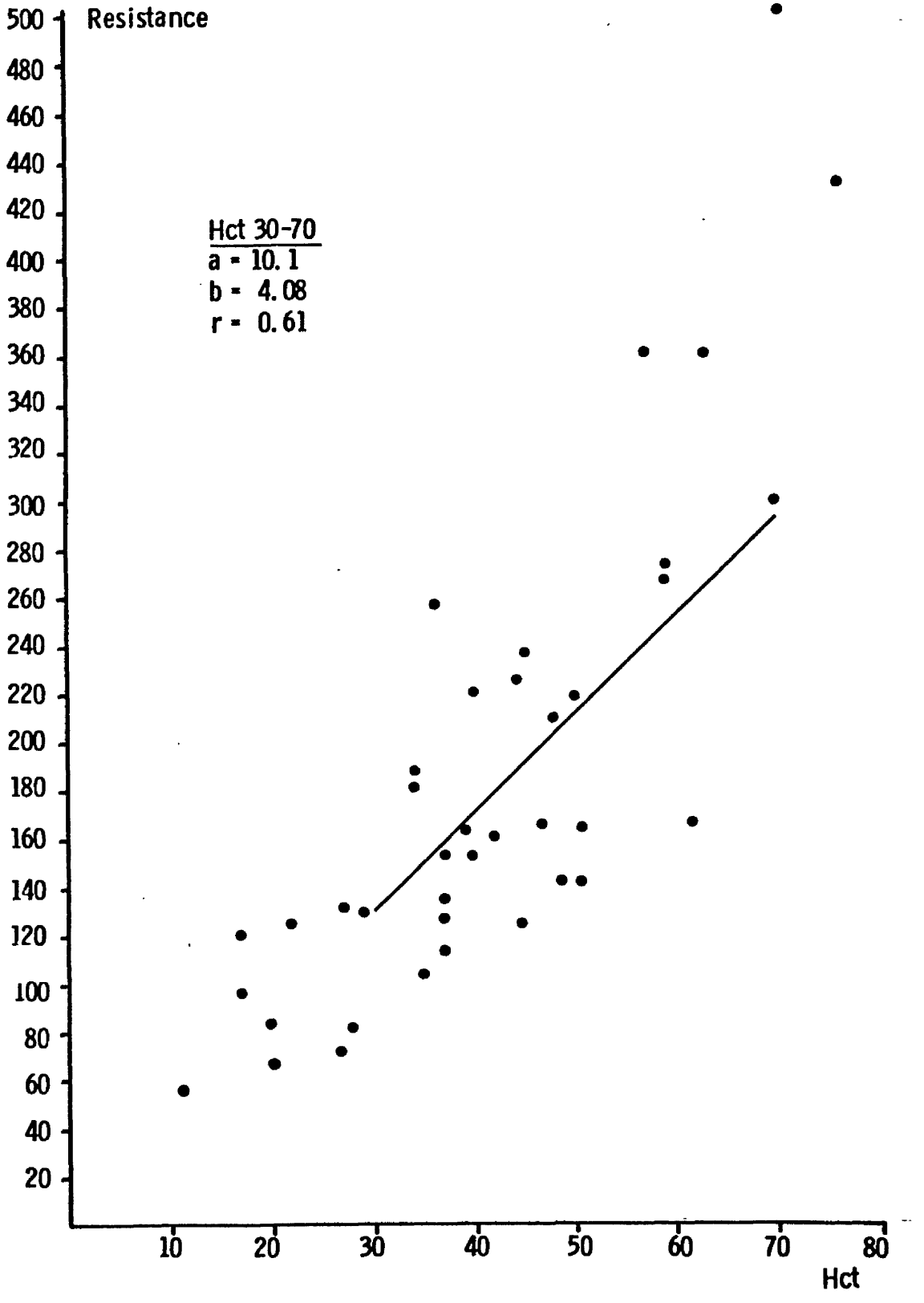


FIG 24: The relationship between resistance (arbitrary units) and haematocrit, ΔP = 10 mmHg. Experiments 1-13

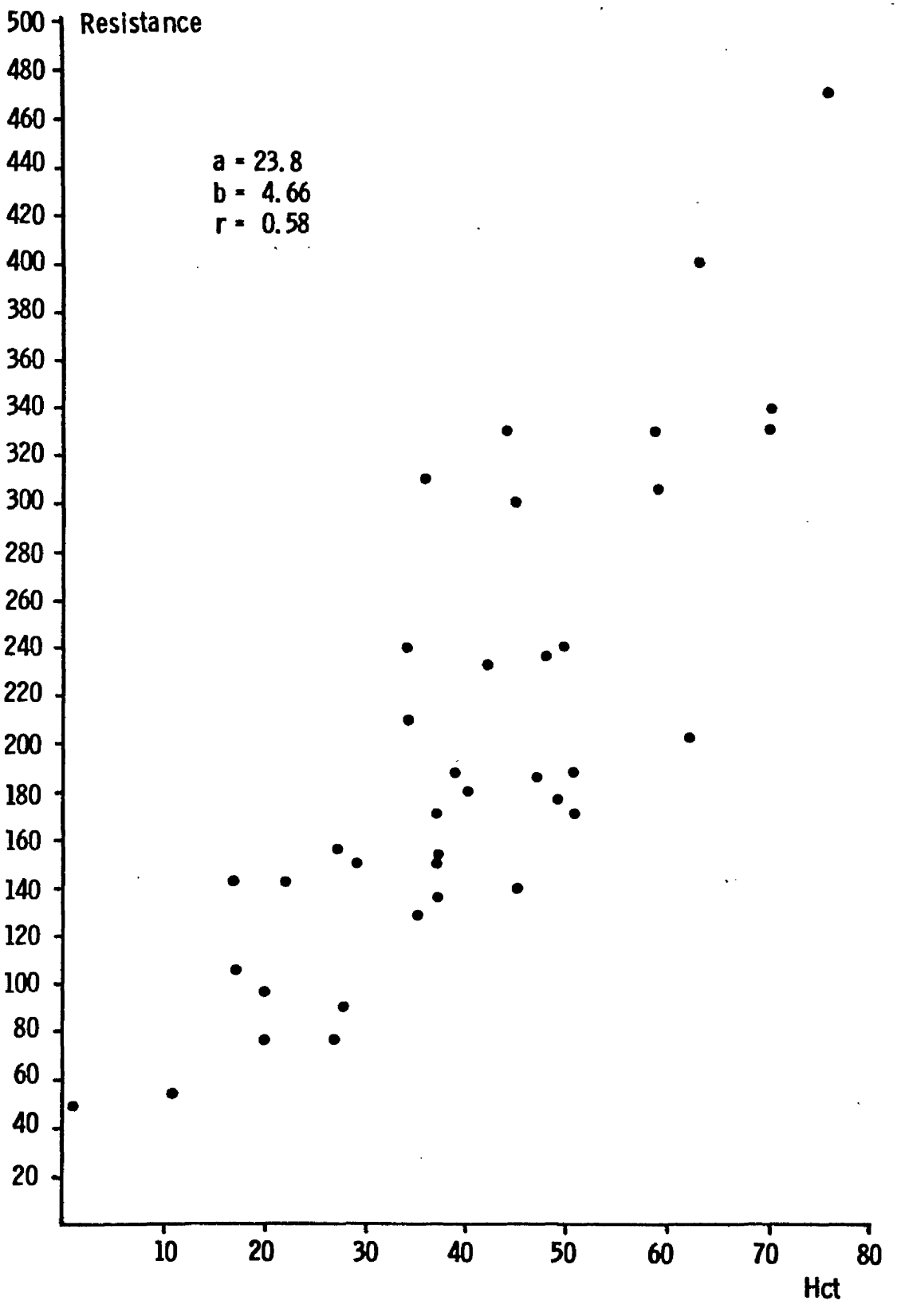


FIG 25: The relationship between resistance (arbitrary units) and haematocrit, $\Delta P = 5$ mmHg. Experiments 1-13

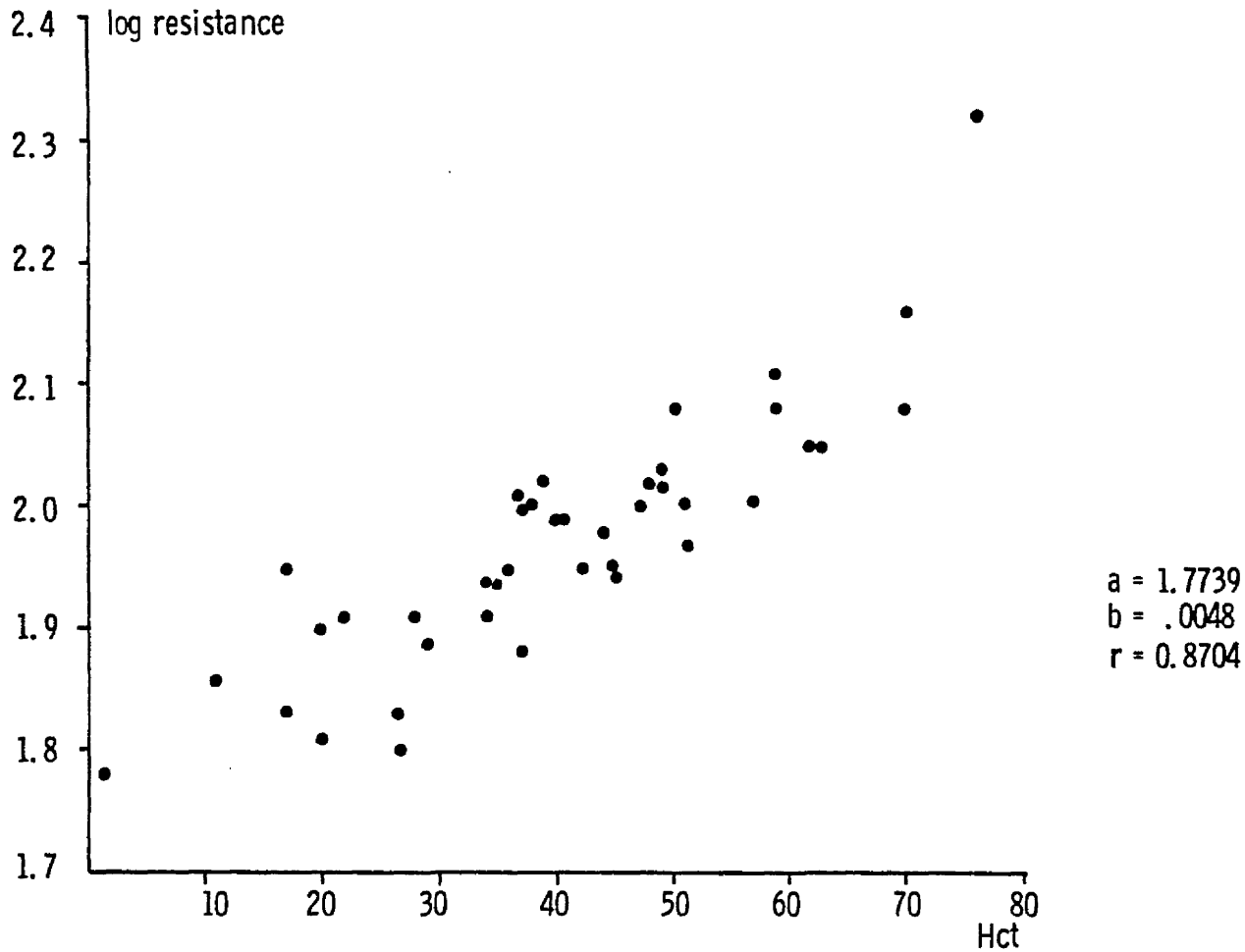


FIG 26: The relationship between log resistance and haematocrit, $\Delta P > 80$ mmHg
The equivalent values for $\Delta P = 10$ mmHg, and $\Delta P = 5$ mmHg are

a = 1.7456	a = 1.78
b = 0.0112	b = 0.0120
r = 0.8334	r = 0.82

TABLE THREE

SHEAR RATE

	230 sec^{-1}			11.5 sec^{-1}			5.75 sec^{-1}		
	a	b	r	a	b	r	a	b	r
Normal	0.10	0.0113	0.97	0.11	0.0178	0.96	0.08	0.0201	0.95
Ancrod Treated	0.11	0.0115	0.98	0.06	0.0199	0.94	0	0.0230	0.93

Log Viscosity v Haematocrit

(y a + bx)

PERFUSION PRESSURE (mm Hg)

	>80 mm Hg			10 mm Hg			5 mm Hg		
	a	b	r	a	b	r	a	b	r
Normal	1.77	0.0048	0.87	1.75	0.0112	0.83	1.78	0.012	0.82
Ancrod Treated	1.79	0.0055	0.83	1.59	0.0175	0.80	1.61	0.0190	0.81

Log Resistance v Haematocrit

(y a + bx)

increase in viscosity with haematocrit is however considerably greater in vitro than in vivo.

When the results of these thirteen experiments are grouped according to haematocrit, viscosity can be plotted against shear rate, and resistance against perfusion pressure (Figures 27 and 28). Since these represent the results of different experiments, the standard deviations are large but the same trend is shown by the individual experiments. It is clear that resistance is considerably less affected by haematocrit than viscosity, and that the shape of the viscosity/shear rate and resistance/perfusion pressure curves are determined by haematocrit. At the lower haematocrits,Group A, these are almost flat while at the higher values, Group F, the asymptotic values are not quite reached at the highest shear rates and perfusion pressures shown.

Correlation between Viscosity and Resistance

It is evident from the preceding figures, e.g. Figures 27 and 28, that there is a strong correlation between viscosity as measured with the Wells-Brookfield Viscometer in vitro and resistance as measured in the canine hind limb preparation. Figure 42 shows the correlation at shear rate 230 sec^{-1} and $\Delta P > 80$ mm Hg, while Figure 43 shows the correlation at shear rate 11.5 sec^{-1} and $\Delta P = 10$ mm Hg. Not surprisingly the scatter of results is wider at the lower shear rate and perfusion pressure. However the coefficient of correlation are 0.88 and 0.82 respectively.

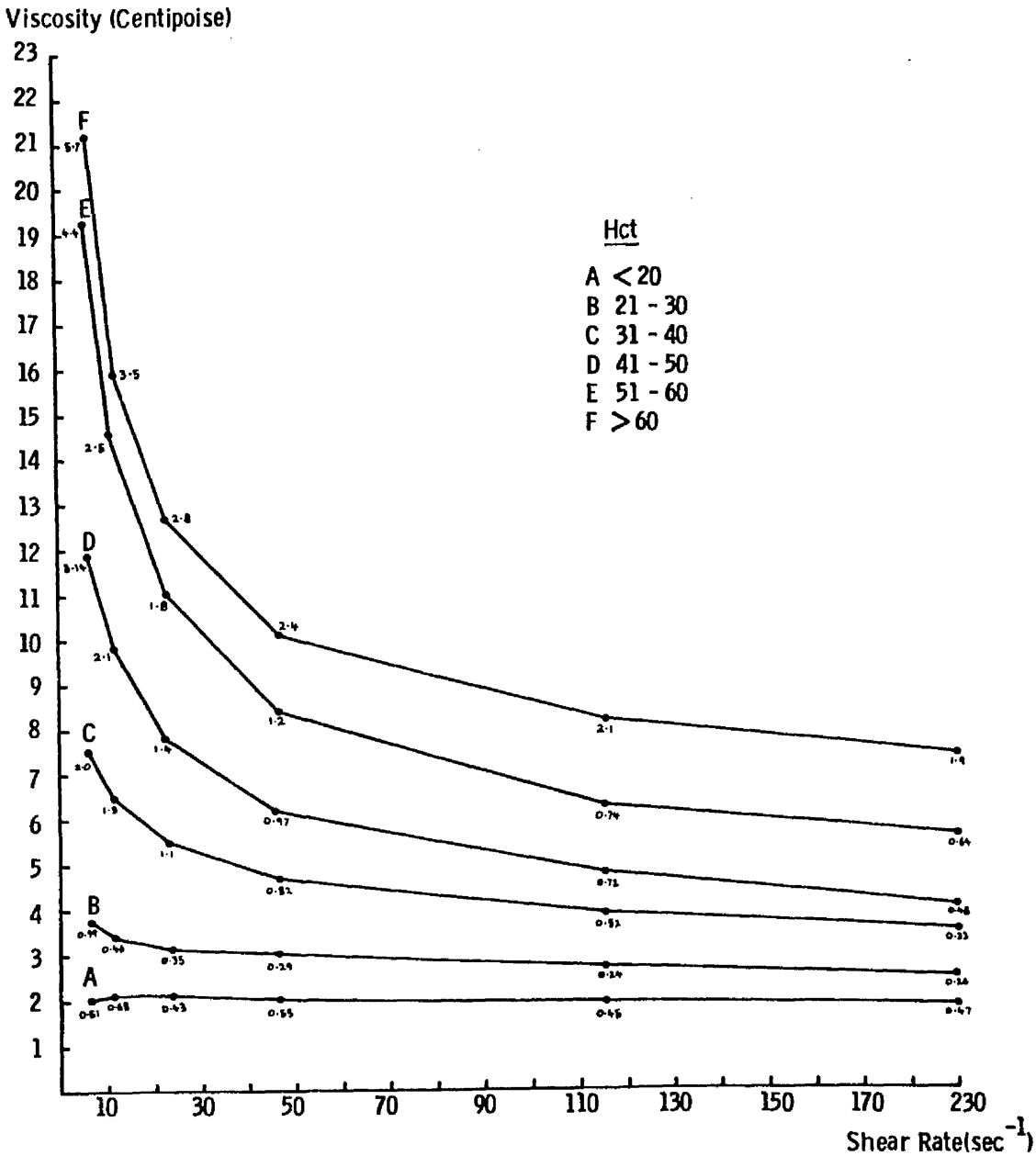


FIG 27: The relationship between viscosity, shear rate and haematocrit, Experiments 1-13, Wells-Brookfield Viscometer. The small numbers represent one standard deviation.

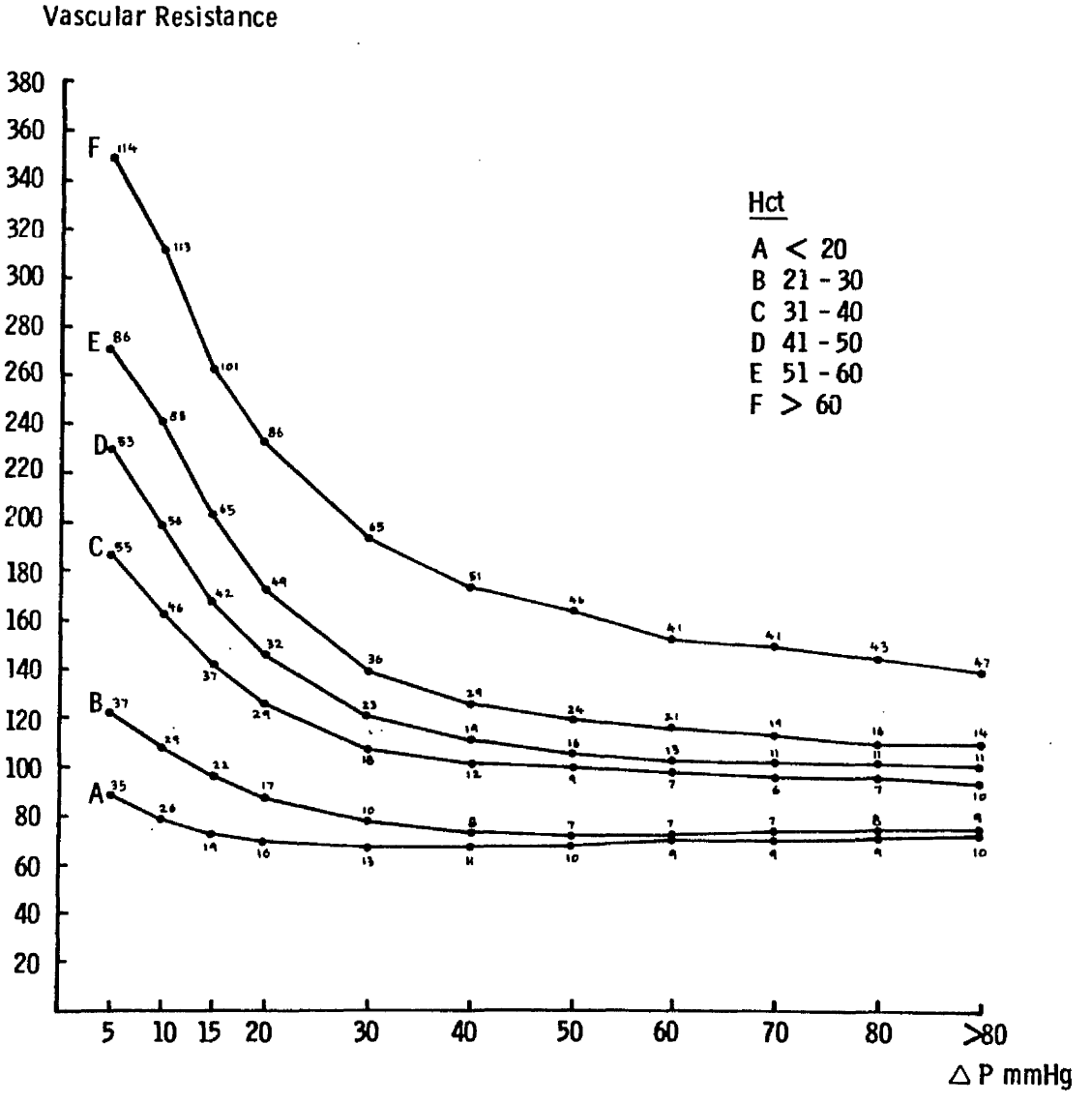


FIG 28: The relationship between vascular resistance, perfusion pressure and haematocrit, Experiments 1-13, Limb perfusion model. The small numbers represent one standard deviation.

EFFECT OF DEFIBRINATION WITH ANCROD

Eight preparations were perfused with blood taken from dogs defibrinated by pre-treatment with ancrod. Any ancrod remaining in the blood was neutralised by the addition of anti-ancrod so that any slight mixing with the limb donor dog's normal blood did not result in the formation of insoluble fibrinogen breakdown products, which in an intact animal would be cleared by the reticulo-endothelial system. In other respects the experimental procedure was the same as for the experiments with normal blood described above.

The results of eight such experiments (Nos. 14,15,16,17, 18,19,20,21) are shown here. The same method of calculation was used as in the previous section.

Effect of Haematocrit

As with normal blood, viscosity and resistance correlated with each other, increased with haematocrit, and increased at low shear rate and perfusion pressures. The behaviour of the defibrinated blood, which in all cases had undetectable levels of fibrinogen, was very similar to that of normal blood but both viscosity and resistance were slightly higher. Figures 29 and 30 show the relationship between viscosity and haematocrit at shear rate 230 sec^{-1}, and resistance and haematocrit at $\Delta P > 80$ mm Hg respectively. Figures 31-34 show the same relationships at shear rates 11.5 sec^{-1} and 5.75 sec^{-1}, and perfusion pressures 10 mm Hg and 5 mm Hg. As with the normal blood when log viscosity and log resistance are plotted against haematocrit, reasonably linear relationships result. The values of the intercept, a, slope, b, and coefficient of correlation, r, for the normal and ancrod treated blood are shown in Table 3 and Figure 22. The gradients

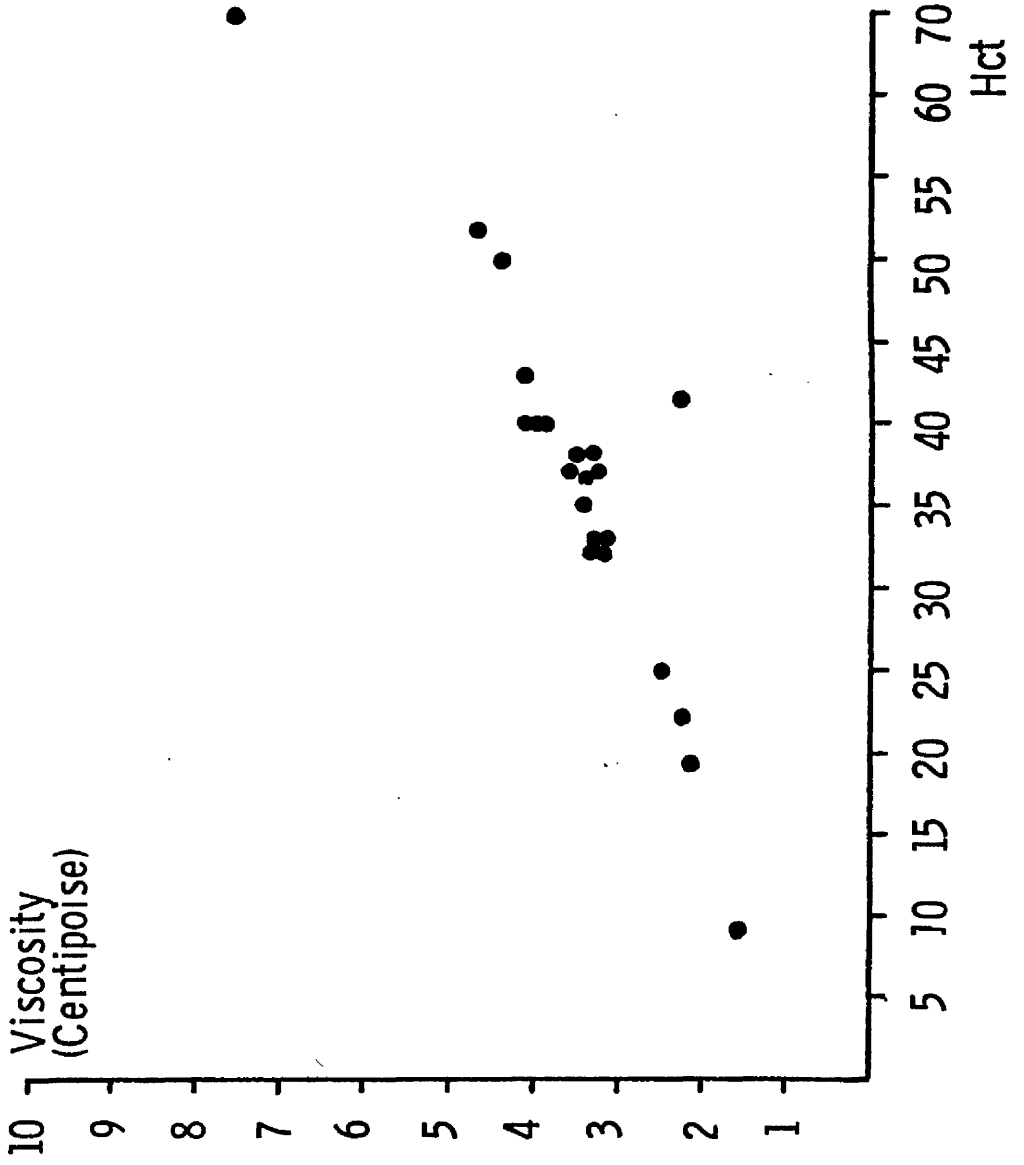


FIG 29: The relationship between viscosity and haematocrit at shear rate = 230 sec^{-1}, defibrinated blood.

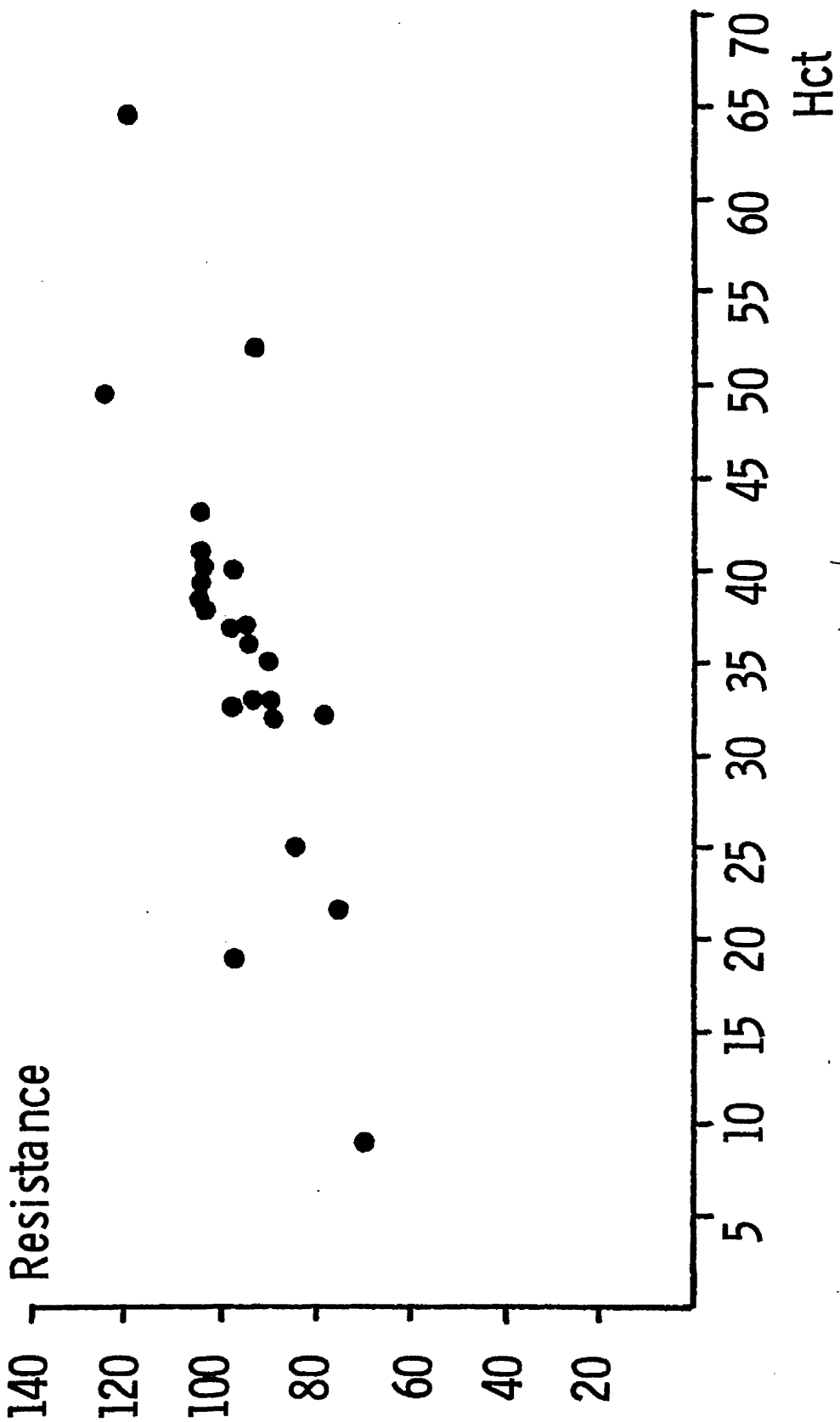


FIG 30: The relationship between ~~viscosity~~ haematocrit and haematocrit perfusion pressure > 80 mmHg defibrinated blood.

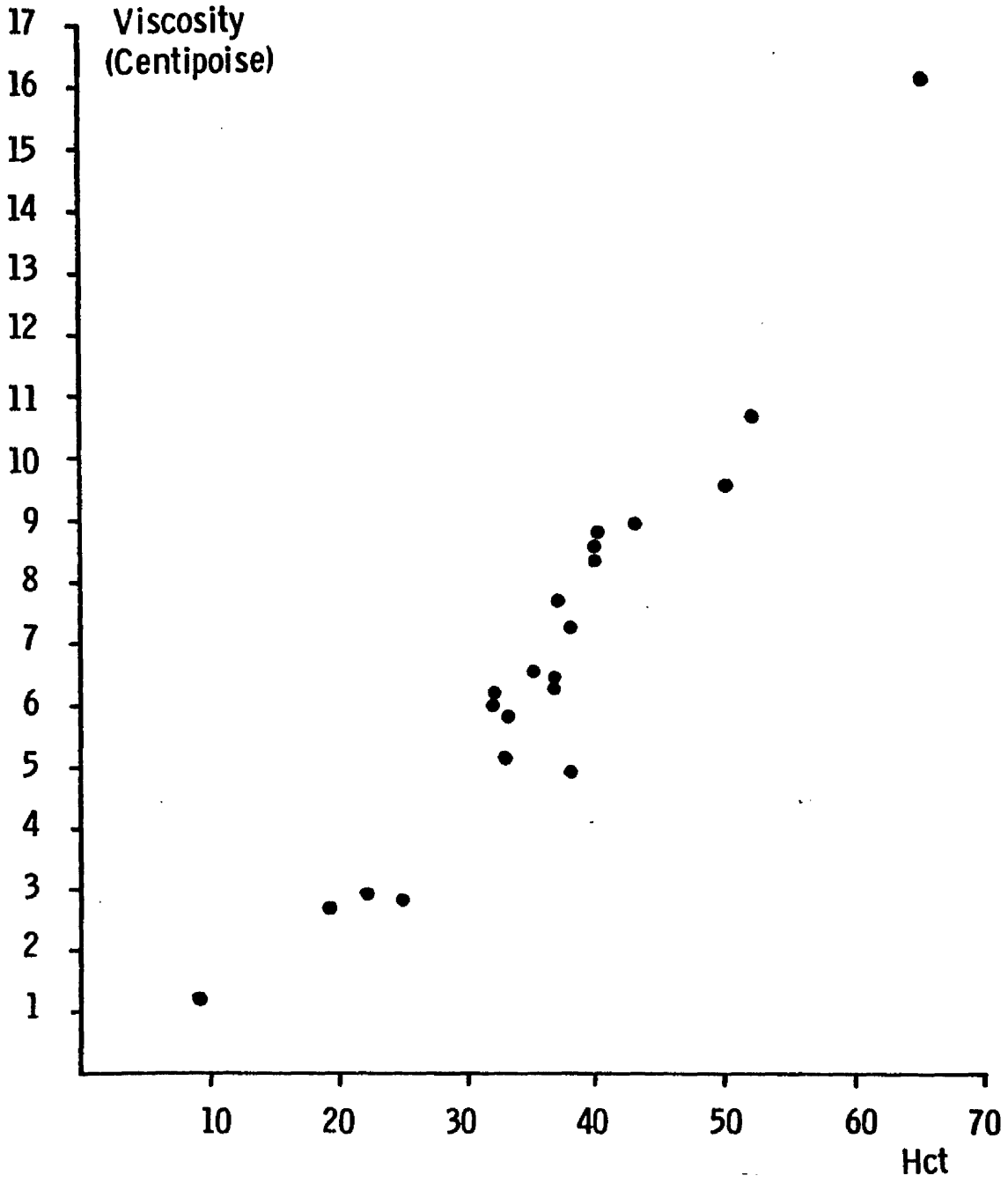


FIG 31: The relationship between viscosity and haematocrit (defibrinated blood) at shear rate 11.5 sec^{-1}

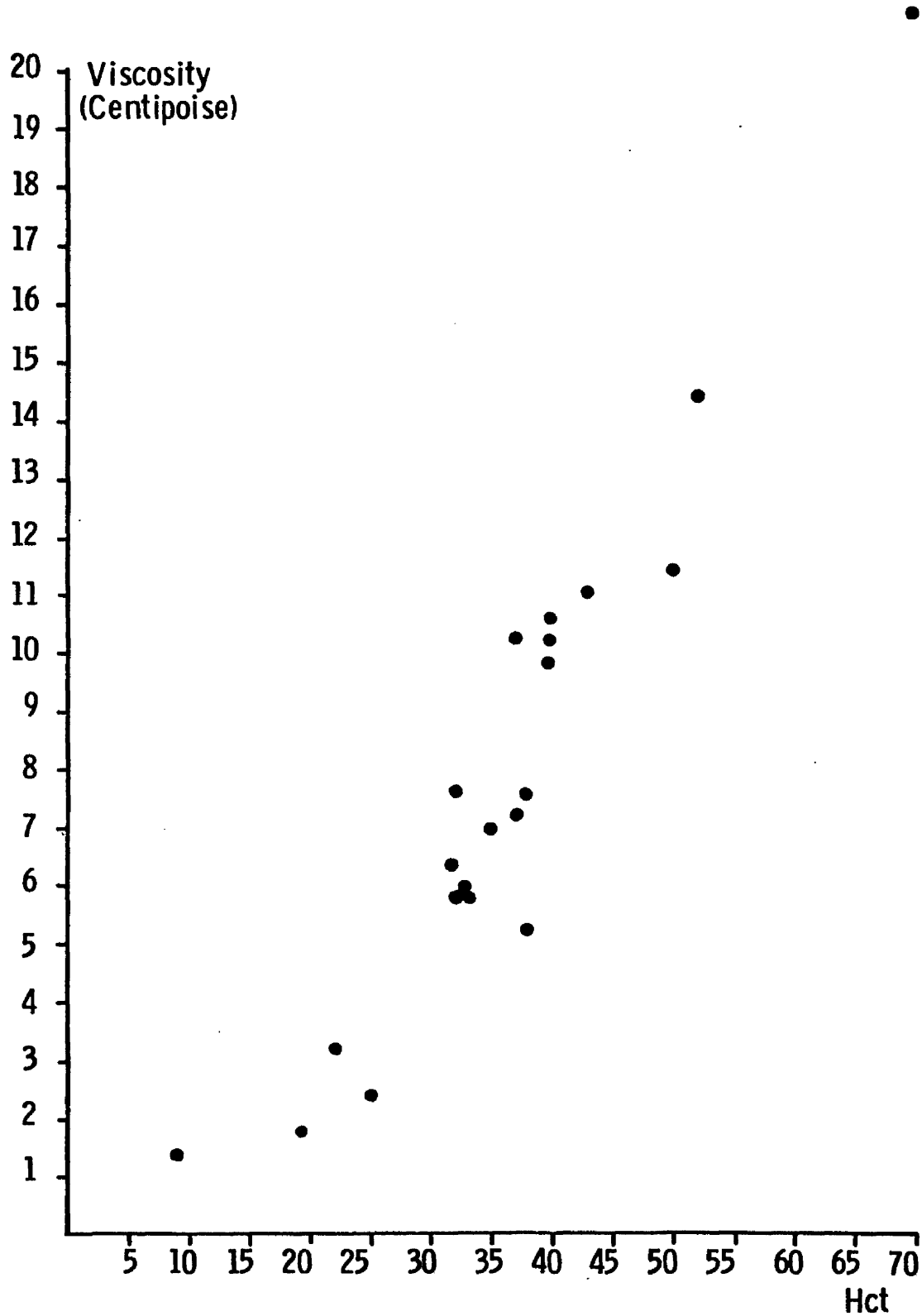


FIG 32: The relationship between viscosity and haematocrit (defibrinated blood) at shear rate 5.75 sec^{-1}

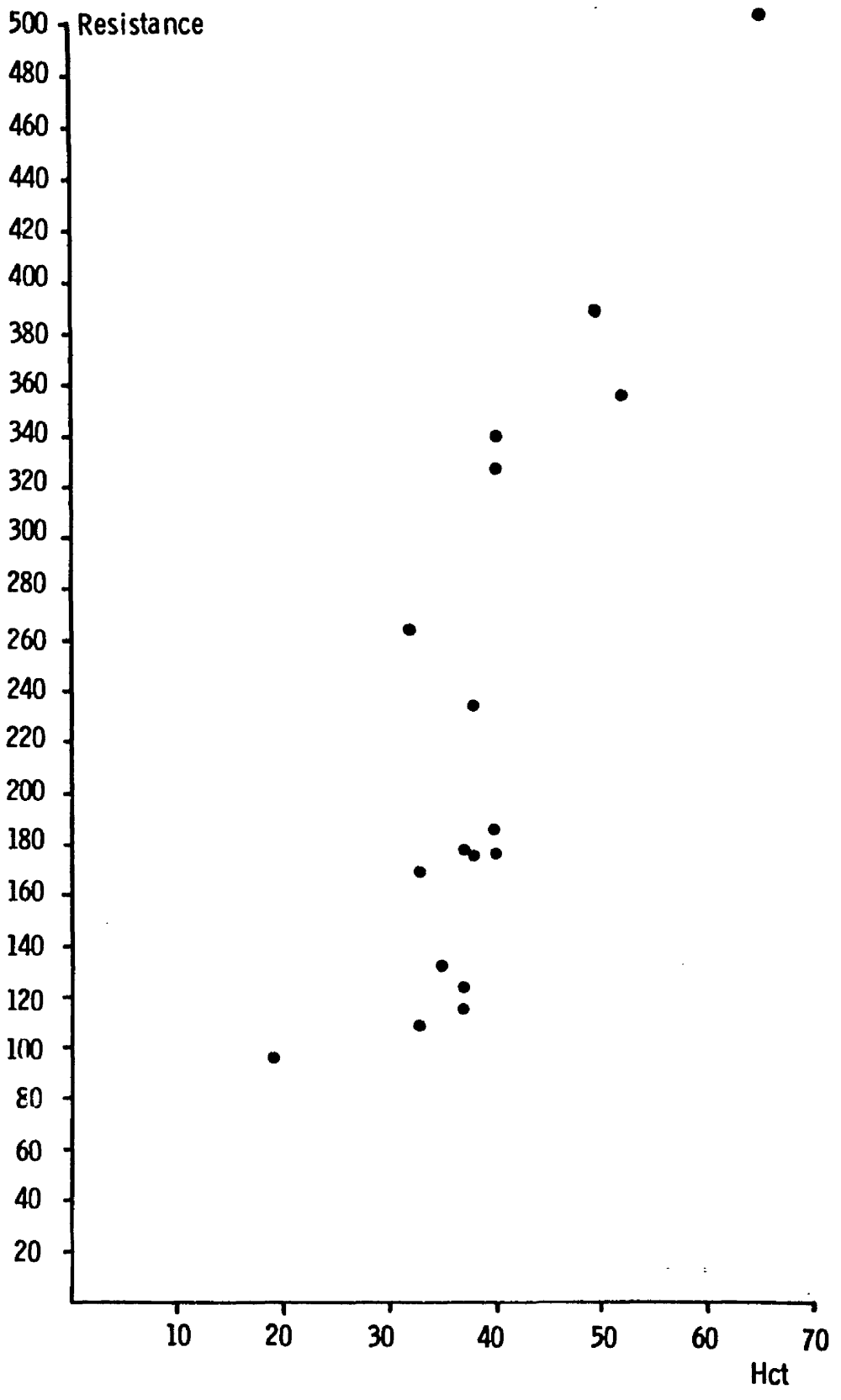


FIG 33: Relationship between resistance and haematocrit (defibrinated blood) at $\Delta P \simeq 10$ mmHg

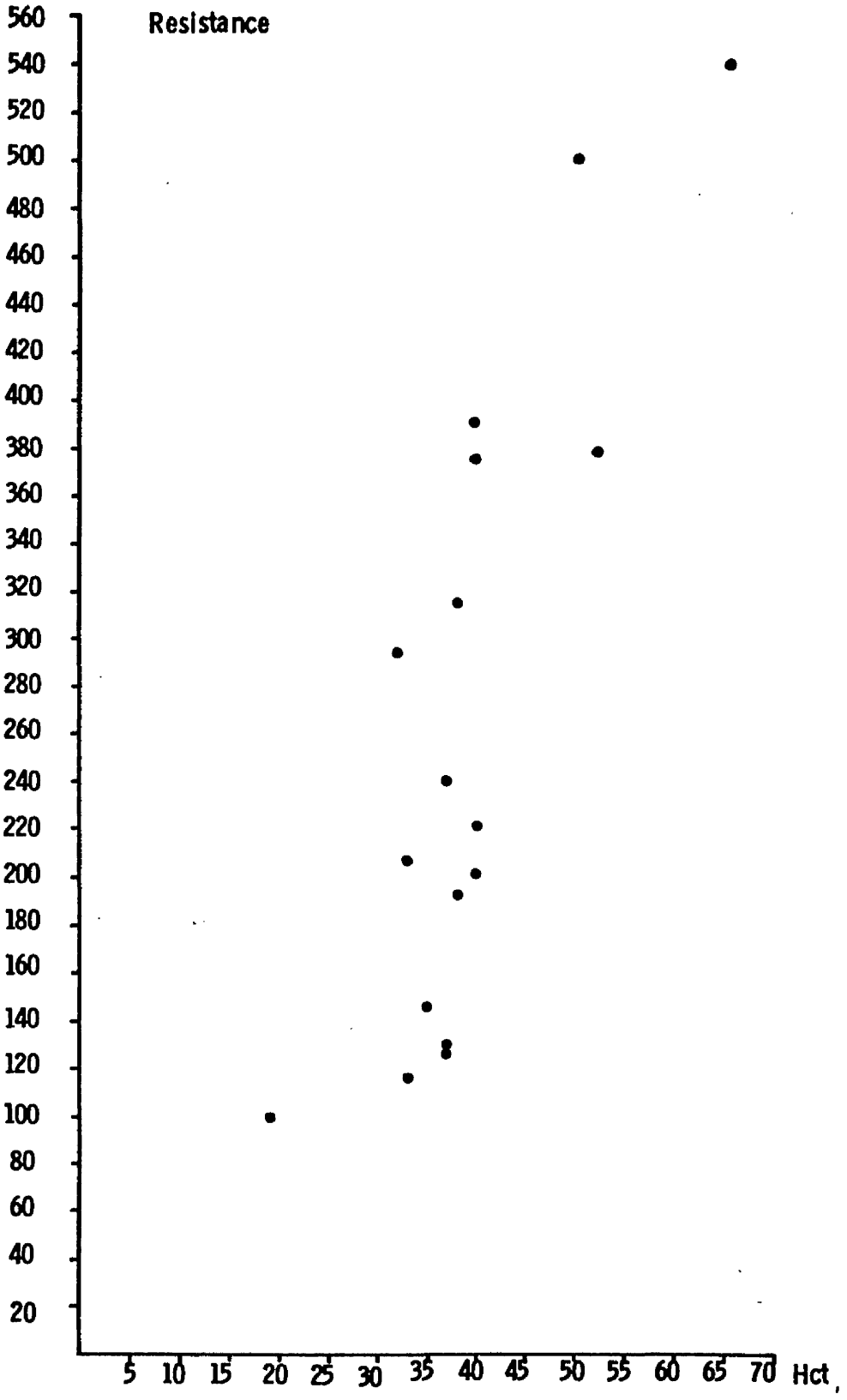


FIG 34: Relationship between resistance and haematocrit (defibrinated blood) at ΔP = 5mmHg

of the ancrod lines are persistently slightly steeper than those of normal blood, although individually when compared by multilinear regression analysis, the differences fall short of statistical significance. (Fig 35.)

Relationship of Fibrinogen to Viscosity and Resistance

In the experiments using normal canine blood, fibrinogen was measured routinely. In order to utilise this information to compare the effect of fibrinogen on viscosity and resistance it was necessary to 'correct' the values to a standard haematocrit. In this section the regression lines for viscosity and resistance at each shear rate and perfusion pressure respectively are used to 'correct' the viscosity and resistance values to haematocrit 45. If fibrinogen exerted a large effect in increasing viscosity one would expect a strong positive correlation between fibrinogen and viscosity, and fibrinogen and resistance. In fact this does not happen. Figures 36 and 37 show no significant correlation between either viscosity at 230 sec^{-1} or resistance at $\Delta P \simeq 80$ mm Hg and fibrinogen concentration. Again at shear rate 11.5 sec^{-1} (Figure 38) and perfusion pressure $\Delta P \simeq 10$ mm Hg (Figure 39) there is little, if any, correlation between viscosity and fibrinogen concentration or resistance and fibrinogen concentration.

Addition of canine fibrinogen to normal blood

In four experiments (experiments 22-25) purified autologous canine fibrinogen was added in increments to the blood being perfused, in otherwise identical experiments to those already described. During the addition of fibrinogen, the test blood was

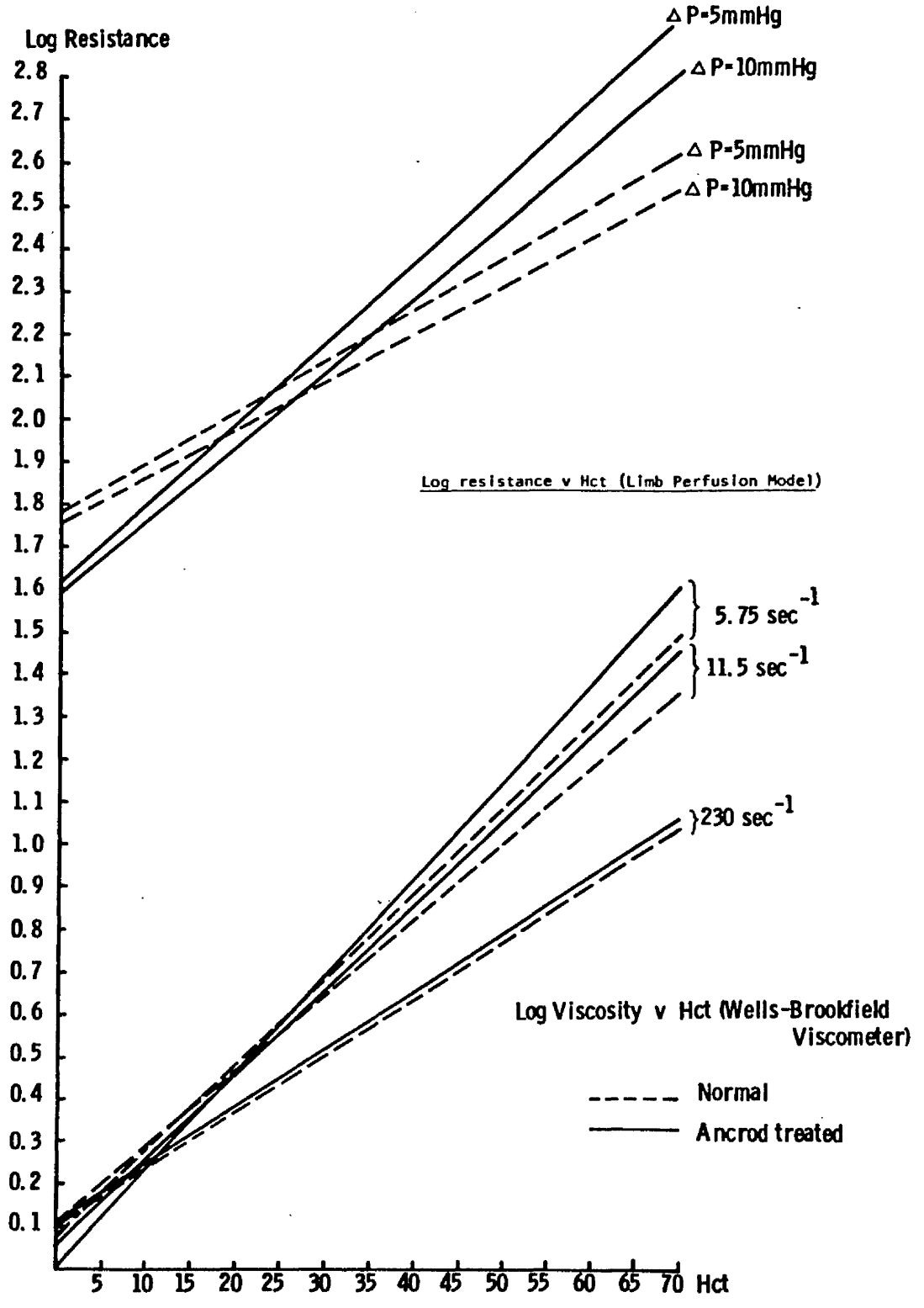


FIG 35: Regression lines for log viscosity v haematocrit (lower diagram) and log resistance v haematocrit (upper diagram) for normal and ancrod treated blood

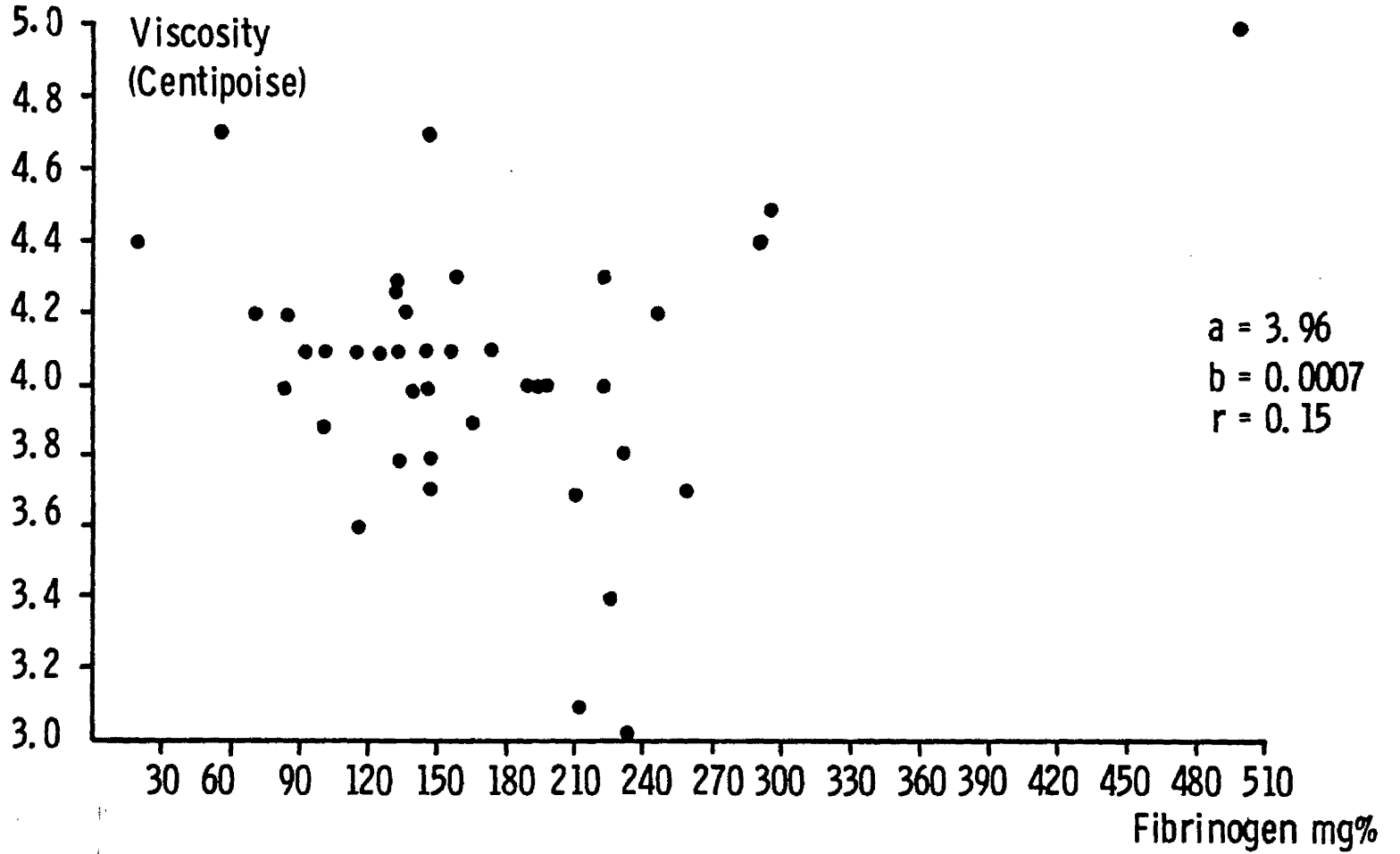


FIG 36: Relationship between viscosity (corrected to Hct 45) at 230 sec^{-1} and fibrinogen concentration (experiments 1-13, 26-30)

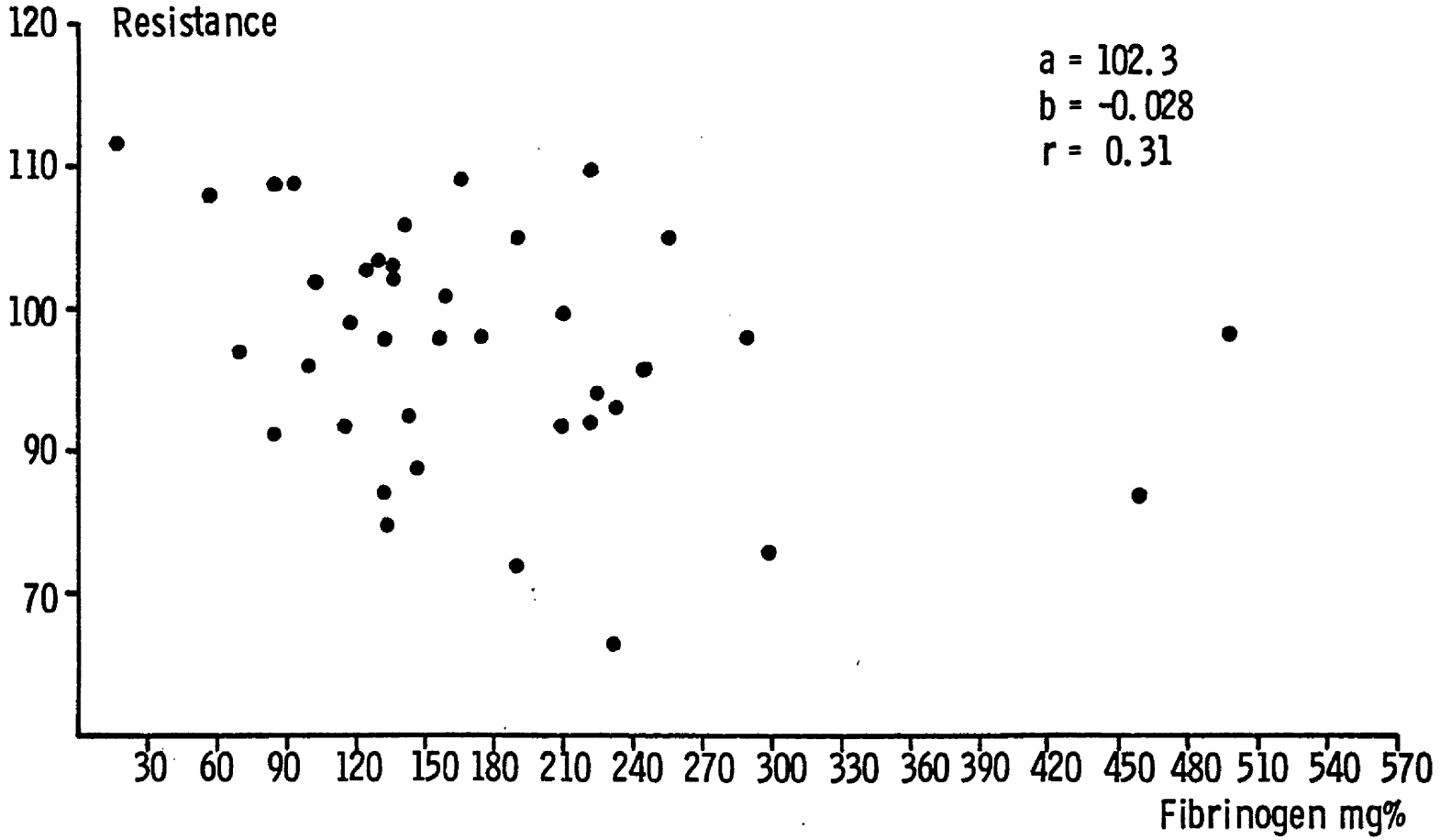


FIG 37: Relationship between reistance (corrected to Hct 45) at ΔP 80 mmHg. and fibrinogen concentration (experiments 1-13, 26-30)

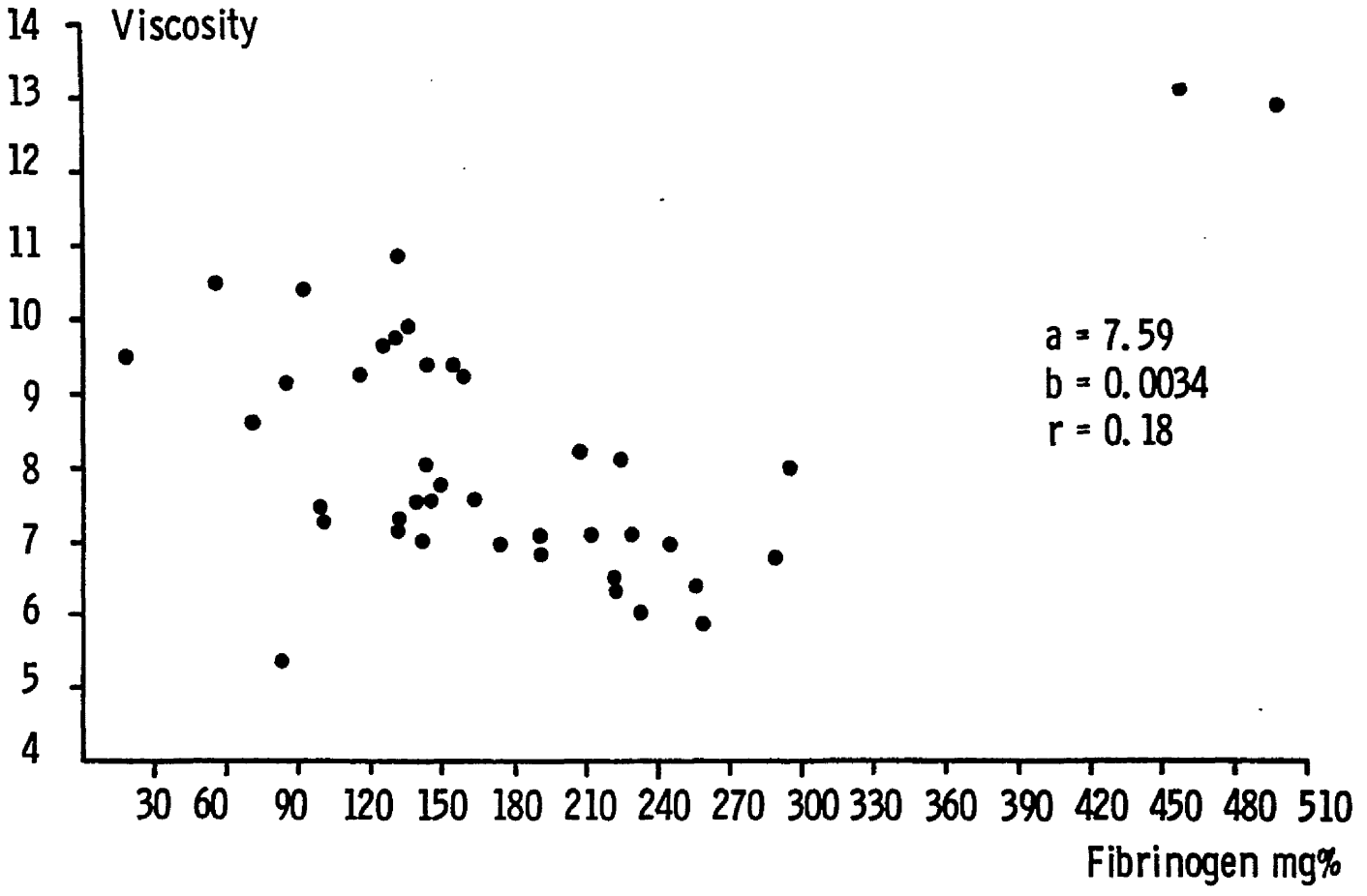


FIG 38: Relationship between viscosity(corrected to Hct 45) at 11.5 sec^{-1} and fibrinogen concentration (experiments 1-13,26-30).

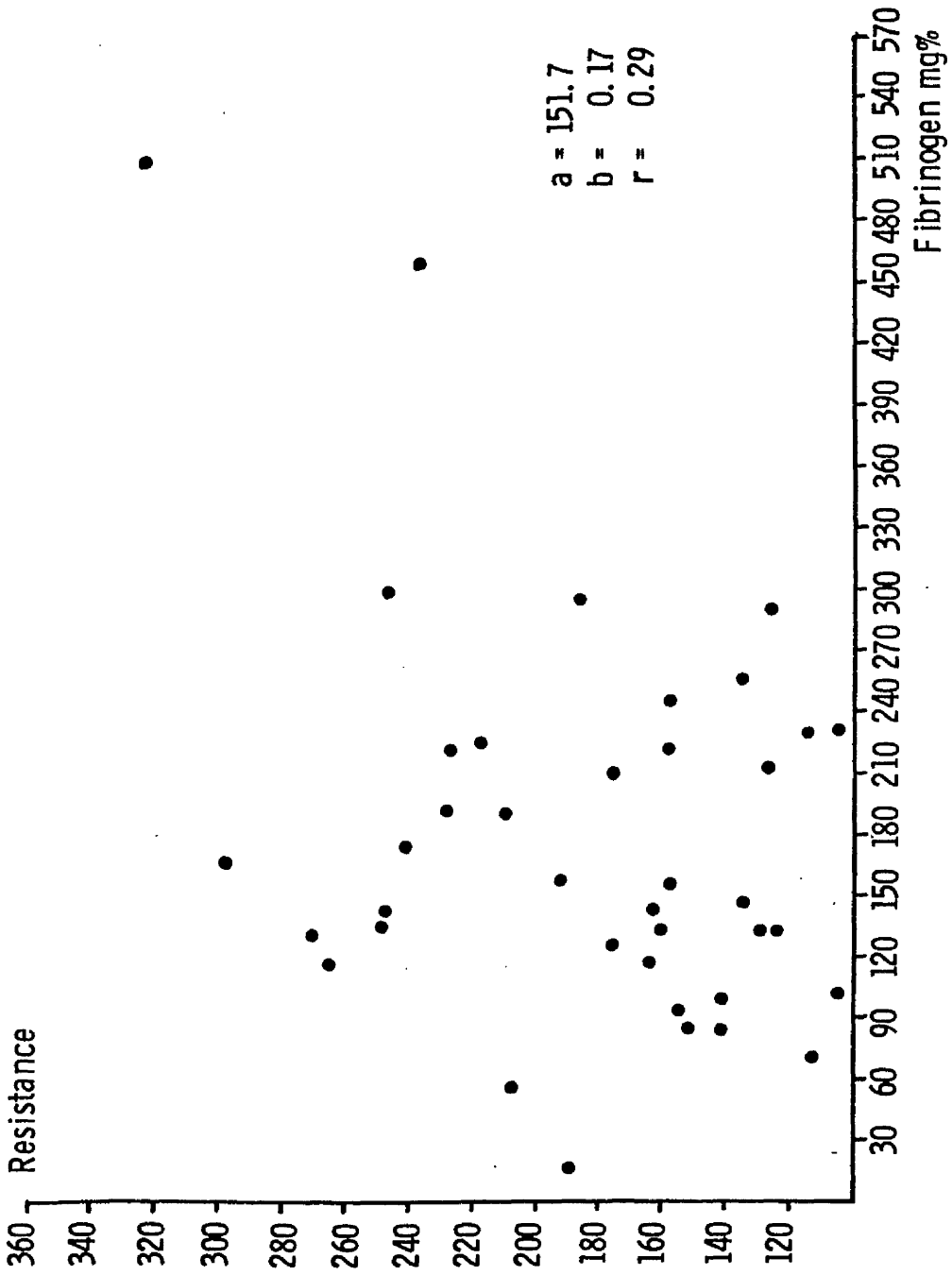


FIG 39: Relationship between resistance (corrected to Hct 45) at $\Delta P = 10$ mmHg and fibrinogen concentration (experiments 1-13, 26-30).

perfused at a constant Δ P of approximately 60 mm Hg, while the fibrinogen mixed, and then perfused at different pressures to obtain a pressure/flow curve, as in the previous experiments. It was observed that the perfusion pressure and flow rate did not alter significantly as the fibrinogen was added, indicating that at constant perfusion pressure resistance remained unaltered. However, when the results from the pressure/flow curves are corrected to haematocrit 45 and plotted against fibrinogen, in the same way as for 'normal' blood, there is a positive correlation between viscosity at 230 sec^{-1} and fibrinogen concentration (Figure 40). The slope (0.0022) however is not steep (note that Figure 40 shows viscosity,not log viscosity, versus fibrinogen) and the correlation (0.70) is not particularly strong. Indeed this relationship may be coincidental since for the same experiments at shear rate 11.5 sec^{-1} there is no correlation at all (Figure 41) between viscosity and fibrinogen concentration and, at neither perfusion pressure $\Delta P > 80$ mm Hg or $\Delta P \leq 10$ mm Hg is there a significant correlation between resistance and fibrinogen concentration ($r \simeq 0.47$ and $r \simeq 0.10$ respectively.)

Relationship between Viscosity, Resistance, and Plasma Protein Concentration

The effect of the total plasma protein concentration on viscosity in vitro and resistance in vivo can be considered in a similar fashion to that employed for fibrinogen. The same regression lines are used to 'normalise' haematocrit to 45, and these normalised values of viscosity and resistance are plotted

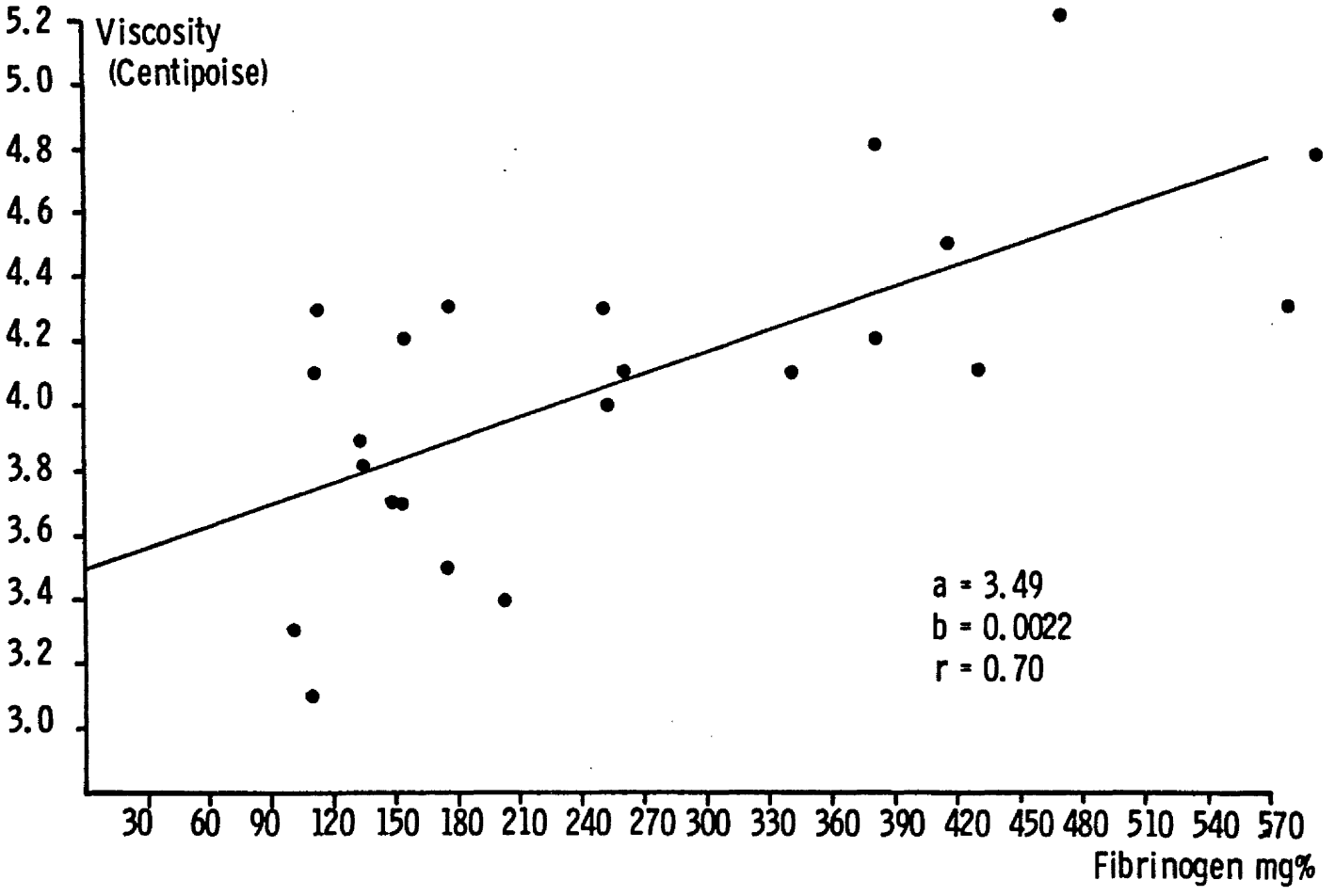


FIG 40: Relationship between viscosity at 230 sec^{-1} and fibrinogen concentration, experiments 22-25.

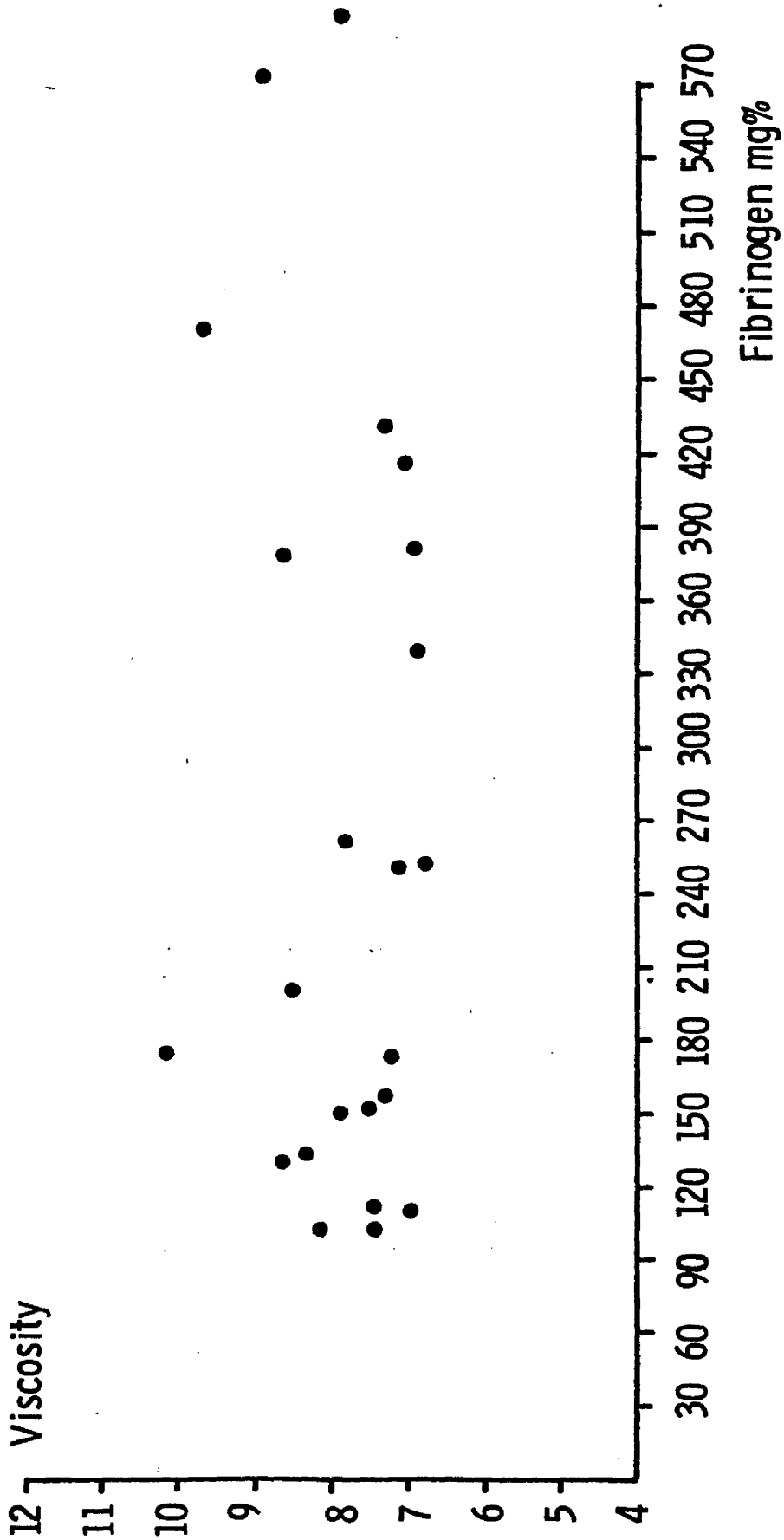


FIG 41: Relationship between viscosity at 11.5 sec^{-1} and fibrinogen concentration, experiments 22-25

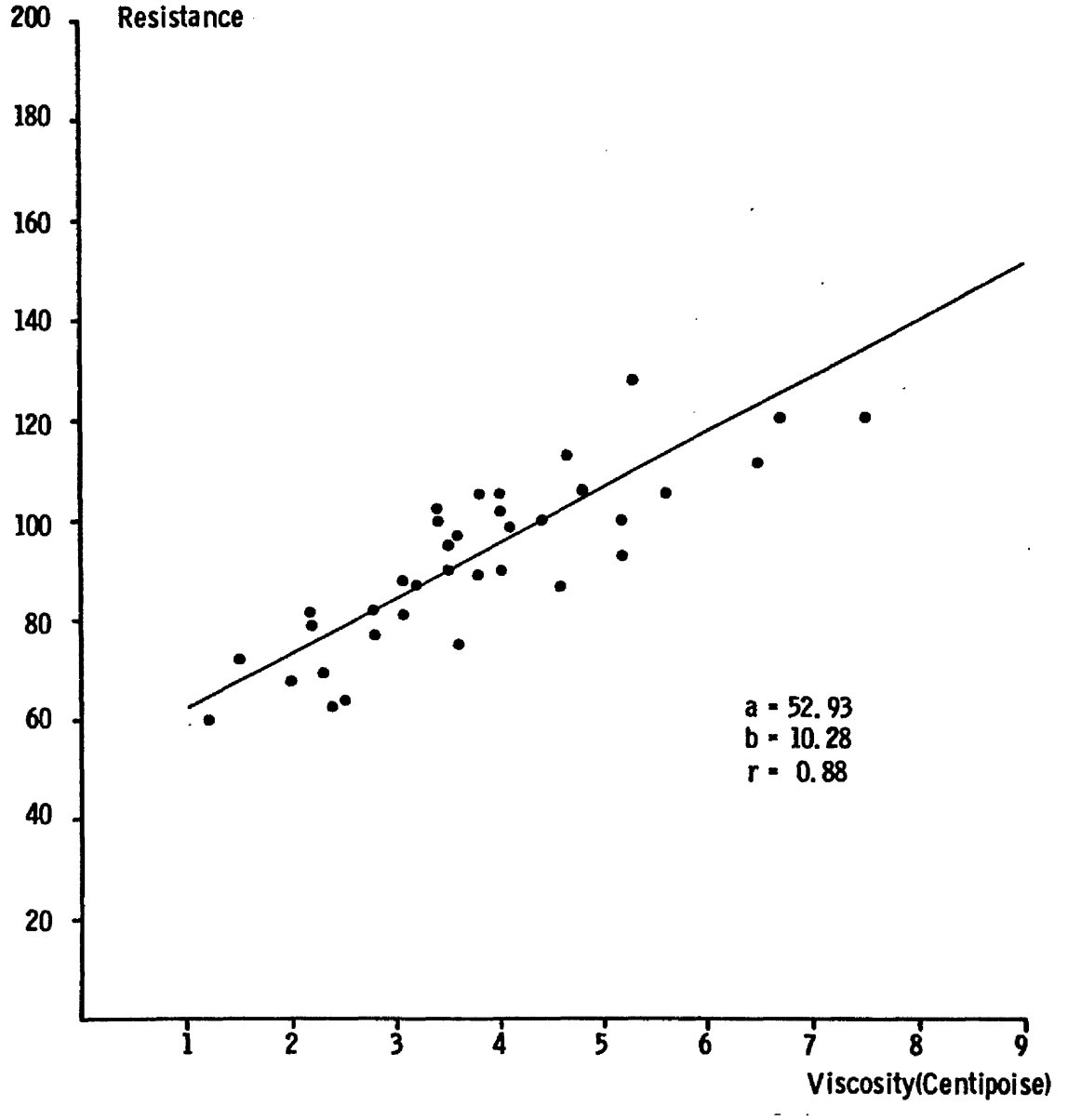


FIG 42: Correlation between resistance, $\Delta P = 80$ mmHg and viscosity, shear rate = 230 sec^{-1}, experiments 1-13

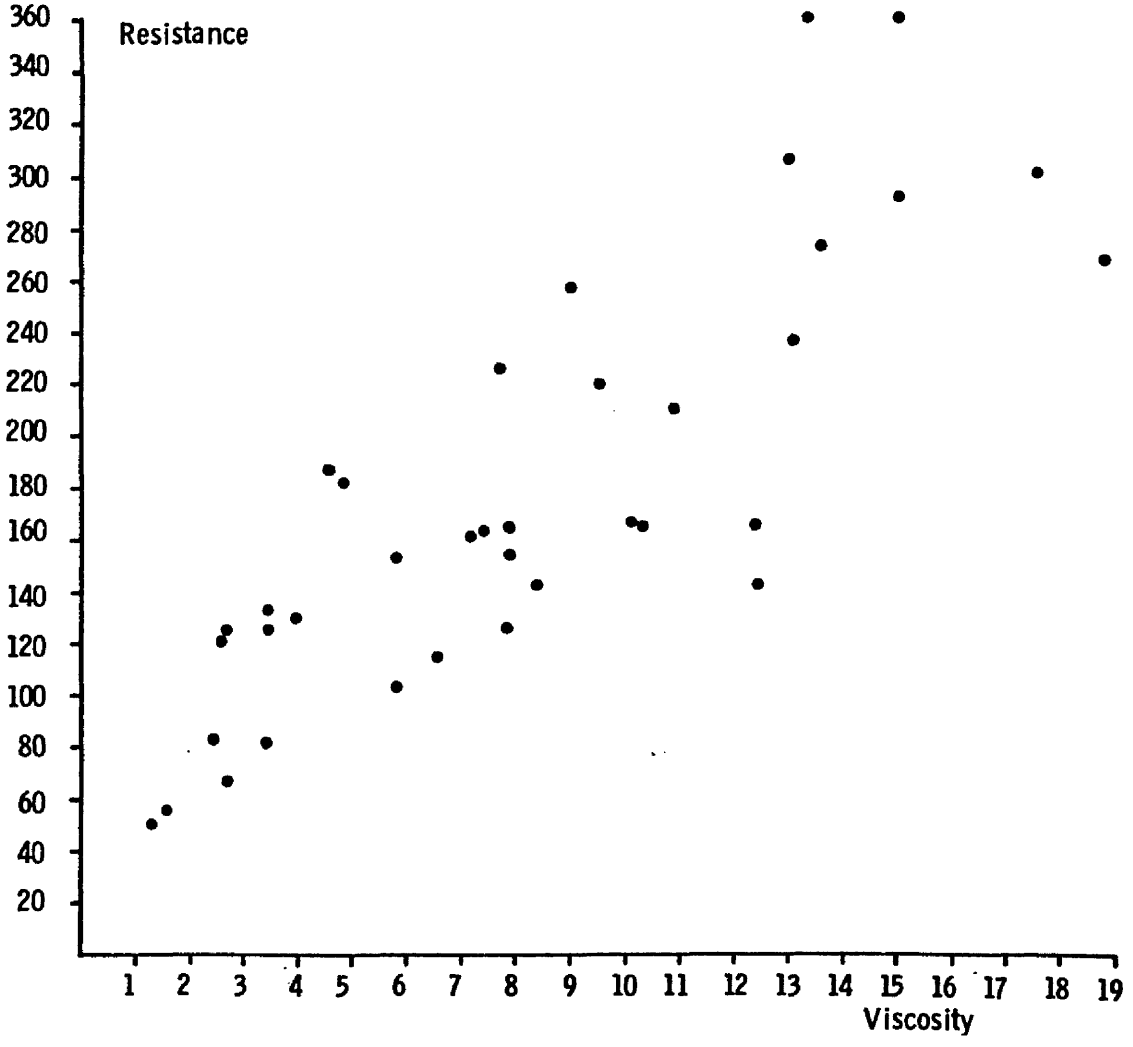


FIG 43: Correlation between resistance, $\Delta P = 10$ mmHg and viscosity, shear rate = 11.5 sec^{-1}, experiments 1-13

against plasma protein concentration. It is evident that there is no clear correlation between whole blood viscosity, or resistance, of normal blood or ancrod treated blood at either high or low shear rates or perfusion pressures (Figures 44-51).

Effect of Temperature

Two experiments (Experiments 28 and 29) were carried out to show the effect of alteration in temperature on viscosity and resistance. The procedure was that of the previous experiments except that the test blood was perfused at temperatures of approximately 25°C and 15°C in addition to 37°C. The temperature of the circulating blood was maintained by altering the temperature of the water circulating in the water jacket of the oxygenator/reservoir. As expected viscosity and resistance showed corresponding increases as temperature was reduced, both experiments yielding very similar results. Figure 52 shows the effect of temperature on resistance in Experiment 28.

Suspensions of Red Blood Corpuscles in Dextran or Ringer's Solution

The characteristics of red cells suspended in Dextran and in Ringer's solution were investigated in three experiments (26, 27 and 30). From Experiment 26 it can be seen that (Figure 53) substituting Dextran for plasma does not seem to alter the non-Newtonian characteristics of the red cell suspension, but merely increases its viscosity and resistance. Similarly the substitution of Ringer's solution for plasma (Experiments 27 and 30) results in slightly lower viscosity and resistance but similar non-Newtonian characteristis (Figure 54).

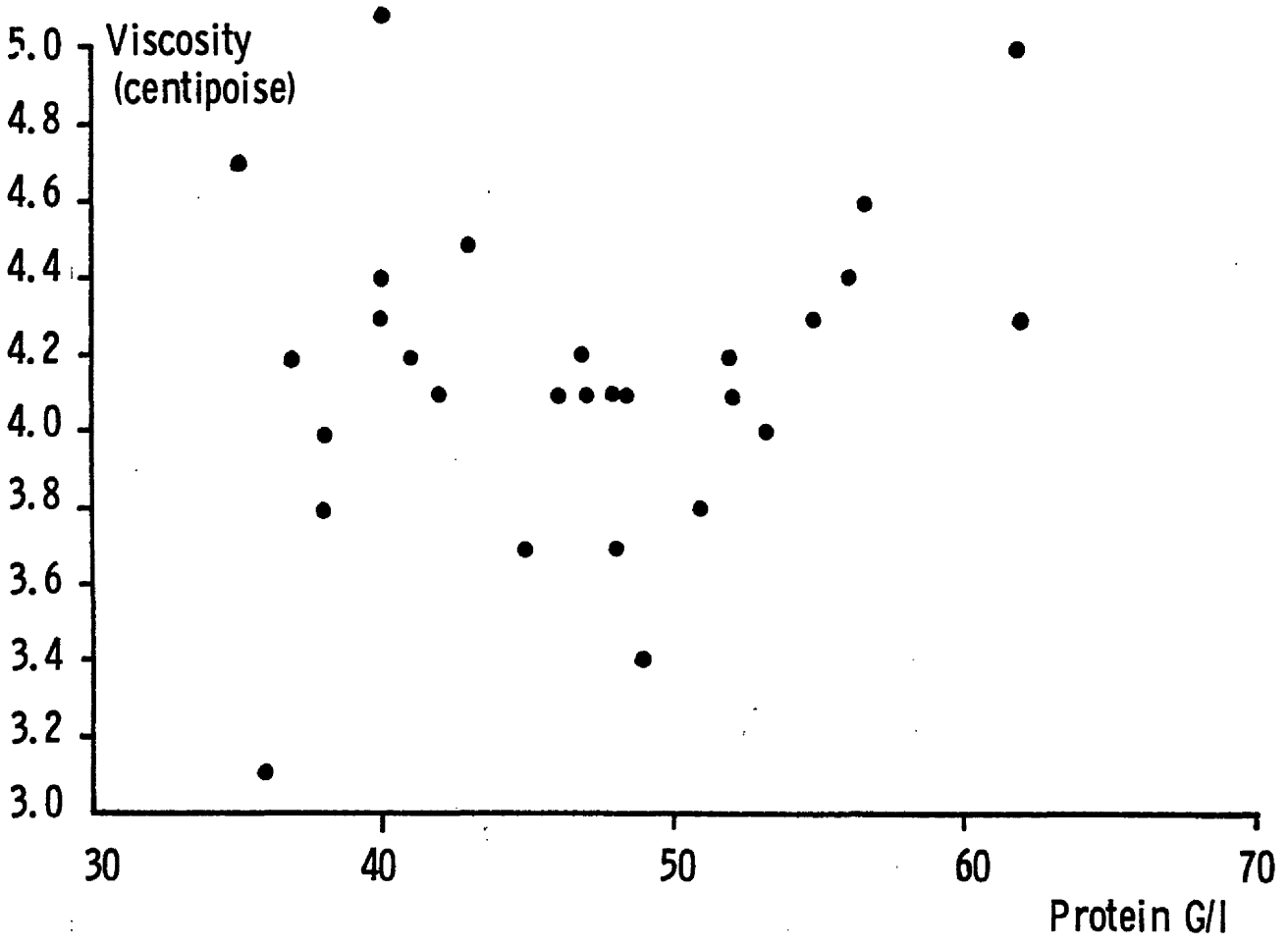


FIG 44: Relationship between viscosity (corrected to Hct 45) and plasma protein concentration, shear rate 230 sec^{-1}

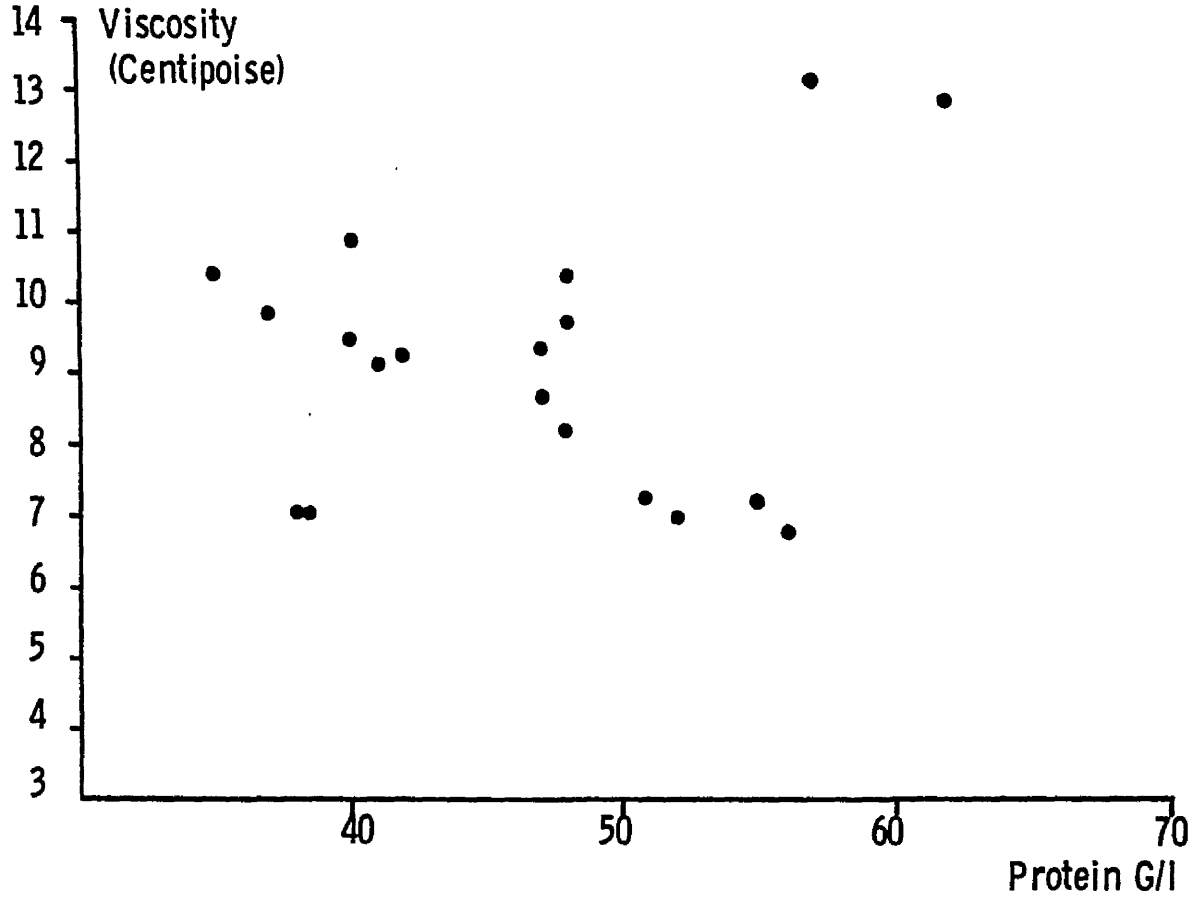


FIG 45: Relationship between viscosity (corrected to Hct 45) and plasma protein concentration, shear rate 11.5 sec^{-1} (experiments 1-13)

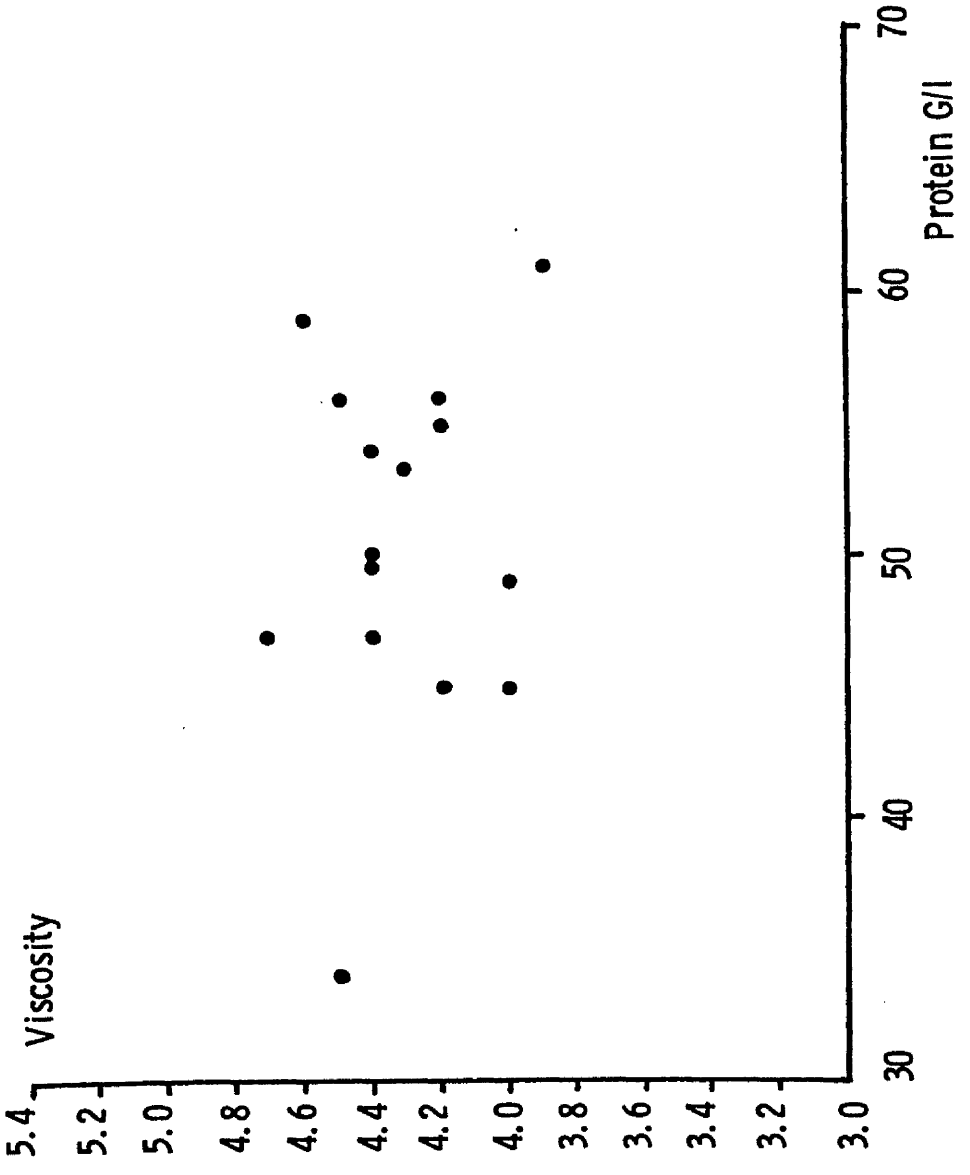


FIG 46: Ancrod treated. Relationship between viscosity (corrected to Hct 45) and plasma protein concentration, shear rate = 230 sec^{-1}

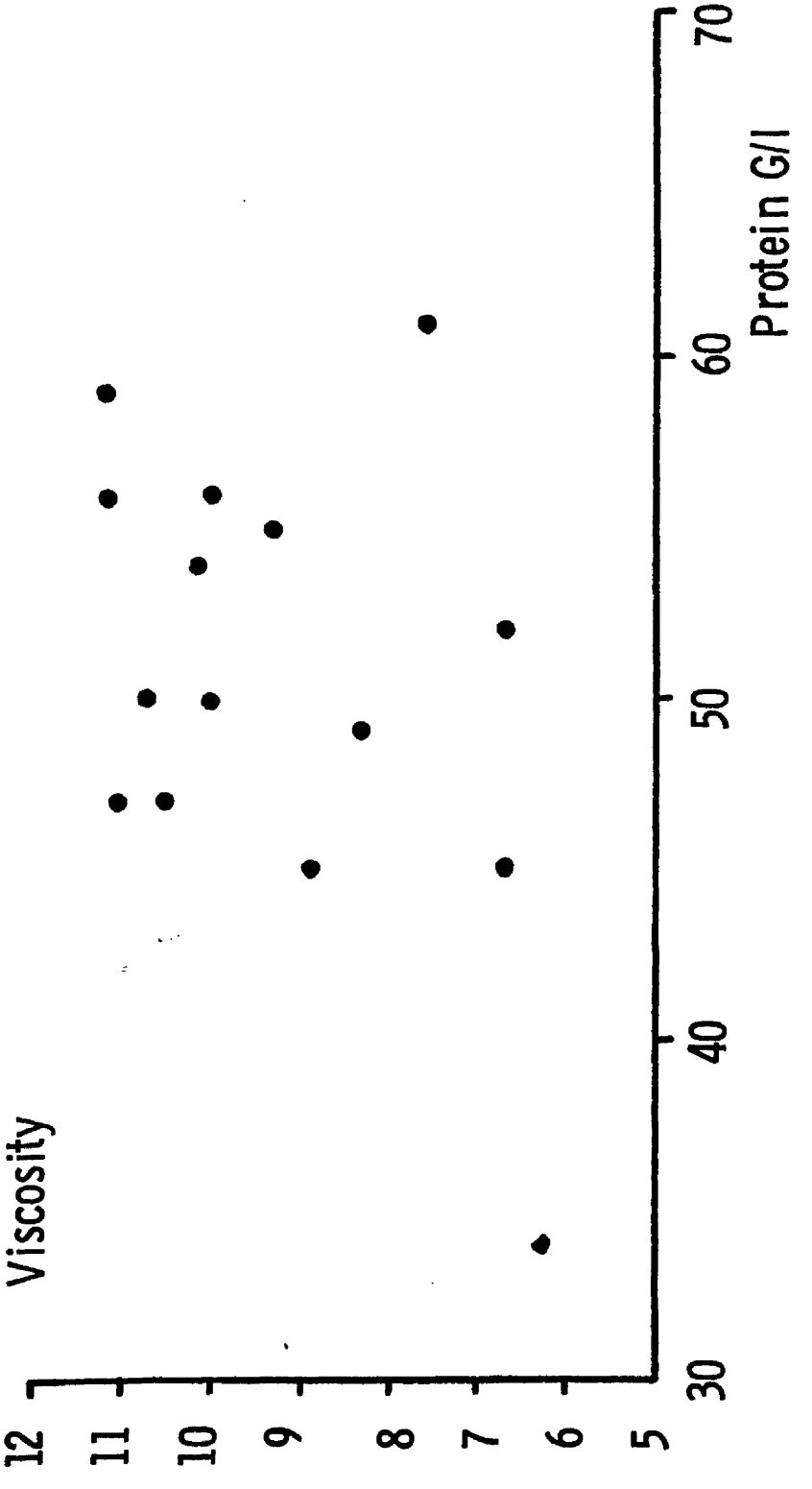


FIG 47: Ancrod treated. Relationship between viscosity (corrected to Hct 45) and plasma protein concentration, shear rate 11.5 sec^{-1}

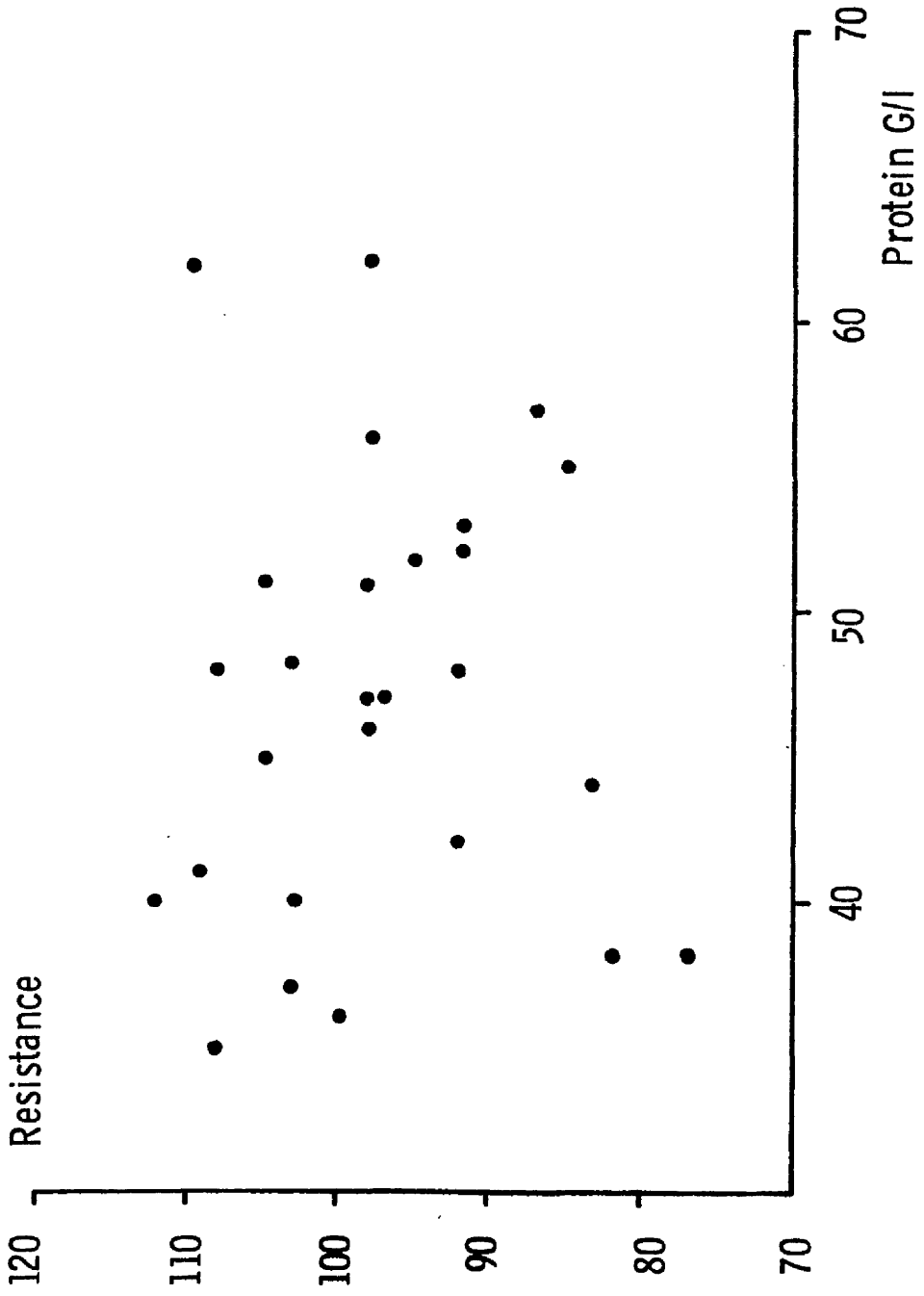


FIG 48: Relationship between resistance (corrected to Hct 45) and plasma protein concentration at $\Delta P > 80$ mmHg.

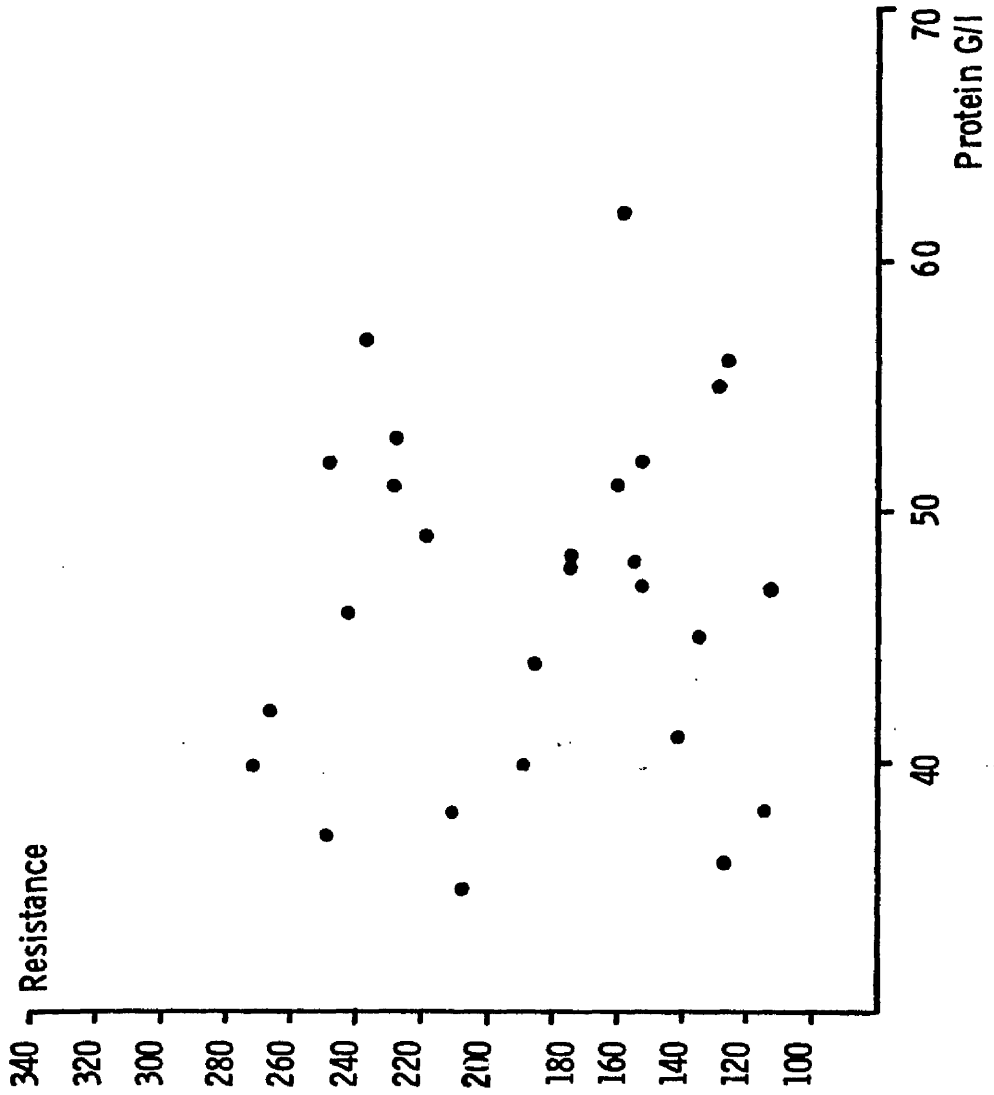


FIG 49: Relationship between resistance (corrected to Hct 45) and plasma protein concentration. ΔP = 10 mmHg.

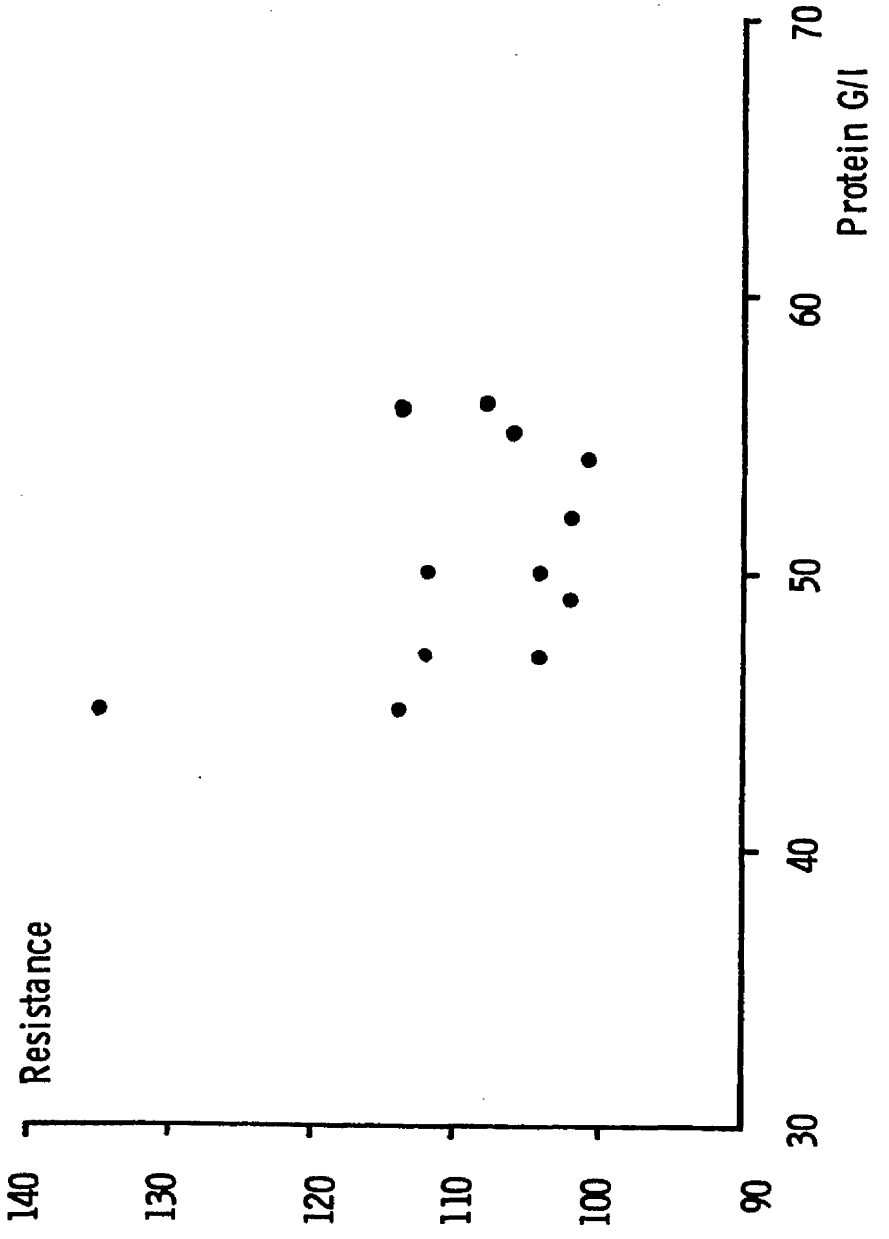


FIG 50: Ancrod treated. Relationship between resistance (corrected to Hct 45) and plasma protein concentration at $\Delta P > 80$ mmHg.

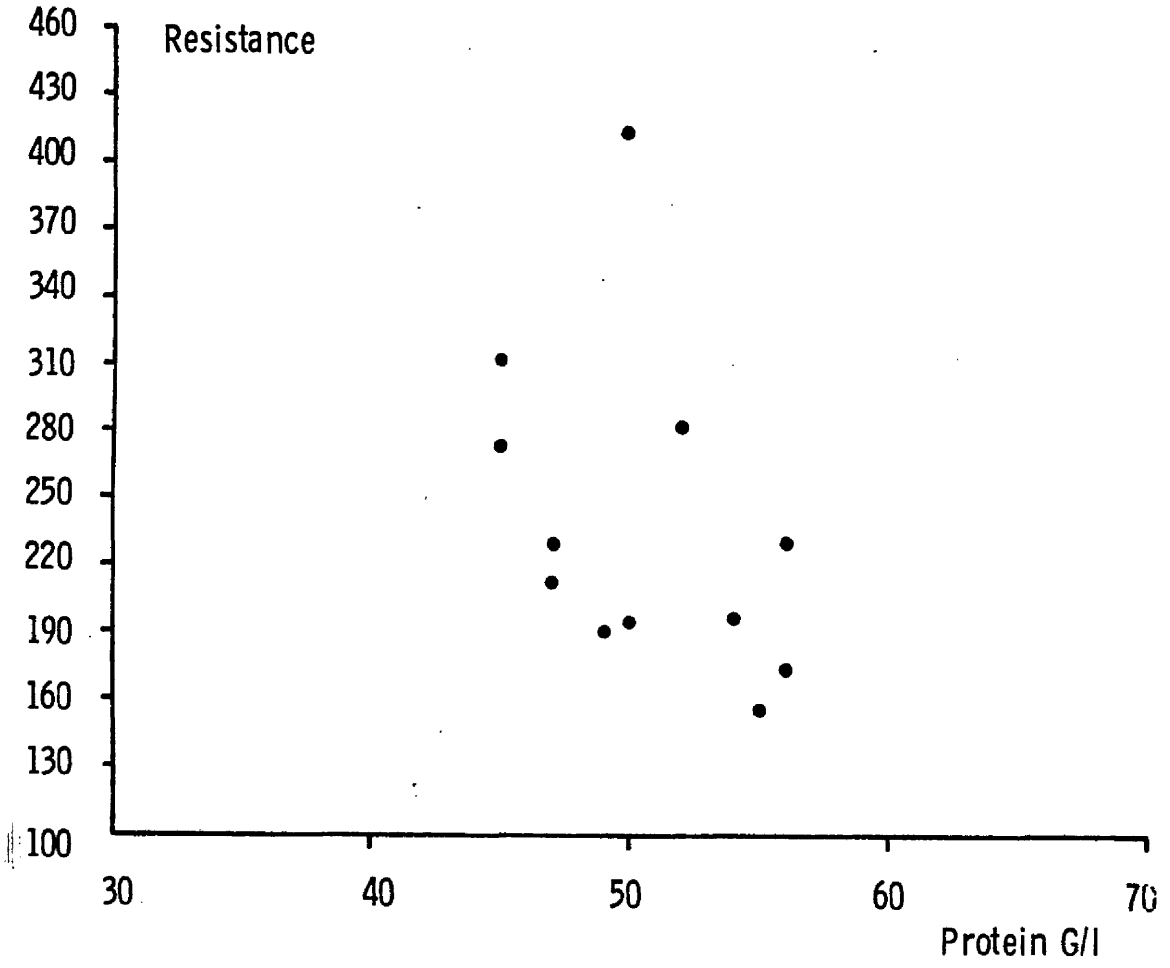


FIG 51: Ancrod treated. Relationship between resistance (corrected to Hct 45) and plasma protein concentration at ..P - 10 mmHg.

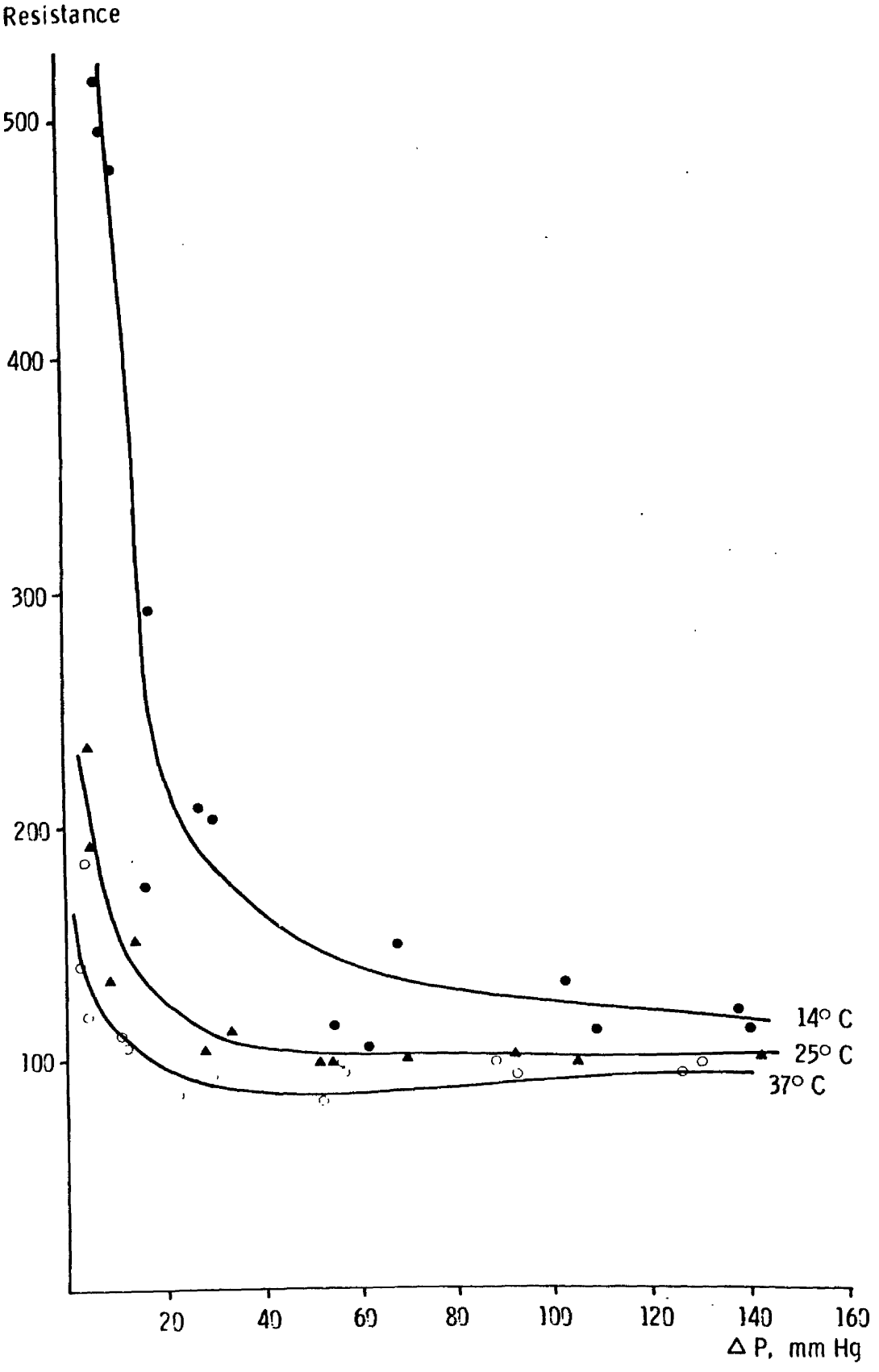

Resistance
500
400
300
200
100
20
40
60
80
100
120
140
160
Δ P, mm Hg
14° C
25° C
37° C

FIG 52

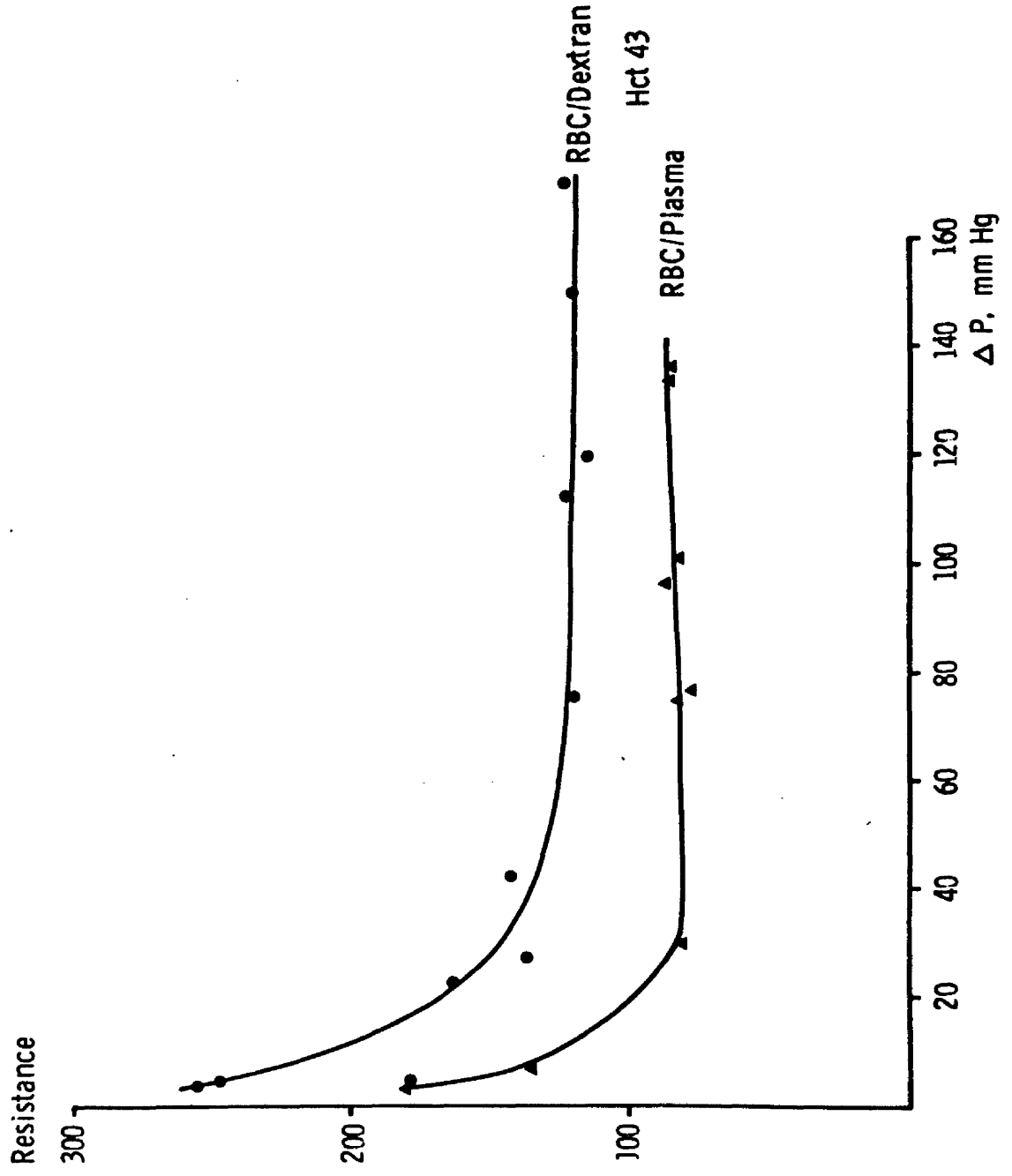

Resistance
300
200
100
RBC/Dextran
Hct 43
RBC/Plasma
20
40
60
80
100
120
140
160
Δ P, mm Hg

FIG 53

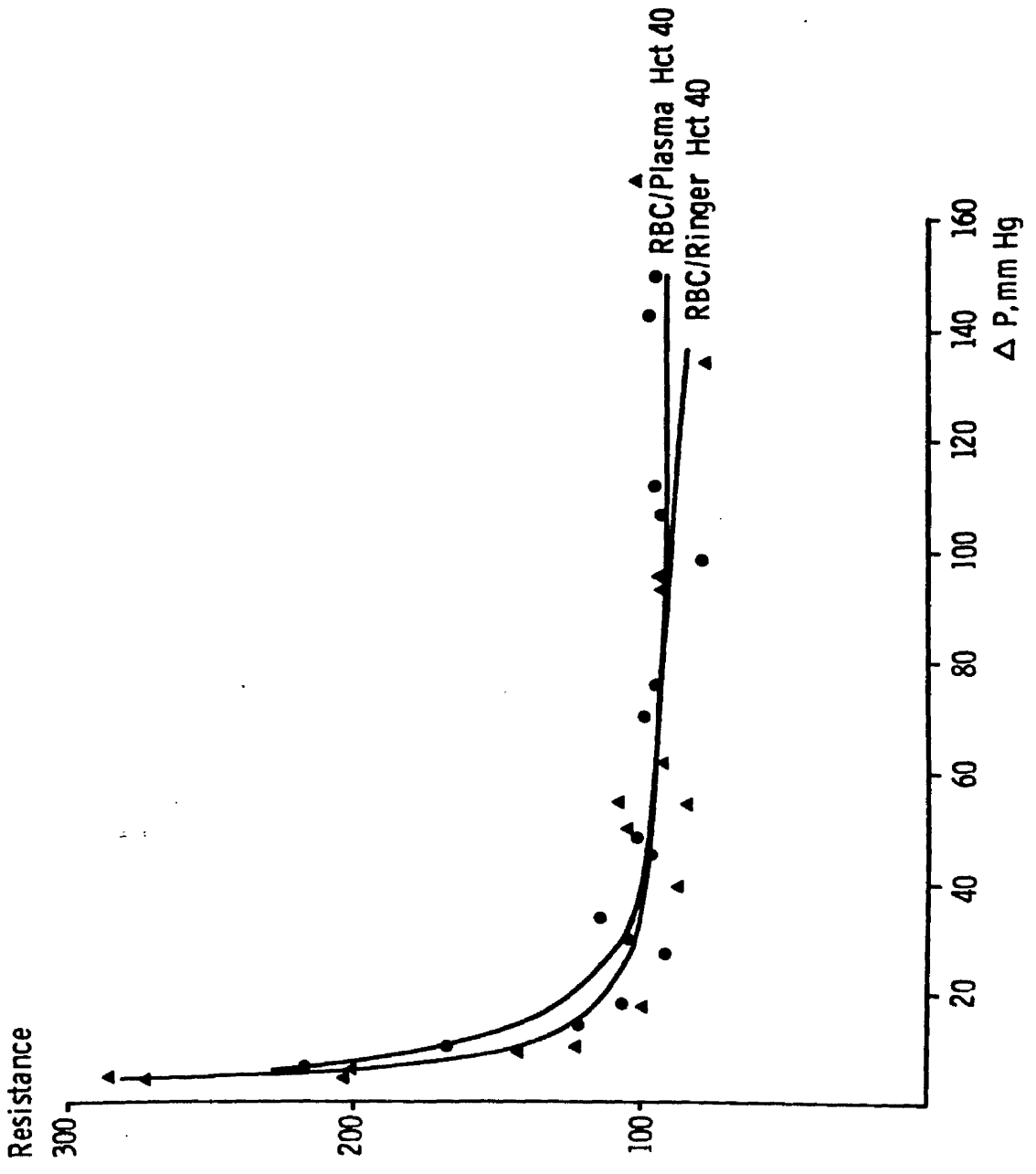

Resistance
300
200
100
20
40
60
80
100
120
140
160
Δ P, mm Hg
RBC/Plasma Hct 40
RBC/Ringer Hct 40

FIG 54

Summary

1. Viscosity measurements in vitro,using the Wells-Brookfield microviscometer, confirm previous work and indicate that:-

   a) haematocrit is the main determinant of blood viscosity, the relationship between log viscosity and haematocrit being virtually linear.

   b) blood viscosity is not constant, but increases at low rates of shear. The magnitude of the increase, and the shear rate below which the increase takes place is related to haematocrit level.

   c) the slope of the haematocrit/log viscosity relationship is a function of shear rate.

2. Vascular resistance in this isolated limb preparation is a measure of "in vivo viscosity". At normal perfusion pressures (i.e.> 80 mm Hg) it is determined mainly by haematocrit. The relationship between resistance and haematocrit is approximately linear in the range haematocrit 30-70, below which resistance is much less influenced by haematocrit. Vascular resistance is not constant at a given blood viscosity, but below a certain perfusion pressure, increases with decreasing perfusion pressure. The magnitude of increase, and the perfusion pressure below which the increase takes place vary with haematocrit.

3. Although vascular resistance in the isolated limb preparation correlates well with in vitro viscosity for a given set of samples, the magnitude of the change in vascular resistance with haematocrit or perfusion pressure is considerably less than that of the change in vitro blood viscosity.

4. Neither fibrinogen nor plasma proteins, in the concentrations studied, had a significant effect on either blood viscosity in vitro, or vascular resistance in the isolated limb.

5. In the isolated limb, suspensions of red blood cells in protein free solutions (Dextran 40/Normal Saline, Ringer's Solution) had similar non Newtonian properties to blood, while Dextran perfused alone was almost Newtonian. (A slight increase in resistance occurred at the extreme ends of the perfusion pressures employed).

6. The effect of cooling the perfused blood in the isolated limb preparation was to increase vascular resistance, and to increase blood's non Newtonian behaviour.

CHAPTER SEVEN

DISCUSSION

The work in this thesis was prompted by the realisation that a large number of patients with disabling peripheral vascular disease are unsuitable for reconstructive surgery and that no other form of therapy has been proven effective. It is well established in theory and in practice that attempts to improve blood flow by vasodilator drugs are unlikely to succeed and similarly sympathectomy has limited application. Since the component of resistance to flow in addition to the size of the resistance vessels (mainly terminal arterioles and venules) is the intrinsic resistance or viscosity of blood, it is reasonable to consider therapeutic manipulation of blood flow properties.

As discussed earlier, a great deal is known about blood rheology in laboratory instruments but much less of its relevance to the intact circulation. The experiments described in this thesis are an attempt to assess the main properties of blood flow in an intact circulation, and to what extent they are predicted by laboratory viscometry.

The experiments can be legitimately criticised on several grounds, mainly the relevance of canine results to humans, and of an isolated circulation to in vivo conditions.

As far as blood rheology is concerned canine blood appears to be very similar to human and it is likely that the physiology of the peripheral circulation is reasonably similar.

Extrapolating the results of isolated organ perfusion experiments to the intact circulation requires caution however, but seems the only way that rheological effects on vascular resistance can be distinguished from changes in dimensions of the

peripheral vessels. It is unfortunate that extracorporeal circulation in itself may cause damage to the blood by the mechanical action of the pump and by artificial oxygenation. Fragmentation of red cells and denaturation of proteins may occur, particularly when a bubble oxygenator is used. Despite this, haemolysis sufficient to discolour plasma was not observed in our experiments. The plasma protein levels in our samples were perhaps lower than expected and this may be accounted for by deposition of protein in the oxygenator. However, if this were exerting a significant rheological effect one would have expected an alteration in resistance at a given flow rate, or perfusion pressure, as the experiment progressed. This did not occur to a significant extent.

Despite these reservations it is reasonable to draw general conclusions from these experiments on the relationship between blood viscosity in vitro and resistance in vivo.

Blood viscosity in vitro and in vivo increases steadily with haematocrit and with decreasing shear rate. However, the magnitude of the changes involved seems to be very much less in vivo than in vitro. It has been generally accepted that blood viscosity is lower in vivo than in vitro.(Whittaker & Winton, 1933; Djojosugito, 1970). This is based on a comparison of ratios of blood/plasma viscosity in vitro, and blood/plasma resistance in vivo. The lower relative viscosity was attributed to the redistribution of red cells (Fahraeus, 1931). However, from our experiments it is seen that although vascular resistance declined steadily as haematocrit is reduced from high levels this curve levels out as haematocrit goes below 30. Thus at low levels of

haematocrit, red cell concentration does not make an appreciable difference to vascular resistance (this was previously noted, in a quite different preparation by Levy, 1973), but it does affect in vitro viscosity, the in vitro viscosity/haematocrit curve being reasonably smooth down to very low haematocrit levels. It seems that at low haematocrit levels vascular resistance has a large component which is not dependent on blood's viscous properties. Therefore, to base a calculation of in vivo viscosity on the relative resistance of blood to plasma will lead to an artificially low value of in vivo viscosity. The idea that blood viscosity is lower in vivo was first mooted by Whittaker and Winton (1933), who attributed this to an effective reduction in haematocrit by the increasingly large cell free zone at the periphery of the vessel as vessel size is reduced. This idea is still widely accepted and Strandness (1976) notes that the reduction by one third of viscosity in vivo found by Whittaker and Winton is consistant with that predicted by the Fahraeus-Lindqvist effect for vessels of 50 microns, approximately the size of the 'resistance' vessels - the terminal arterioles and venules.

An alternative explanation, suggested originally by Benis (1970), for the apparently low in vivo blood viscosity, that the discrepancy is due to non-linear inertial pressure losses in the low haematocrit blood seems more likely in the light of our results. In 1970 Benis reported experiments in which canine hind paws were perfused under conditions of steady flow with blood of varying haematocrit and suspensions of albumen-Ringer solution. In common with our experiments they found that blood

and RBC/ringer's solution behaved similarly at a particular haematocrit, and that flow resistance increased markedly with haematocrit. They considered that non Newtonian viscosity, as well as vessel distensibility contributed to the non-linearity of pressure/flow curves. By perfusing their preparation with two Newtonian cell free fluids of different viscosity (Pameg-Ringer Solution, viscosity 4.51 cp; Albumen/Ringer, viscosity 0.972 cp) inertial losses were calculated under conditions of steady flow. At physiological pressures inertial losses accounted for about 40% of the total pressure drop for the cell free albumen Ringer solution, but only 5% for blood of haematocrit 50.

The same authors expanded on this work (Benis,1973) by repeating Whittaker and Winton's experiments. They perfused canine hind limbs with suspensions of erythrocytes in albumen/ Ringer solution and in addition perfused both freshly excised limbs and limbs fixed in 10% formaldehyde (whose vascular dimensions could not alter) with cell free plasma expanders of different viscosities. They confirmed Whittaker and Winton's basic findings that the ratio of blood viscosity to plasma viscosity is less in the limb than in the viscometer, but showed that the same discrepancy occurred between cell free Newtonian fluids of high and low viscosity. The explanation therefore cannot lie in the Fahraeus-Lindqvist effect (which depends on redistribution of erythrocytes), but is likely to be due to non-linear (inertial) pressure losses in the large vessels of the hind limb.

In our experiments, if in vivo viscosity were calculated by comparing the ratio of resistance of normal blood in the isolated perfusion system to plasma then it would appear as if viscosity were lower in vivo than in vitro, since in the viscometer blood of haematocrit 40 has a viscosity of about three times that of plasma while in the isolated limb its resistance is about twice. However, a similar calculation using Dextran-40 instead of blood shows that both in the viscometer and in the isolated limb Dextran had a viscosity similar to that of blood of haematocrit 40. Thus the ratio of the viscosity of Dextran to plasma is also considerably less in the isolated limb than in the viscometer. Clearly the effect cannot be related to the redistribution of red cells but can be explained by disproportionately higher inertial losses for plasma.

It appears from our experiments that fairly large changes of viscosity, as measured in the laboratory viscometer, are required to exert a noticeable effect in vivo. This may account for the failure of fibrinogen concentration to exert a significant effect on vascular resistance in the limb perfusion model. This finding is disappointing since from the in vitro low shear rate work of Merrill(1969) and others, it would be anticipated that reduction of fibrinogen levels would have lowered vascular resistance, at very low rates of flow. Ancrod, which selectively and safely reduces plasma fibrinogen to a very low level, would then have been a major advance in the treatment of inoperable vascular disease. There are now a number of reports of ancrod's use in obstructive arterial disease which have shown that controlled therapeutic defibrination is feasible (Dormandy,1977)

and safe in the per-operative period (Barrie, 1975) but the benefit seems marginal. The drug has however shown promise in improving the patency rates of synthetic arterial grafts in dogs (Postlethwaite, 1977), although, as in the experiment described in Chapter 4 the benefit may have been derived from an anti-coagulant effect rather than an alteration in rheological properties.

A number of other drugs have been claimed to improve blood flow by among other actions, reducing blood viscosity. The vast majority are quite uncontrolled observations and do not merit serious consideration. Two drugs, Cinnerazine (trade name in UK Stugeron) and Oxpentifylline (trade name in UK Trental) have shown some promise as active rheological agents. Cinnerazine, a piperazine derivative, was synthesised in 1958. It has been reported to increase resting blood flow and prolong hyperaemic flow (Ellis, 1977) and to benefit patients with intermittent claudication, muscular cramps, cold extremities and vascular spasms, (Staessen, 1977). Di Perri (1977) studied the drug's action on viscosity and showed an 11-18% reduction in whole blood viscosity but no significant change in haematocrit, plasma viscosity or fibrinogen concentration. This implies that the characteristics of the red cell - such as internal viscosity, aggregation, or deformability are influenced, of which the last is the most likely.

Naftidrofuryl (Praxilene) is also reported to lower blood viscosity and enhance metabolism in ischaemic tissues. It is claimed that its effect in treating obstructive arterial disease depends on these actions but a properly controlled trial of the drug in patients with claudication failed to show any definite benefit

(Ruckley, 1978).Similarly Charlesworth's group (Mashiah, 1978) measured the viscosity and limb blood flow in claudicants before and after treatment with three drugs - all said to reduce blood viscosity - Tetranicotinoyl Fructose (Bradilan), Oxpentifylline (Trental) and Cinnerazine (Stugeron). After treatment there was little or no change in blood viscosity and no change in blood flow.

No attempt was made in our experiments to assess red cell flexibility but it is likely that the ability of the red cells to deform is fundamental to flow in the micro-circulation. At present there is no generally accepted method of measuring red cell flexibility, although Reid(1976b) has recently suggested a test involving filtration of blood through a poly-carbonate sieve containing cylindrical channels 5 μ in diameter under a pressure of 20 cms of water. Interestingly red cell flexibility appears to be directly related to fibrinogen concentration (Sirs, 1968) and it is tempting to postulate that the disappointing results of defibrination are due to the theoretical reduction in whole blood viscosity being compensated by a corresponding decrease in red cell flexibility. Further evidence for the importance of this topic is to be found in recent papers showing decreased red cell flexibility in patients with vascular disease (Reid,1976a) and in diabetes (Macmillan, 1978).

Our studies have shown that whole blood viscosity as measured in the Wells-Brookfield Viscometer will provide an indication of blood's behaviour in the circulation, but that the changes of resistance or apparent viscosity in vivo are much less than the corresponding changes in in vitro viscosity. The

combination of a reduced flow rate and raised haematocrit is particularly disadvantageous and it may well be worth treating patients with raised haematocrit levels which fall well short of polycythaemia. In 1963 Cranley showed some benefit from repeated phlebotomy in such patients, although more recently in a small pilot trial Ford (1978) could not confirm this. However an important study by Marshall and his colleagues at the Institute of Neurology (Thomas, 1977) showed that in a group of 19 patients cerebral blood flow increased by 50% as haematocrit was reduced (by venesection) from a mean value of 49.3 to 42.6. In the same journal Nicolaides (1977) reported blood viscosity studies on patients with angina. Whole blood viscosity, haematocrit, red cell flexibility and plasma fibrinogen concentration were all higher in the 22 patients than in the controls, but when viscosity was corrected to a standard haematocrit (45%) there was no significant difference in the mean viscosity of the two groups. This indicated that the raised viscosity in patients with angina is a result of higher haematocrit, and not of raised plasma fibrinogen concentration.

If one is going to treat obstructive arterial disease by improving flow properties the experiments described in this thesis and the recent work on cerebral blood flow by Thomas (1977) suggest that reduction of even modestly raised haematocrit levels may be the most effective method. Clearly, reducing the red cell concentration of blood reduces its oxygen carrying capacity, in addition to improving its fluidity, and one could well ask if the one will not cancel the other in terms of benefit to the patient. Thomas (1977) calculated that in his

patients the 50% increase in cerebral blood flow obtained by reduction in haematocrit (from 49 to 42) was offset by a less than 15% reduction in oxygen carrying capacity. Theoretically there should be an optimum haematocrit at which the oxygen carrying capacity is greatest allowing for the opposing factors of increasing oxygen carrying capacity with increasing haematocrit and decreasing flow caused by the increased viscosity of the blood, although this concept has been disputed (Halmagyi, 1977).

In experiments on conscious dogs Von Restorff (1975a,b) examined the effect of normo-volaemic haemo-dilution with dextran 70 on oxygen consumption at rest and during exercise. Cardiac output increased but not enough to compensate for the reduced arterial oxygen content, possibly due to the increased inertial losses of low haematocrit blood. However, Fowler (1975) on the basis of similar animal experiments concluded that relatively small decreases of blood viscosity may significantly increase resting cardiac output, and in a comprehensive review article Messmer (1975) considered that in the haematocrit range 25-30% the increase in flow rate is able to compensate fully for the diminished oxygen content of the blood. Horstman (1974) studied the effects of haemoglobin reduction on maximum oxygen consumption in normo-volaemic dogs which had been previously bled to 75% of the normal haemoglobin value. Cardiac output, cardiac work and central blood volume did not limit maximum oxygen consumption and cardiac output was a function of, and largely regulated by, peripheral resistance. Similarly Sunder-Plassmann (1975) showed, in dogs, that the predominant adaptional mechanism in acute dilutional anaemia (haematocrit 20%) is a marked increase

in nutritional blood flow to vital organs and tissues.

It seems likely that the optimum haematocrit is in the low forties or less (Crowell, 1958) and Dormandy may be correct in his suggestion (Dormandy, 1978) that the optimum concentration of a biological variable - such as haematocrit - may not necessarily be the normal value. With regard to haematocrit and haemoglobin concentration, in evolutionary terms the major threat to survival would have been traumatic haemorrhage and hence a high volume of red cell concentration would have been advantageous. Now that degenerative arterial narrowing is so much more a cause of mortality than traumatic exsanguination, we may have inherited a biological level of red cell concentration which is set too high. If a method could be found safely and permanently to fix the haematocrit of patients with obstructive arterial disease at an optimum level - say 35-40% - this might contribute substantially to the symptomatic treatment of arterial disease.

Experiment No. 1

Hct = 17

ΔP	Q	R	R^N	C	R^{NC}
102	231	0.44	99	98	97
89	192	0.46	103	102	105
68	158	0.43	96	104	99
52	126	0.41	92	104	96
40	97	0.41	92	99	91
24	62	0.39	87	99	86
15	36	0.42	94	89	84
8	18	0.44	99	83	82
3	5.25	0.57	128	83	106
8	14	0.57	128	83	106
16	39	0.41	92	89	82
21	60	0.35	78	99	77
34	92	0.37	83	99	82
45	135	0.33	74	104	77
53	158	0.34	76	104	79
69	200	0.35	78	104	81
101	287	0.35	78	98	76

Experiment No. 1

Hct = 37

ΔP	Q	R	R^N	C	R^{NC}
95	200	0.48	107	102	109
79	166	0.48	107	104	111
57	130	0.44	99	104	103
37	84	0.44	99	99	98
24	52	0.46	103	99	102
15	30	0.50	112	89	100
14	24	0.58	130	89	116
5.5	8.25	0.67	150	83	125
4	4	1.0	224	83	186
10	12	0.83	186	83	154
17	26	0.65	146	89	130
21	46	0.46	103	99	102
31	71	0.44	99	99	98
45	111	0.41	92	104	96
59	150	0.39	87	104	90
70	176	0.40	90	104	94

Experiment No. 1

Hct = 50

ΔP	Q	R	R^N	C	R^{NC}
76	130	0.58	130	1.04	135
69	102	0.68	152	1.04	158
50	61	0.82	183	1.04	191
28	28	1.0	224	0.99	221
12	11	1.09	244	89	217
7	5	1.4	314	83	261
3	2.8	1.07	240	83	199
3	3	1.0	224	83	186
19	19.5	0.97	217	89	193
21	29	0.72	161	99	159
35	60	0.58	130	99	129
48	91	0.53	119	104	124
59	120	0.49	110	104	114
72	167	0.43	96	104	100
87	200	0.44	99	102	101

Experiment No. 1

Hct = 76

Fibrinogen = 260

Protein = 5.7

ΔP	Q	R	R^N	C	R^{NC}
83	76	1.09	244	102	249
49	48	1.02	229	104	238
38	32	1.19	267	99	264
14	12	1.17	262	89	233
8	4.5	1.78	399	83	331
22	10	2.20	493	99	488
27	16	1.69	379	99	375
36	32	1.13	253	99	250
47	60	0.78	175	104	182
60	77	0.78	175	104	182
78	98	0.80	179	104	186
51	53	0.96	215	704	224
34	30	1.13	253	99	250
25	17.5	1.43	321	99	318
12	7.25	1.66	372	89	331
4	3.25	1.23	276	83	229
20	7.75	2.58	578	89	514

Viscosity	Shear Rate (sec^{-1})
	5.75 30
	11.5 21
	23 16.6
	46 13.1
	115 10.8
	230 9.7

Experiment No. 1

Dextran

ΔP	Q	R	R^N
36	68	0.53	119
22	51	0.43	96
9	24	0.37	83
2	8	0.25	56
2	5	0.40	90
12	18	0.67	150
33	70	0.47	105
57	135	0.42	94
75	185	0.41	92
95	280	0.40	90

Experiment No. 2

Hct = 27

Fibrinogen = 230

Protein = 3.8

ΔP	Q	R	R^N	C	R^{NC}
111	326	0.34	62	98	61
88	265	0.33	61	102	62
70	213	0.33	61	104	63
53	158	0.34	62	104	64
38	103.4	0.37	68	99	67
26	65.9	0.39	72	99	71
16.5	40	0.41	75	89	67
12	29	0.41	75	89	67
8.5	18.2	0.47	86	83	71
6	15	0.40	73	83	61
5.5	10.9	0.50	92	83	76
6	10.9	0.55	101	83	84
8.5	17.6	0.48	88	83	73
12.5	27.8	0.45	83	89	74
17	38	0.44	81	89	72
27	69	0.39	72	99	71
40	107	0.37	68	99	67
54	158	0.34	62	104	64
74	207	0.36	66	104	69
91	261	0.35	64	102	65
123	333	0.37	68	96	65

Viscosity	Shear Rate (sec $^{-1}$)	
	5.75	3.2
	11.5	3.4
	23	3.2
	46	3.0
	115	2.6
	230	2.4

Experiment No. 2

Hct =27

Fibrinogen = 190

Protein = 3.8

ΔP	Q	R	R^N	C	R^{NC}
117	328	0.36	66	98	65
88	250	0.35	64	102	65
66	194	0.34	62	104	64
55	146	0.38	70	104	73
40	97	0.41	75	99	74
28.5	60	0.48	99	99	87
20.5	37	0.55	101	99	100
16.5	26	0.63	115	89	102
13	17	0.76	139	89	124
10	9.1	1.10	202	83	168
8.5	10.2	0.83	152	83	126
13	17	0.76	139	89	124
18	27	0.67	123	89	109
21	36.5	0.57	105	99	104
27.5	59	0.47	86	99	85
37	94	0.39	72	99	71
53	143	0.37	68	104	71
69	200	0.35	64	104	67
87	244	0.36	66	102	67
110	306	0.36	66	98	65

Viscosity	Shear Rate (sec^{-1})	
	5.75	5.4
	11.5	3.4
	23	2.9
	46	3.1
	115	2.7
	230	2.5

Experiment No. 2

Hct = 32

Fibrinogen = N.A.

Protein = N.A.

ΔP	Q	R	R^N	C	R^{NC}
118	333	0.35	64	98	63
89	259	0.34	62	102	63
67	196	0.34	62	104	65
50	149	0.34	62	104	65
39	100	0.39	71	99	70
24	60	0.40	73	99	72
17.5	37.5	0.47	86	89	77
14.5	28	0.52	95	89	85
12	18	0.67	123	89	97
8.5	10.5	0.81	148	83	123
10.5	11.1	0.94	172	83	143
13	17	0.76	139	89	124
17	28	0.61	112	89	100
21	36.6	0.57	105	99	104
28	59	0.48	88	99	87
39	100	0.39	72	99	71
53.5	146	0.37	68	104	70.1
74	201	0.37	68	104	71
94.5	250	0.38	70	104	73
122	326	0.37	68	104	71

Viscosity	Shear Rate (sec^{-1})
5.75	2.6
11.5	2.8
23	3.1
46	3.4
115	3.7
230	4.2

Experiment No. 2

Dextran

P	Q	R	R^N
183	312	0.59	108
154	268	0.57	104
127	207	0.61	112
98	162	0.60	110
71	114	0.62	114
47	75	0.63	115
25	48	0.52	95
17	32	0.53	97
10.5	17.6	0.60	110
8	13	0.61	112
6.5	10	0.65	119
11	15.5	0.70	128
18	25	0.72	132
23	36	0.64	117
37	68	0.54	99
53	107	0.49	90
74	158	0.47	86
95	211	0.45	83
120	261	0.46	84
147	319	0.46	84

Experiment No. 3

Hct = 37

Fibrinogen = 84

Protein = 4.1

P	Q	R	R^N	C	R^{NC}
112	263	0.43	104	98	102
90	211	0.43	104	102	106
67	157	0.43	104	104	108
48	112	0.43	104	104	108
33	71	0.46	111	99	110
21	45	0.47	113	99	112
9.6	17	0.5	135	83	112
7.5	12	0.62	149	83	124
3.5	5.4	0.65	156	83	129
7.5	10.8	0.69	166	83	138
9.3	17.6	0.53	128	83	106
13	29	0.45	108	89	96
17.8	42	0.43	104	89	93
29	72	0.40	96	99	95
40	107	0.37	89	99	88
56.5	156	0.36	87	104	90
79	208	0.38	91	104	95
102	259	0.39	94	98	96
130	323	0.40	96	96	92

Viscosity	Shear Rate (sec^{-1})
5.75	7.8
11.5	6.6
23	5.5
46	4.7
115	3.9
230	3.4

Experiment No. 3

Hct = 37

Fibrinogen = 92

Protein = 4.8

P	Q	R	R^N	C	R^{NC}
133	306	0.43	104	96	100
105	248	0.42	101	98	99
88	200	0.44	106	102	108
66	150	0.44	106	106	110
41.5	104	0.40	96	104	100
28	68	0.41	99	99	98
18	42	0.43	104	89	93
14	28	0.50	120	89	107
10.5	12.5	0.84	202	89	227
8	12.5	0.64	154	83	128
5	6.7	0.75	180	83	149
9.4	13.4	0.70	169	83	140
11	19	0.58	140	83	116
14.7	30	0.49	118	89	98
19.5	42	0.47	113	89	101
29.1	68	0.43	104	99	103
41.7	106	0.39	94	104	98
57	152	0.38	91	104	95
80	200	0.40	96	104	100
103	250	0.41	99	98	97
133	303	0144	106	96	102

Viscosity	Shear Rate (sec $^{-1}$)
5.75	12.4
11.5	10.0
23	8.0
46	6.3
115	4.9
230	4.0

Experiment No. 3

Dextran

P	Q	R	R^N
154	326	0.47	113
119	254	0.47	113
97.4	214	0.45	108
73	165	0.44	106
48	115	0.42	101
30.4	76	0.40	96
19.4	47	0.41	99
13	30.6	0.42	101
9	20	0.45	108
6	14	0.44	106
3	7.4	0.41	99
7	11.5	0.61	147
9.7	17	0.57	137
13.7	26.7	0.51	123
18	38	0.47	113
28	65	0.43	104
41	103	0.40	96
58.5	156	0.37	89
78	210	0.37	89
101	259	0.39	94
128	326	0.39	94

Experiment No. 4

Hct = 36

Fibrinogen = 500

Protein = 6.2

P	Q	R	R^N	C	R^{NC}
169	333	0.51	101	96	97
132	278	0.48	95	96	91
106	227	0.47	93	98	91
86	176	0.49	97	102	99
65	129	0.50	99	104	103
45	77	0.59	117	104	122
35	56	0.63	125	99	124
31	41	0.76	150	99	149
25	26	0.97	192	99	190
19	19.5	0.98	194	89	173
15	13.6	1.1	218	89	194
11	5.9	1.86	369	89	328
11.25	5.6	2.01	398	89	354
16	12.5	1.28	254	89	226
19	18.2	1.05	208	89	185
23	30	0.77	153	99	151
27.2	39.5	0.69	137	99	136
36	62.5	0.58	115	99	114
52	103	0.50	99	104	103
68	158	0.43	85	104	88
89	205	0.43	85	102	87
125	250	0.50	99	96	95
148	329	0.45	89	96	85

Viscosity	Shear Rate (sec^{-1})
5.75	11.0
11.5	9.0
23	7.2
46	5.9
115	4.7
230	4.0

Experiment No. 4

Hct = 45

Fibrinogen = 460

Protein = 5.7

P	Q	R	R^N	C	R^{NC}
160	323	0.50	99	96	95
124	263	0.47	93	96	89
99.5	200	0.50	99	102	101
74	150	0.49	97	104	101
51	103	0.49	97	104	101
36	65.8	0.55	109	99	108
26	41	0.63	125	99	124
21.5	30	0.71	140	99	139
15	21	0.7	139	89	124
9.5	6.2	1.53	304	83	252
10	5.9	1.69	335	83	278
15	13	1.15	228	89	203
18.7	19.5	0.96	190	89	169
22.7	30	0.76	151	99	149
27	40	0.63	125	99	124
35.5	62.5	0.57	113	99	112
49	97	0.50	99	104	103
71	150	0.47	93	104	97
96	200	0.48	95	102	97
118	250	0.47	93	98	91
152	316	0.48	95	96	91

Viscosity	Shear Rate (sec $^{-1}$)
5.75	16.4
11.5	13.1
23	9.9
46	7.6
115	5.7
230	4.6

Dextran

P	Q	R	R^N
153	300	0.51	101
114	238	0.48	95
88	188	0.47	93
57	130	0.44	87
45	102	0.44	87
32	73	0.44	87
19.5	49	0.40	79
11	16	0.69	137
7.4	15	0.49	97
4.4	8	0.55	109
4.4	6.5	0.68	134
8.4	10.8	0.78	154
12.7	18	0.71	141
18	27	0.67	133
23.7	39	0.61	121
38	64	0.60	119
57	102	0.56	111
80	150	0.53	105
105.5	207	0.51	101
134	250	0.53	105
169	316	0.54	107

Experiment No. 5

Hct = 40

Fibrinogen = 131

Protein = 3.7

Shear Rate (sec^{-1})	
5.75	11.6
11.5	9.5
23	7.8
46	6.4
115	4.9
230	4.1

P	Q	R	R^N	C	R^{NC}
154.5	322	0.48	101	96	97
120	268	0.45	95	96	91
99	213	0.47	99	102	101
79.7	158	0.50	106	104	110
55	106	0.52	110	104	114
31.7	62.5	0.51	108	99	107
20	34	0.59	125	89	111
16.2	20.5	0.79	167	89	149
14.5	12.6	1.15	243	89	216
10.5	7.8	1.35	285	83	237
14.5	12.2	1.19	251	89	223
17.2	18.2	0.95	201	89	179
20	27	0.73	154	89	137
25	43	0.58	123	99	122
33.5	58.8	0.57	120	99	119
48	96.8	0.50	106	104	110
69	142	0.49	103	104	107
90	194	0.47	99	102	101
118	250	0.47	99	98	97
155	316	0.49	104	96	100

Experiment No. 5

Hct = 49

Fibrinogen = 132

Protein = 4.0

P	Q	R	R^N	C	R^{NC}
166	320	0.52	110	96	106
130	261	0.50	106	96	102
109	214	0.51	108	98	106
85	156	0.54	114	102	116
62	112	0.55	116	104	121
44	73	0.60	127	104	132
29	45	0.64	135	99	134
23.4	33.3	0.70	148	99	147
19	20	0.95	201	89	179
15.7	14	1.13	239	89	213
12	7.6	1.58	334	89	297
16	12.3	1.30	274	89	244
18.7	18.1	1.03	218	89	194
23	29	0.79	167	99	165
26	38	0.68	144	99	143
35.5	62	0.57	120	99	119
51	100	0.51	108	104	112
71	148	0.48	101	104	105
101	207	0.49	104	98	102
136.5	238	0.57	120	96	115
163	326	0.50	106	96	102

Viscosity	Shear Rate (sec $^{-1}$)			
	5.75	17.2	46	7.5
	11.5	13.0	115	6.0
	23	10.0	230	4.8

Experiment No. 5

Hct = 57

Fibrinogen = 116

Protein = 4.2

P	Q	R	R^N	C	R^{NC}
193	349	0.55	116	96	111
148.5	286	0.52	110	96	106
109	214	0.51	108	98	106
84	161	0.52	110	102	112
63	114	0.55	116	104	121
40	72	0.56	118	99	117
24	31	0.77	162	99	160
18.9	21	0.91	192	89	171
14.5	10	1.45	306	89	272
12.5	7	1.79	378	89	336
15	10	1.5	317	89	282
19	16	1.19	251	89	223
23.7	29	0.81	171	99	169
29	42	0.70	148	99	147
42	71	0.59	125	104	130
56	106	0.53	112	104	116
84	167	0.50	106	102	108
103.7	214	0.48	101	98	99
136	261	0.52	110	96	106
177	333	0.53	112	96	108

Viscosity	Shear Rate (sec^{-1})
	5.75 19.8
	11.5 15.0
	23 11.4
	46 8.7
	115 6.4
	230 5.6

Experiment No. 5

Dextran

P	Q	R	R^N
166	319	0.52	110
131	286	0.46	97
101.5	229	0.44	93
74	180	0.41	87
52	136	0.38	80
31	86	0.36	76
21	41	0.51	108
17	39	0.43	91
13	25	0.52	110
9.7	17.6	0.55	116
7.5	10	0.75	158
7	8	0.88	186
10.5	12	0.88	186
14	16.2	0.86	182
18.5	26	0.7	148
22	37	0.59	125
32	56	0.58	123
47	91	0.52	110
68	139.5	0.49	104
89	200	0.45	95
116	245	0.47	99
150	300	0.50	106

Experiment No. 6

Hct = 1

Fibrinogen = 134

Protein = 5.1

P	Q	R	R^N	C	R^{NC}
92	316	0.29	64	1.02	65
66	259	0.26	57	104	59
50	200	0.25	55	104	57
7	23	0.30	66	83	55
4.5	16.4	0.27	60	83	50
4	9.8	0.41	90	83	75
3.3	17.6	0.19	42	99	42
11	50	0.22	49	89	44
21.2	91	0.23	51	99	50
36	142	0.25	55	99	54
56	205	0.27	60	104	62
82	273	0.30	66	102	67
107	395	0.27	60	98	59

Viscosity	Shear Rate (sec $^{-1}$)
	5.75 not done
	11.5 1.3
	23 1.3
	46 1.2
	115 1.3
	230 1.2

Experiment No. 6

Hct = 20

Fibrinogen = 132

Protein = 5.5

P	Q	R	R^N	C	R^{NC}
116	357	0.32	71	98	70
84	288	0.29	64	102	65
66	221	0.30	66	104	69
46	174	0.26	57	104	59
31	122	0.25	55	99	54
16	65	0.25	55	89	49
9.5	35	0.27	60	83	50
5.5	17.6	0.31	68	83	56
3.8	10.5	0.36	79	83	66
1.8	5.1	0.35	77	83	64
3.5	10	0.35	77	83	64
6	16.7	0.36	79	83	95
9.7	32	0.30	66	83	80
19.5	65	0.30	66	89	59
31	107	0.28	62	99	61
45	164	0.27	60	104	62
62	217	0.29	64	104	67
83	273	0.30	66	102	67
108.5	326	0.33	73	98	72
147	405	0.36	79	96	76

Viscosity	Shear Rate (sec^{-1})
5.75	2.1
11.5	2.7
23	2.8
46	2.5
115	2.4
230	2.3

Experiment No. 6

Hct = 35

Fibrinogen = 70

Protein = 4.7

P	Q	R	R^N	C	R^{NC}
138	341	0.40	88	96	85
107.5	273	0.39	86	98	84
82	214	0.38	84	102	86
60	162	0.37	82	104	85
39	117	0.33	73	99	72
25	77	0.35	77	99	76
15.2	37	0.42	93	89	83
11.5	27	0.43	95	89	85
9.0	16.7	0.54	119	83	99
6.7	10.2	0.66	146	83	121
4.0	55	0.72	159	83	132
6.5	9.7	0.67	148	83	123
9.2	15.4	0.60	132	83	110
17.4	44	0.40	88	89	78
24.5	61	0.40	88	99	87
38.5	107	0.36	79	99	78
58	160	0.36	79	104	82
78	211	0.37	82	104	85
100	258	0.39	86	102	88
133	333	0.40	88	96	85

Viscosity	Shear Rate (sec^{-1})
5.75	6.6
11.5	5.8
23	5.1
46	4.4
115	3.6
230	3.2

Experiment No. 6

Hct = 40

Fibrinogen = 126

Protein 4.8

P	Q	R	R^N	C	R^{NC}
139	319	0.44	97	96	93
107	250	0.43	95	98	93
86	193	0.44	97	102	99
63	143	0.44	97	104	101
42	103.4	0.41	90	104	94
26.2	71.4	0.37	82	99	81
17.7	42	0.42	93	89	83
14	27.2	0.51	113	89	101
10	17.14	0.58	128	83	106
7	9.5	0.74	163	83	196
45	4.5	1.0	221	83	266
10.3	15.7	0.66	146	83	176
18.6	36	0.52	115	89	102
32	79	0.41	90	99	89
50.5	126	0.40	88	104	92
73	176	0.41	90	104	94
108.5	229	0.47	104	98	102
134	300	0.44	97	96	93
178	375	0.47	104	96	100

Viscosity	Shear Rate (sec^{-1})
	5.75 9.2
	11.5 7.9
	23 6.5
	46 5.3
	115 4.2
	230 3.6

Experiment No. 6

Dextran

P	Q	R	R^N
176	366	0.48	106
146	294	0.50	110
117	248	0.47	104
90	196	0.46	101
67	146	0.46	101
43.5	103	0.42	93
19	29	0.66	146
9	20.3	0.44	97
5	10.9	0.46	101
3	6.6	0.45	100
5	8.8	0.57	126
8.7	15	0.58	128
12.5	22.5	0.55	121
24	45	0.53	117
36	79	0.46	101
50	111	0.45	100
70.5	171	0.41	90
91	217	0.42	93
125	275	0.45	99
152	330	0.46	101

Experiment No. 7

Hct = 39

Fibrinogen = 17

Protein = 4.0

P	Q	R	R^N	C	R^{NC}
166.5	405	0.41	107	96	103
14	24	0.58	151	89	134
5.2	5.9	0.88	229	83	190
3.8	5.0	0.76	198	83	164
112	15.5	0.72	188	98	184
174	423	0.41	107	96	103
123	329	0.37	96	96	92
104.5	259	0.40	104	98	102
171	370	0.46	119	96	114
16.7	32.6	0.51	133	89	118
6.7	8.9	0.75	195	83	162
2.5	2.6	0.96	250	83	208
118	315	0.37	96	98	94
57	143	0.40	104	104	108
34	92	0.37	96	99	95
53	54	0.98	106	104	110

Viscosity	Shear Rate (sec^{-1})
5.75	8.7
11.5	7.4
23	6.5
46	5.4
115	4.4
230	3.8

Experiment No. 7

Hct = 48

Fibrinogen = 55

Protein = 3.5

P	Q	R	R^N	C	R^{NC}
152	353	0.43	112	96	108
149.5	341	0.44	115	96	110
87	200	0.44	115	102	117
23.7	27.6	0.86	224	99	222
4.7	4.4	1.07	279	83	232
4.7	4.0	1.18	307	83	255
7	6.7	1.04	271	83	225
147	366	0.40	104	96	100

Viscosity	Shear Rate (sec^{-1})	
	5.75	13.2
	11.5	10.9
	23	8.7
	46	6.9
	115	5.0
	230	4.0

Experiment No. 7

Hct = 59

Fibrinogen = 55

Protein = 3.5

P	Q	R	R^N	C	R^{NC}
131	273	0.48	125	96	120
105	222	0.47	122	98	120
44.5	75	0.59	154	104	160
16.5	16.5	1.0	260	89	231
7.2	5.5	1.31	341	83	283
6.2	4.5	1.38	359	83	298
70	160	0.44	115	104	120
74	162	0.46	120	104	125
151	330	0.46	120	96	115

Viscosity	Shear Rate (sec^{-1})
	5.75 26.6
	11.5 18.8
	23 13.9
	46 10.3
	115 7.6
	230 6.7

Experiment No. 7

Dextran

P	Q	R	R^N
79	176	0.45	117
5	12.7	0.39	101
2.8	9	0.31	81
3	8.9	0.34	89
41	136	0.30	78
40	140	0.28	73
180	370	0.49	114
137	306	0.45	112

Experiment No. 8

Hct = 20

Fibrinogen = 246

Protein = 5.2

P	Q	R	R^N	C	R^{NC}
151	390	0.39	83	96	80
86.5	244	0.35	75	102	77
7.2	16	0.45	96	83	80
4.2	6.8	0.61	130	83	108
1.7	3.1	0.55	117	83	97
4.2	6.2	0.68	145	83	120
39.2	119	0.33	70	99	69
160	417	0.38	81	96	78
108.5	278	0.39	83	98	81

Viscosity	Shear Rate (sec $^{-1}$)
5.75	2.4
11.5	2.5
23	2.5
46	2.3
115	2.2
230	2.2

Experiment No. 8

Hct = 28

Fibrinogen = 290

Protein = 5.6

P	Q	R	R^N	C	R^{NC}
159	385	0.41	87	96	84
122	300	0.41	87	96	84
37.5	125	0.3	64	99	63
4.2	9	0.47	100	87	83
3.5	6.8	0.51	109	83	90
1.7	3.2	0.53	113	83	94
7.0	13.2	0.53	113	83	94
64	186	0.34	72	104	75
159	394	0.40	85	96	82

Viscosity	Shear Rate (sec^{-1})
5.75	3.6
11.5	3.4
23	3.3
46	3.0
115	2.9
230	2.8

Experiment No. 8

Hct = 45

Protein = 5.6

P	Q	R	R^N	C	R^{NC}
165	337	0.49	104	96	100
157.5	345	0.46	98	96	94
92	215	0.43	91	102	93
15.5	28.3	0.55	117	89	104
6.2	8	0.78	166	83	138
3.5	4.5	0.78	166	83	138
5.8	7.4	0.78	166	83	138
14.4	23.5	0.61	130	89	116
95.5	252	0.38	81	102	83
159.5	370	0.43	91	96	87
98.7	229	0.43	91	102	93

Viscosity	Shear Rate (sec $^{-1}$)
5.75	9.2
11.5	7.8
23	6.4
46	5.3
115	4.3
230	3.8

Experiment No. 8

Hct = 51

Fibrinogen = 132

Protein = 5.6

P	Q	R	R^N	C	R^{NC}
153	312	0.49	104	96	99
92.3	208	0.44	94	98	92
17.25	28.4	0.61	130	89	116
6.5	7.5	0.87	185	83	154
4	3.6	1.11	236	83	196
5.7	6.4	0.89	189	83	157
100	265	0.38	81	102	83
151	323	0.47	100	96	96
166	331	0.50	106	96	102
106.5	216	0.49	104	98	102
18.5	25	0.74	157	89	140
6.2	7.1	0.86	183	83	152
4.7	4	1.18	251	83	208
12.5	13.6	0.92	196	89	174
90	200	0.45	96	102	98
142	291	0.49	104	96	100

Viscosity	Shear Rate (sec $^{-1}$)
5.75	15.8
11.5	12.5
23	9.6
46	7.5
115	5.9
230	5.2

Experiment No. 8

Dextran

P	Q	R	R^N
189	375	0.50	106
114	245	0.47	100
10.8	15	0.72	153
10.5	16	0.66	140
3	4	0.75	160
2.6	3.8	0.68	145
1.2	1.5	0.80	170
15.9	34	0.46	98
143	278	0.51	109
187	368	0.51	109

Experiment No. 9

Hct = 22

Fibrinogen = 192

Protein = 5.1

P	Q	R	R^N	C	R^{NC}
121	278	0.44	86	96	83
157	340	0.45	88	96	84
73	179	0.41	80	104	83
19.7	33	0.60	117	89	104
19.1	35	0.55	107	89	95
5.9	8.1	0.73	143	83	119
4.0	3.9	1.03	201	83	167
5.7	6	0.95	186	83	154
74	217	0.34	66	104	69
129.5	300	0.42	82	96	79
104	239	0.44	86	98	84

Viscosity	Shear Rate (sec^{-1})
5.75	2.8
11.5	2.7
23	2.7
46	2.5
115	2.4
230	2.2

Experiment No. 9

Hct = 29

Fibrinogen = N.A.

Protein = N.A.

P	Q	R	R	R^N	C	R^{NC}
78.5	184	0.43	0.51	84	104	87
110.5	300	0.37		72	98	71
22.3	43	0.52		102	99	101
19.7	37.5	0.53		104	89	93
6.5	8.8	0.74		145	83	120
4.5	3.9	1.15		225	83	187
6.8	6.8	1.0		195	83	162
39.2	136	0.29		57	99	56
91.5	259	0.35		68	102	69
152	333	0.46		90	96	86

Viscosity	Shear Rate (sec -1)
5.75	3.8
11.5	4.0
23	3.6
46	3.3
115	3.0
230	2.8

Experiment No. 9

Hct = 42

Fibrinogen = 210

Protein = 4.8

P	Q	R	R^N	C	R^{NC}
136	273	0.50	98	96	94
157	326	0.48	94	96	90
63.5	119	0.53	104	104	108
13.3	19.4	0.69	135	89	120
11.7	13.5	0.87	170	89	151
7.5	7	1.07	209	83	173
5.2	3.7	1.41	275	83	228
5.6	3.5	1.6	3.3	83	260
7.8	6.1	1.28	250	83	208
32.2	79.4	0.41	80	99	79
162	339	0.48	94	96	90
121	250	0.48	94	96	90

Viscosity	Shear Rate (sec^{-1})	
	5.75	7.8
	11.5	7.2
	23	6.0
	46	5.0
	115	4.0
	230	3.5

Experiment No. 9

Hct = 47

Fibrinogen = 156

Protein = 4.7

P	Q	R	R^N	C	R^{NC}
136.5	242	0.56	109	96	105
159	228	0.57	111	96	107
26.2	42	0.63	123	99	122
8.2	11	0.75	146	83	121
5.2	4.4	1.20	234	83	194
7.9	6.9	1.14	223	83	185
32.5	67	0.49	96	99	95
89	202	0.44	86	102	88
152	297	0.51	100	96	96

Viscosity	Shear Rate (sec^{-1})
5.75	12.4
11.5	10.3
23	7.9
46	6.2
115	4.9
230	4.4

Experiment No. 9

Hct = 70

Fibrinogen = 84

Protein = N.A.

P	Q	R	R^N	C	R^{NC}
122.3	187.5	0.65	129	96	122
59	81	0.73	143	104	149
23	24.5	0.94	184	99	182
10.3	7.5	1.37	268	83	222
8	4.9	1.63	318	83	264
7.6	3.4	2.24	438	83	364
11.5	6	1.92	375	89	334
23.4	20	1.17	229	99	227
95	150	0.64	125	102	128
137	226	0.61	119	96	114

Viscosity	Shear Rate (sec^{-1})
5.75	19
11.5	15
23	12.5
46	10.3
115	8.4
230	7.5

Experiment No. 9

Dextran

P	Q	R	R	R^N
149.5	265	0.56	0.51	109
53.7	113	0.47		92
9.2	24	0.38		74
5	88	0.57		111
3.2	6	0.53		104
3.1	5	0.60		117
5.3	7.3	0.73		143
63	120	0.53		104
107	250	0.43		84
168	294	0.57		111
122	260	0.47		93
129	243	0.53		104
135	255	0.53		104
148	265	52		102

Experiment No. 10

Hct = 17

Fibrinogen = 144

Protein = 5.2

P	Q	R	R	R^N	C	R^{NC}
59	200	0.30	0.45	66	104	69
95	303	0.31		69	102	70
45.5	107	0.43		95	104	99
4.1	6	0.68		150	83	125
4.1	4.6	0.89		197	83	164
2.6	3	0.87		192	83	159
4.1	4	1.03		228	83	189
48.5	162	0.30		66	104	69
99	353	0.28		62	102	63
81	250	0.32		71	102	72
20	65	0.31		96	89	85

Viscosity	Shear Rate (sec^{-1})
5.75	2.6
11.5	2.6
23	2.3
46	2.3
115	2.1
230	2.0

Experiment No. 10

Hct = 44

Fibrinogen = 225

Protein = 4.9

P	Q	R	R^N	C	R^{NC}
134	286	0.46	102	96	98
58	136	0.42	93	104	97
12	13	0.92	203	89	181
7.8	5.7	1.36	301	83	250
6.2	3.4	1.82	402	83	334
55.4	151	0.36	80	104	83
133	300	0.43	95	96	91

Viscosity	Shear Rate (sec^{-1})
	5.75 8.8
	11.5 7.7
	23 7.0
	46 5.4
	115 4.0
	230 3.5

Experiment No. 10

Hct = 63

Fibrinogen = 222

Protein = 5.3

P	Q	R	R^N	C	R^{NC}
114	225	0,51	113	98	111
41.5	55.5	0.75	166	104	173
9	5.4	1.67	369	83	306
8.4	3.3	2.55	564	83	468
10.5	4	2.62	579	83	481
33.8	42.5	0.80	177	99	175
72	125	0.58	128	104	133
127.5	234	0.54	119	96	114

Viscosity	Shear Rate (sec $^{-1}$)
5.75	16.4
11.5	13.4
23	10.9
46	8.8
115	7.1
230	6.5

Experiment No. 10

Dextran

P	Q	R	R^N
131	263	0.50	110
69	159	0.43	95
16.5	31	0.54	119
3.7	7.2	0.51	112
2.3	4.6	0.50	110
133	283	0.47	104
75	185	0.41	91

Experiment No. 11

Hct = 11

Fibrinogen = 256

Protein = 4.5

P	Q	R	R^N	C	R^{NC}
61	214	0.28	99	61	104
87.5	254	0.34	121	74	102
30.3	123	0.25	89	54	99
1.3	4	0.33	117	72	83
6.2	23	0.27	96	59	83
30.4	136	0.22	78	48	99
95.3	294	0.32	113	70	102

Viscosity	Shear Rate (sec^{-1})	
	5.75	1.8
	11.5	1.6
	23	1.6
	46	1.6
	115	1.6
	230	1.5

Experiment No. 11

Hct = 49

Fibrinogen = 2.2

Protein = 3.6

P	Q	R	R^N	C	R^{NC}
96	20	0.48	104	102	106
38.1	86	0.44	96	99	95
4.2	3.4	1.24	270	83	224
7.3	10	0.73	159	83	132
51	136	0.38	83	104	86
54	136	0.40	87	104	90
132	273	0.48	104	96	100
93	195	0.48	104	102	106

Viscosity	Shear Rate (sec^{-1})
5.75	9.8
11.5	8.4
23	6.5
46	5.0
115	3.9
230	3.4

Experiment No. 11

Hct = 62

Fibrinogen = 232

Protein = 5.0

P	Q	R	R^N	C	R^{NC}
57	100	0.57	124	104	129
120	234	0.54	117	96	112
62	120	0.52	113	104	118
17.8	31	0.57	124	89	110
7	7.5	0.93	202	83	168
4.8	4	1.2	261	8.3	217
7.3	6.8	1.07	233	83	193
32	71	0.45	98	99	97
83	179	0.46	100	102	102
126	231	0.55	120	96	115

Viscosity	Shear Rate (sec^{-1})
5.75	16.8
11.5	12.4
23	9.3
46	6.9
115	5.3
230	4.7

Experiment No. 11

Dextran

P	Q	R	R^N
122.5	240	0.51	110
53	128	0.42	91
16.2	41	0.40	87
5.2	8	0.65	141
6.5	7.0	0.93	202
9.6	15	64	139
72	176	0.41	89
125	250	0.50	109

Experiment No. 12

Hct = 37

Fibrinogen = 296

Protein = 4.3

P	Q	R	R	R^N	C	R^{NC}
114.5	226	0.51	0.64	80	98	78
117	254	0.46		72	98	71
99.3	200	0.50		78	102	80
81.5	150	0.54		84	102	86
59	100	0.59		92	104	96
40	62	0.65		101	99	100
19.7	27	0.73		114	89	101
5.7	7	0.81		126	83	105
4.8	3	1.6		250	83	208
10.2	9	1.13		176	83	146
16.5	13.5	1.22		190	89	169
22.3	29.4	0.76		119	99	118
32.7	56	0.59		92	99	91
52	90	0.58		90	104	94
78.5	148	0.53		83	104	86
98	200	0.49		76	102	77.5
116.3	261	0.45		70	98	69
95.6	190	0.50		78	102	80
76.7	131.6	0.58		90	104	94
52.2	80	0.65		101	104	105
33	43.1	0.77		120	99	119
20.6	20	1.03		161	89	143
11.7	10	1.17		182	89	162
5.6	4.4	1.27		198	83	164

Viscosity	Shear Rate (sec^{-1})
5.75	6.7
11.5	5.8
23	5.0
46	4.4
115	4.0
230	3.6

Experiment No. 12

Dextran

P	Q	R
211	326	0.65
171.4	254	0.67
132.4	214	0.62
93	156	0.60
63	107	0.59
29.4	41	0.72
8.1	15.8	0.51
3.2	6	0.53
8.5	12	0.70
14.8	19.4	0.76
30.7	45	0.68
63.2	94	0.67
96.2	149	0.65
132.5	205.5	0.64

Experiment No. 13

Hct = 34

Fibrinogen = 174

Protein = 4.6

P	Q	R	R^D	R^N	C	R^{CN}
114	233	0.42	0.52	81	98	79
90	195	0.46		89	102	91
64	139	0.46		89	104	93
47	91	0.51		98	104	102
25	41	0.60		116	99	115
154.7	20	0.74		143	89	127
10.5	10	1.05		203	83	168
6.6	4,3	1.53		295	83	245
11	10	1.1		212	89	189
15.5	19.5	0.80		154	89	137
27.7	58	0.48		93	99	92
46	104	0.44		85	104	88
73	172	0.42		81	104	84
100	234	0.42		81	102	83
141	319	0.44		85	96	82

Viscosity	Shear Rate (sec^{-1})
5.75	5.4
11.5	4.9
23	4.1
46	3.5
115	3.2
230	3.1

Experiment No. 13

Hct = 34

Fibrinogen = 174

Protein = 4.6

P	Q	R	R^D	R^N	C	R^{CN}
49	78	0.63	0.52	122	104	127
138	300	0.46		89	96	85
99	210	0.47		91	102	93
70	140	0.50		96	104	100
18	21	0.86		166	89	148
11	10	1.1		212	89	189
23	34	0.68		131	99	130
44	77	0.58		112	104	116
63	130	0.48		93	104	97
89	196	0.45		87	102	89
124	273	0.45		87	96	84

Viscosity	Shear Rate (sec^{-1})	
	5.75	5.2
	11.5	4.5
	23	4.3
	46	3.8
	115	3.3
	230	3.1

Experiment No. 13

Dextran

P	Q	R
103	188	0.55
65	128	0.51
15.5	22	0.70
9.4	10.7	0.88
5.5	6	0.92
8	8.3	0.96
19.4	28.3	0.69
46	86	0.54
75	150	0.50
111	222	0.50
149	291	0.51

Experiment No. 14

Hct = 33

Fibrinogen = 0

Protein = 5.5

P	Q	R	R^N	C	R^{NC}
50.5	103	0.49	112	104	116
33	75	0.44	101	99	100
21.5	45	0.47	108	99	107
14	26	0.54	124	89	110
9	15.8	0.57	131	83	109
6	11.8	0.51	117	83	97
4.5	7.5	0.6	138	83	115
6.5	10.9	0.60	138	83	115
9	14.3	0.63	147	83	122
13	25	0.52	119	89	106
17.5	35	0.50	115	89	102
30.5	70	0.44	101	99	100
40	105	0.38	87	99	86
56.5	150	0.38	87	104	90
75	196	0.38	87	104	90
93.5	234	0.40	92	102	94
116	293	0.40	92	98	90

Viscosity	Shear Rate (sec^{-1})
5.75	5.8
11.5	5.1
23	4.5
46	4.0
115	3.5
230	3.3

Experiment No. 14

Hct = 33 (2nd run)

Protein = 5.5

P	Q	R	R^N	C	R^{NC}
100.5	231	0.435	101	102	103
73	185	0.395	92	104	96
57	143	0.40	92	104	96
40	94	0.425	99	99	98
28.5	62.5	0.46	106	99	105
16.5	33	0.50	115	89	102
14	23	0.61	140	89	125
11.5	13	0.88	202	89	180
8	9	0.89	204	83	169
7	7	1.0	229	83	190
8.75	9	0.97	223	83	185
11.5	15	0.77	177	89	158
15	24	0.652	145	89	129
28	64	0.44	101	99	100
39	100	0.39	90	99	89
56	150	0.37	85	104	88
74	188	0.39	90	104	94

Viscosity	Shear rate (sec^{-1})
5.75	5.9
11.5	5.2
23	4.5
46	4.1
115	3.5
230	3.4

Experiment No. 14

Dextran

P	Q	R	R^N
81	214	0.38	87
62	158	0.39	90
38	97	0.39	90
13.0	41	0.32	73
7.0	20	0.35	80
5	12	0.42	96
21	40	0.53	122
8.5	20	0.43	99
13	33	0.39	90
19.5	55	0.35	90
27.5	68	0.40	92
39.5	111	0.36	83
56.5	158	0.36	83
74.5	200	0.37	85
121	275	0.44	101
129	300	0.43	99
135	293	0.46	106
142	346	0.41	94

Experiment No. 15

Hct = 37

Fibrinogen = 0

Protein = 5.5

Shear Rate (sec^{-1})	
5.75	7.2
11.5	6.4
23	5.5
46	4.5
115	3.8
230	3.4

P	Q	R	R^N	C	R^{NC}
90	250	0.36	94	102	96
72	207	0.35	91	104	95
54	158	0.34	89	104	93
38.5	111	0.35	91	99	90
27	75	0.36	94	99	93
18	44	0.41	107	89	95
13	34	0.38	99	89	88
9.5	19	0.50	130	83	108
7	13.3	0.53	138	83	115
6	10.9	0.55	143	83	119
7.5	12.8	0.58	151	83	125
9.5	19.5	0.48	125	83	104
13.5	28	0.48	125	89	111
17.5	41	0.43	112	89	100
27.5	71	0.39	102	99	101
37.5	107	0.35	91	99	90
53.5	154	0.35	91	104	95
72	211	0.34	88	104	92
89	250	0.36	94	102	96
114	310	0.37	96	98	94

Experiment No. 15

Hct = 37 (2nd run)

Fibrinogen = 0

Protein = 5.5

Shear Rate (sec^{-1})	
5.75	7.2
11.5	6.4
23	5.5
46	4.5
115	3.8
230	3.4

P	Q	R	R^N	C	R^{NC}
107	285	0.38	99	98	97
84	234	0.36	94	102	96
69	120	0.36	94	104	98
51	150	0.34	89	104	93
38	103	0.37	96	99	95
28	65	0.43	112	99	111
17.5	34	0.51	133	89	118
13.5	23	0.59	154	89	137
10	13.3	0.75	195	83	162
8.75	11.3	0.78	203	83	168
8.25	8	1.03	268	83	222
9	10.9	0.83	213	83	177
11.5	15.25	0.75	195	89	174
15	23.5	0.63	164	89	146
18	31.5	0.57	148	89	132
27	65	0.42	109	99	108
36.5	100	0.37	96	99	95
52	150	0.35	91	104	95
69.5	190	0.37	96	104	100
82	230	0.36	94	102	96
106	290	0.37	96	98	94

Experiment No. 15

Dextran

P	Q	R	R^N
130	312	0.42	109
103	250	0.41	107
84	209	0.40	104
62	155	0.40	104
41	107	0.38	100
28	75	0.37	96
22	51	0.43	112
15	34	0.44	114
8.5	19.7	0.43	112
6	15	0.40	104
5.8	10.5	0.55	143
6.7	12.77	0.60	156
8.5	18	0.47	122
14	32	0.44	114
18	45	0.40	104
26	71	0.37	96
36.5	103	0.35	91
53	150	0.35	91
69	194	0.36	94
88	231	0.38	99
109	278	0.39	102

Experiment No. 16

Hct = 30

Protein = 5.6

Viscosity · Shear Rate (sec $^{-1}$)

5.75 - 7.6
11.5 - 7.2
23 - 6.0
46 - 5.0
115 - 4.1
230 - 3.5

P	Q	R	R^N	C	R^{NC}
137	319	0.43	100	96	96
116	250	0.46	107	98	105
90.7	200	0.45	104	102	106
71.5	146	0.49	114	104	119
50	100	0.50	116	104	121
33.7	65.2	0.52	121	99	120
22	42	0.53	123	99	122
14.8	28.3	0.52	121	89	108
12.5	18	0.69	160	89	142
10.7	13	0.82	190	83	158
6.3	6.4	0.98	227	83	188
12	14	0.86	200	89	178
16	20	0.80	186	89	166
20.7	29	0.72	167	89	149
26	42	0.62	144	99	143
36.8	66.7	0.55	128	99	127
52.3	100	0.52	121	104	126
69	150	0.45	104	104	108
92.8	200	0.46	107	102	109
116	254	0.46	107	98	105
150	316	0.48	111	96	107

Experiment No. 10

Hct = 35

Fibrinogen = 0

Protein = 5.4

P	Q	R	R^N	C	R^{NC}
138	319	0.43	100	96	96
109	270	0.40	93	98	91
88.5	211	0.42	97	102	99
65	163	0.40	93	104	97
46.5	104	0.45	104	104	108
32.7	69	0.47	109	99	108
22.5	45	0.50	116	99	115
17	31	0.54	125	89	111
12	22	0.55	128	89	114
9.5	15	0.63	146	83	121
4.5	6.2	0.73	169	83	140
9.5	14	0.68	158	83	131
12	18	0.66	153	89	136
17	29	0.59	137	89	122
20.6	40.5	0.51	118	89	105
31.5	69	0.46	107	99	106
44	107	0.41	95	104	99
60.5	154	0.39	90	104	94
81	207	0.39	90	102	92
100	270	0.37	86	102	88

Viscosity	Shear Rate (sec $^{-1}$)
	5.75 7.0
	11.5 6.5
	23 5.3
	46 4.6
	115 3.8
	230 3.4

Experiment No. 16

Hct = 37

Protein = 5.6

Viscosity	Shear Rate (sec $^{-1}$)
5.75	10.2
11.5	7.7
23	6.3
46	5.2
115	4.2
230	3.6

P	Q	R	R^N	C	R^{NC}
134	326	0.41	95	96	91
106.5	260	0.41	95	98	93
84	200	0.42	97	102	99
65	150	0.43	100	104	104
45	102	0.44	102	104	106
30.5	63	0.48	111	99	110
21	41	0.51	118	99	117
15.7	28	0.55	128	89	114
11.5	20.4	0.56	130	89	116
9.5	14.9	0.64	148	83	123
4.8	7.25	0.66	153	83	127
9.5	14.3	0.67	155	83	129
11.9	19.4	0.61	142	89	126
16.2	28.8	0.56	130	89	116
24.5	53.6	0.46	107	99	106
34.4	78.9	0.44	102	104	106
48	127	0.38	88	104	92
66.7	165	0.40	93	104	97
88	234	0.38	88	102	90
110	268	0.41	95	98	93
137.5	303	0.44	102	96	98

Experiment No. 16

Dextran

P	Q	R	R^N
147.5	316	0.47	109
125	254	0.49	114
103.5	203	0.51	118
82	158	0.52	121
46	110	0.42	97
30	71	0.42	97
17	45	0.38	88
13	31	0.42	97
8.5	20	0.43	100
6.8	14	0.49	114
3.2	7.2	0.44	102
6.8	12.2	0.55	128
9.7	18	0.53	123
12.5	28	0.45	104
16.4	38	0.43	100
24.5	65	0.38	88
37	100	0.37	86
53.3	146	0.36	84
74.5	200	0.37	86
95	246	0.39	90
126.5	309	0.41	95

Experiment No. 17

Hct = 40

Fibrinogen = 0

Protein = 5.0

P	Q	R	R^N	C	R^{NC}
119	272	0.44	93	98	91
100.4	214	0.47	99	102	101
77	158	0.49	104	104	108
54.8	107	0.51	108	104	112
46.4	81	0.57	120	104	125
36	52	0.70	148	99	147
30	38	0.79	167	99	165
20.5	18.75	1.09	230	89	205
14	6	2.3	486	89	433
22	16.7	1.32	279	99	276
31.5	33.3	0.94	199	99	197
43.4	57.7	0.75	158	104	164
56	83.3	0.67	142	104	148
74	122	0.61	129	104	134
95	165	0.58	123	102	125
123	214	0.57	120	96	115
147	273	0.54	114	96	109

Viscosity	Shear Rate (sec $^{-1}$)
5.75	10.6
11.5	8.5
23	7.1
46	5.7
115	4.6
230	3.9

Experiment No. 17

Hct = 40 (2nd run)

Protein = 5.0

P	Q	R	R^N	C	R^{NC}
132.5	273	0.49	104	96	100
91	167	0.55	116	102	118
45	75	0.6	127	104	132
22.5	24	0.94	199	99	197
20	15.8	1.26	266	89	237
17	11.5	1.48	313	89	279
31	35	0.85	188	99	186
66	115	0.57	120	104	125
108	200	0.54	114	98	112
154	300	0.51	108	96	104

Experiment No. 17

Dextran

P	Q	R	R^N
150	324	0.46	97
105	231	0.46	97
61	150	0.41	87
42	95	0.44	93
22	52	0.42	89
16.5	37	0.45	95
12.3	14.4	0.85	179
44	82	0.54	114
65	145	0.45	95
93	191	0.49	104
118	250	0.47	99
170	316	0.54	114

Experiment No. 18

Hct = 40

Fibrinogen = 0

Protein = 4.7

P	Q	R	R^N	C	R^{NC}
154	333	0.46	111	96	107
129	261	0.49	118	96	113
86	167	0.52	125	102	128
60	111	0.54	130	104	135
41.5	75	0.55	133	104	138
23	47	0.49	118	99	117
18.8	28	0.67	161	89	143
12.7	16	0.79	190	89	169
8.5	8.4	1.01	243	83	202
8.5	8.6	0.99	239	83	198
11	15	0.73	176	89	157
15	26.3	0.57	137	89	122
20	39	0.51	123	89	109
38	85.7	0.44	106	99	105
53	117	0.45	108	104	112
80	176	0.45	108	104	112
130	300	0.43	104	96	100

Viscosity	Shear Rate (sec^{-1})
	5.75 10.2
	11.5 8.4
	23 7.0
	46 5.6
	115 4.5
	230 3.9

Experiment No. 18

Hct = 40 (2nd run)

Protein = 4.7

P	Q	R	R^N	C	R^{NC}
124	288.5	0.43	128	96	123
98	231	0.42	101	102	103
91	190	0.48	116	102	118
71.5	136	0.52	125	104	130
51	80	0.62	149	104	155
27	39.5	0.68	164	99	162
18.5	27	0.68	164	89	146
13.5	17	0.78	188	89	167
11.6	13	0.89	214	89	190
7.6	7.0	1.09	263	83	218
9.7	12	0.81	195	83	162
13.2	18.5	0.71	171	89	152
16.2	27.2	0.59	142	89	126
20.5	38.5	0.53	128	89	114
32.7	73	0.45	108	99	107
47	104	0.45	108	104	112
68	158	0.43	104	104	108
88	210	0.42	101	102	103
106	26.3	0.40	96	98	94
138	333	0.41	99	96	95
172	400	0.43	104	96	100

Experiment No. 18

Dextran

P	Q	R	R^N
144	306	0.47	113
106	254	0.42	101
79	205	0.39	94
59	157	0.38	92
38	108	0.35	84
26	68	0.38	92
13.5	41	0.33	80
10	26.7	0.38	92
6	15	0.40	96
2.5	6.8	0.37	89
6	12.8	0.47	113
10	23	0.43	104
15.5	36	0.43	104
29	68	0.43	104
44	105	0.42	101
66.7	158	0.42	101
87	207	0.42	101
144	316	0.46	110

Experiment No. 19

Hct = 19

Protein = 4.5

P	Q	R	R^N	C	R^{NC}
138	333	0.41	87	96	84
101.5	259	0.39	83	98	81
80	212	0.38	81	104	84
60.5	158	0.38	81	104	84
43	113	0.38	81	104	84
31	71	0.43	91	99	90
21	44	0.47	100	99	99
15	30	0.50	106	94	94
7.5	15.1	0.50	106	83	88
5	8.8	0.57	121	83	100
2	3.9	0.51	121	83	100
5.5	8.6	0.64	136	83	113
7.7	14	0.55	117	83	97
11	25	0.44	94	89	84
23	56	0.41	87	99	86
32.5	83	0.39	83	99	82
55	136	0.40	85	104	88
76	188	0.41	87	104	90
102.5	234	0.44	94	98	92
130	300	0.43	91	96	87

Viscosity	Shear Rate (sec^{-1})
	5.75 1.8
	11.5 2.7
	23 2.0
	46 2.2
	115 2.1
	230 2.1

Experiment No. 19

Hct = 38

Protein = 4.5

P	Q	R	R^N	C	R^{NC}
109.5	234	0.47	100	98	98
40	88	0.45	96	99	95
31	62	0.50	106	99	105
22	34	0.64	136	99	135
13.5	13	1.04	221	89	197
9	6.6	1.36	289	83	240
5.5	3	1.83	389	83	323
9	7	1.28	272	83	226
14.5	14	1.03	219	89	195
19.5	24	0.81	172	89	153
26	37	0.71	151	99	149
41	62	0.66	140	104	146
59	107	0.55	117	104	122
102.5	194	0.53	113	98	111

Viscosity	Shear Rate (sec^{-1})
5.75	5.2
11.5	4.9
23	4.8
46	4.2
115	3.5
230	3.3

Experiment No. 19

Hct = 50

Protein = 4.5

P	Q	R	R^N	C	R^{NC}
176	312	0.56	119	96	114
137	231	0.59	126	96	121
105	185	0.57	121	98	119
80	149	0.54	115	104	120
41.5	62	0.66	140	104	146
30	34	0.87	185	99	183
24	22	1.07	228	99	226
18	12.6	1.43	304	89	271
12.5	6.9	1.8	383	89	341
8.5	3	2.8	596	83	595
12.5	6	2.08	443	89	394
18	12.7	1.42	302	89	269
31	31.8	0.97	206	99	204
55	71	0.77	164	104	171
79	12.7	0.62	132	104	137
108	179	0.60	128	98	125
147	231	0.64	136	96	131

Viscosity	Shear Rate (sec^{-1})
5.75	11.4
11.5	9.5
23	7.7
46	5.0
115	4.9
230	4.4

Experiment No. 19

Dextran

P	Q	R	R^N
166	312	0.53	112
122	254	0.48	102
91	206	0.44	94
59	140	0.42	89
35	85	0.41	87
8.3	23	0.36	77
4.5	9.5	0.47	100
2	4.2	0.48	102
4	8	0.50	106
8.5	15	0.57	121
17.5	35.2	0.50	106
40	85	0.47	100
66	135	0.49	104
85	181	0.47	100
114	245	0.46	98
161	303	0.53	113

Experiment No. 20

Hct = 25

Fibrinogen = 0

Protein = 5.2

P	Q	R	R^N	C	R^{NC}
137.5	312	0.44	93	96	89
104	259	0.40	85	98	83
86	214	0.40	85	102	87
69	158	0.44	93	104	97
50.5	109	0.46	97	104	101
26	43	0.60	127	99	126
17.5	27	0.64	136	89	121
9.8	15.1	0.65	138	83	115
5.5	7.5	0.73	155	83	129
3.5	6	0.58	123	83	102
6.5	8.2	0.79	167	83	139
9.2	14	0.67	142	83	118
21	37	0.57	121	99	120
33	65	0.51	108	99	107
47	105	0.45	95	104	99
63.5	152	0.42	89	104	93
85	207	0.41	87	102	89
104.5	261	0.40	85	98	83
134	326	0.41	87	96	84

Viscosity	Shear Rate (sec^{-1})
5.75	2.4
11.5	2.7
23	2.8
46	2.6
115	2.6
230	2.5

Experiment No. 20

Hct = 25

P	Q	R	R^N	C	R^{NC}
134	330	0.41	87	96	84
99	263	0.38	80	102	82
82	210	0.39	83	102	85
63	167	0.38	80	104	83
46.5	109	0.42	89	104	93
34	68	0.50	106	99	105
22	41	0.54	114	99	113
16.5	28.6	0.58	123	89	109
9.5	17	0.56	118	83	98
5.4	8.7	0.62	131	83	109
2.2	4.2	0.52	110	83	91
5.1	7.1	0.72	152	83	126
8.9	13	0.68	144	83	120
19	33	0.57	121	89	108
28	62	0.45	95	99	94
43	100	0.43	91	104	95
60	156	0.38	80	104	83
78	208	0.37	78	104	81

Experiment No. 20

Dextran

P	Q	R	R^N
175	323	0.54	114
141	280	0.50	106
111.5	246	0.45	95
91.5	187	0.49	104
62	133	0.47	100
39	89	0.44	93
24	55	0.43	91
12	30	0.39	83
7	15.8	0.44	93
3.5	7.8	0.45	95
1.7	4.4	0.39	83
3.2	5.8	0.55	116
9.2	12.1	0,76	160
18.5	31	0.59	118
31.5	63	0.50	106
47	101	0.47	100
68	150	0.45	95
97	200	0.49	104
134	272	0.49	104
179	353	0.51	108

Experiment No. 21
Hct = 9
Fibrinogen = 0
Protein = 5.2

P	Q	R	R^N	C	R^{NC}
157	417	0.38	80	96	77
91	250	0.36	75	102	77
45	130	0.35	73	104	76
2.2	4.5	0.49	103	83	85
44.5	167	0.27	57	104	59
1.9	8	0.29	60	83	50
1.4	4.2	0.33	69	83	57
2.6	6.5	0.40	84	83	70
31	146	0.21	44	99	44
95	373	0.25	52	102	53

Viscosity	Shear Rate (sec^{-1})
5.75	1.4
11.5	1.2
23	1.6
46	1.7
115	1.6
230	1.6

Experiment No. 21

Hct = 22

Fibrinogen = 0

Protein = 4.9

P	Q	R	R^N	C	R^{NC}
87.5	254	0.34	71	102	72
132	349	0.38	80	96	77
38	100	0.38	80	99	79
7.5	19	0.39	82	83	68
3.4	6.3	0.54	113	83	94
1.9	2.4	0.79	165	83	137
3.7	6.1	0.61	128	83	106
39.4	217	0.18	38	99	38
110	300	0.37	77	98	71

Viscosity	Shear Rate (sec $^{-1}$)
	5.75 3.2
	11.5 2.9
	23 2.8
	46 2.5
	115 2.4
	230 2.2

Experiment No. 21

Hct = 32

Fibrinogen = 0

Protein = 5.0

P	Q	R	R^N	C	R^{NC}
135	299	0.45	94	96	90
36.8	76	0.48	101	99	100
4.5	6.2	0.73	153	83	127
2.8	2.2	1.27	266	83	221
4.4	4.4	1.0	209	83	173
15.7	31.2	0.50	105	89	93
73	196	0.37	77	104	80
105	315	0.33	69	98	68
106.5	273	0.39	82	98	80

Viscosity	Shear Rate (sec^{-1})
	5.75 6.4
	11.5 6.0
	23 5.0
	46 3.7
	115 3.3
	230 3.2

Experiment No. 21

Hct = 33

Fibrinogen = 0

Protein = 5.0

P	Q	R	R^N	C	R^{NC}
156	366	0.46	96	96	92
123	280	0.44	92	96	88
11	38	0.30	63	89	56
10.8	26	0.42	88	83	73
4.2	5	0.84	176	83	146
4.6	5.3	0.87	182	83	151
3.3	2.4	1.38	289	83	240
5.2	5.5	0.95	199	83	165
38.3	148	0.26	54	99	53
162	380	0.43	90	96	86
112	279	0.40	84	98	82
72	159	0.45	94	102	96
48	123	0.39	82	104	85

Viscosity	Shear Rate (sec^{-1})
5.75	6.0
11.5	5.8
23	6.9
46	4.2
115	3.5
230	3.2

Experiment No. 21

Hct = 43

Fibrinogen = 0

Protein = 5.0

P	Q	R	R^N	C	R^{NC}
136	260	0.52	109	96	105
156	300	0.52	108	96	104
91.5	169	0.54	113	102	115
10.3	13.6	0.76	159	83	132
6.1	4	1.55	324	83	269
5.8	3.5	1.66	348	83	289
7.5	5	1.5	314	83	261
86	216	0.40	84	102	86
165	361	0.46	96	96	92
138.5	271	0.51	107	96	103

Viscosity	Shear Rate (sec^{-1})
5.75	11.0
11.5	8.9
23	7.4
46	6.3
115	4.8
230	4.1

Experiment No. 21

Dextran

P	Q	R	R^N
168	300	0.56	117
30.5	79	0.39	82
2.6	4.3	0.60	126
1.7	2	0.85	178
6.3	10.5	0.60	125
69	154	0.45	94
132	273	0.48	101

Experiment No. 22

Hct = 33

Fibrinogen = 415

Protein = 3.3

P	Q	R	R^N	C	R^{NC}
123.8	326	0.38	82	96	79
92	246	0.37	80	102	82
71.8	182	0.39	85	104	88
53.8	133.3	0.40	87	104	90
36.3	83	0.44	95	99	94
16.2	21.8	0.74	161	89	143
10.3	9	1.14	247	83	205
6.7	4	1.68	364	83	302
4.9	2.2	2.23	484	83	401
7.0	3.5	2.0	434	83	360
12	9	1.33	289	89	257
17.1	16.22	1.05	228	89	203
36.2	69	0.53	115	99	114
53.8	130	0.41	89	104	93
73	176	0.41	89	104	93
93.2	231	0.40	87	102	89
116.3	300	0.39	85	98	83

Viscosity	Shear Rate (sec^{-1})
5.75	5.7
11.5	5.0
23	4.3
46	3.9
115	3.6
230	3.3

Experiment No. 22

Hct = 37

Fibrinogen = 150

Protein = 5.0

P	Q	R	R^N	C	R^{NC}
132.5	330	0.40	87	96	84
106	265	0.40	87	98	85
78.7	197	0.40	87	104	90
57.8	143	0.40	87	104	90
41.6	94	0.44	95	104	99
23.2	39.1	0.59	128	99	127
16.4	20	0.82	178	89	158
10	8.8	1.14	247	83	205
5.2	4	1.3	282	83	234
4.9	2.4	2.04	443	83	368
6.2	3.6	1.72	373	83	310
10.3	7.8	1.32	286	83	237
20.6	40	0.52	113	89	101
39.4	94.3	0.42	91	99	90
56	146	0.38	82	104	85
77	194	0.40	87	104	90
99	250	0.40	87	102	89
132,8	319	0.42	91	96	87

Viscosity	Shear Rate (sec^{-1})
	5.75 7.7
	11.5 6.3
	23 5.4
	46 4.5
	115 3.7
	230 3.2

Experiment No. 22

Hct = 39

Fibrinogen = 163

Protein = 4.7

P	Q	R	R^N	C	R^{NC}
120	278	0.43	93	98	91
88.7	221	0.40	87	102	89
68.4	167	0.41	89	104	93
49.2	115	0.43	93	104	97
31.4	67.3	0.47	102	99	101
16.2	20	0.81	176	89	157
10.4	8.8	1.18	256	87	212
6.5	4	1.63	345	83	294
5.3	2.1	2.52	547	83	454
6.7	2.6	2.58	560	83	465
11.9	7.4	1.61	349	89	311
16.7	17.9	0.94	204	89	182
23	35	0.66	143	99	142
41.6	88.24	0.47	102	104	106
62	136	0.45	98	104	102
81	188	0.43	93	102	95
102	246	0.41	89	98	87
127.7	294	0.43	93	96	89
125	300	0.42	91	96	87

Viscosity	Shear Rate (sec^{-1})
5.75	6.4
11.5	5.3
23	4.9
46	4.1
115	3.5
230	3.0

Experiment No. 22

Dextran

P	Q	R	R^N
120.4	250	0.48	104
91.6	195	0.47	102
66.2	146	0.45	98
43	100	0.43	93
21	50	0.43	93
11.0	23	0.48	104
5.3	10.2	0.52	113
3.1	5.2	0.60	130
1.7	3.4	0.50	108
27	3.8	0.71	154
6.1	6.6	0.92	200
17.6	27	0.66	143
40.5	86	0.47	102
60.6	136	0.44	95
84.4	185	0.46	100
108.6	231	0.47	102
140.3	294	0.48	104
120	260	0.46	100
155	352	0.44	96
130	271	0.48	104

Experiment No. 23

Hct = 41

Fibrinogen = 202

Protein = 2.8

P	Q	R	R^N	C	R^{NC}
161	385	0.42	88	96	85
133	326	0.41	86	96	83
53.5	119	0.45	94	104	98
10.4	16.5	0.63	132	83	110
7.1	7.7	0.92	192	83	159
6	3.9	1.54	322	83	267
17.4	35	0.50	105	89	93
172	428	0.40	84	96	81
126	312	0.40	84	96	81
16.2	27.2	0.59	123	89	109

Viscosity	Shear Rate (sec^{-1})
5.75	7.8
11.5	7.2
23	6.1
46	4.9
115	3.9
230	3.1

Experiment No. 23

Hct = 42

Fibrinogen = 101

Protein = 2:8

P	Q	R	R^N	C	R^{NC}
150.5	368	0.41	86	96	83
107	273	0.39	82	98	80
7	8	0.88	184	83	153
3.8	3	1.27	266	83	221
10.3	12.5	0.82	172	83	143
182	435	0.42	88	96	84
140.5	341	0.41	86	96	83
105	273	0.38	79	98	77
28.5	66	0.43	90	99	89
18	32	0.56	117	89	104

Viscosity	Shear Rate (sec^{-1})
5.75	8.2
11.5	7.3
23	5.8
46	4.8
115	3.7
230	3.1

Experiment No. 23

Hct = 44

Fibrinogen = 110

Protein = 2.8

P	Q	R	R^N	C	R^{NC}
168.5	395	0.43	90	96	86
20	44	0.45	94	89	84
7	6	1.16	243	83	202
4	3.2	1.25	262	83	217
14	23.5	0.60	126	89	112
165.5	390	0.42	88	96	84
133	333	0.40	84	96	81

Viscosity	Shear Rate (sec^{-1})
5.75	7.7
11.5	6.7
23	5.5
46	4.7
115	3.6
230	3.0

Experiment No. 23

Dextran

P	Q	R	R^N
164	333	0.49	103
89.4	200	0.45	94
28.5	66	0.43	90
3.8	6	0.63	132
1.7	2.5	0.68	142
1.8	2.4	0.75	157
21.3	39	0.55	115
180.5	370	0.49	103
101.5	222	0.46	96

Experiment No. 24

Hct = 44

Fibrinogen = 656

Protein = 5.3

P	Q	R	R^N	C	R^{NC}
59	107	0.55	128	104	133
82	167	0.49	114	102	116
126.5	273	0.46	107	96	103
45.5	80.2	0.57	133	104	138
19.2	24	0.8	186	89	166
6.7	5	1.34	312	83	259
4.8	7.1	0.68	158	83	131
32	189	0.17	40	99	39
8.5	7.06	1.2	279	83	232
110	250	0.44	102	98	100

Viscosity	Shear Rate (sec^{-1})
5.75	8.8
11.5	7.5
23	7.0
46	6.7
115	5.5
230	4.8

Experiment No. 24

Hct = 48

Fibrinogen = 576

Protein = 5.5

P	Q	R	R^N	C	R^{NC}
113	238	0.47	109	98	107
124.5	273	0.46	107	96	103
70	146	0.48	112	104	116
38	70	0.54	126	99	125
6.4	8.9	0.72	167	83	139
6.0	7.6	0.79	184	83	153
4.2	4.4	0.95	221	83	183
23.7	27	0.88	205	99	203
25.5	30	0.85	198	99	196
72	174	0.41	95	104	99
109	234	0.47	109	98	107

Viscosity	Shear Rate (sec^{-1})
5.75	11.8
11.5	9.9
23	8.1
46	6.7
115	5.5
230	4.7

Experiment No. 24

Hct = 54

Fibrinogen = 132

Protein = 5.5

P	Q	R	R^N	C	R^{NC}
78	149	0.53	123	104	128
116	208	0.57	133	98	130
135.7	278	0.49	114	96	109
77	147	0.52	121	104	126
11.5	15	0.77	179	89	159
4.6	5	0.92	214	83	178
11	14	0.79	184	89	164
98.6	214	0.46	107	102	109
124	261	0.48	112	96	107

Viscosity	Shear Rate (sec^{-1})
5.75	15.4
11.5	12.7
23	9.5
46	7.1
115	5.2
230	4.9

Experiment No. 24

Hct = 60

Fibrinogen = 188

Protein = 6.2

P	Q	R	R^N	C	R^{NC}
122	179	0.68	158	96	152
61.5	85	0.73	170	104	177
33	39	0.84	195	99	193
7.5	5.6	1.34	312	83	259
6.3	3.6	1.75	407	83	338
8.4	5	1.68	391	83	325
137.5	227	0.61	142	96	136
43.7	70	0.63	147	104	153

Viscosity	Shear Rate (sec^{-1})
5.75	17.8
11.5	13.7
23	10.4
46	8.2
115	6.6
230	5.9

Experiment No. 24

Dextran

P	Q	R	R^N
107.5	227	0.47	109
30.4	73	0.42	98
4	8.8	0.45	105
3.8	7.5	0.51	119
50.6	125	0.40	93
116	220	0.43	100

Experiment No. 25

Hct = 37

Fibrinogen = 260

Protein = 4.1

P	Q	R	R^N	C	R^{NC}
99.7	238	0.42	88	102	90
144	313	0.46	96	96	92
98	231	0.42	88	102	90
70.7	176	0.40	84	104	87
46.6	126	0.37	78	104	81
29.5	82	0.36	75	99	74
12.6	24.4	0.52	109	89	97
9	10.2	0.88	184	83	153
5.6	4.4	1.27	266	83	221
4.2	2.4	1.75	367	83	305
7.2	4.6	1.57	329	83	273
10.2	8	1.28	268	83	222
23	50	0.46	96	99	95
38.3	89	0.43	90	99	89
56.7	146	0.39	82	104	85
80.8	194	0.42	88	102	90
111	259	0.43	90	98	88
143	323	0.44	92	96	88

Viscosity	Shear Rate (sec^{-1})
5.75	6.0
11.5	5.3
23	4.4
46	4.0
115	3.6
230	3.4

Experiment No. 25

Hct = 37

Fibrinogen = 392

Protein = 42

P	Q	R	R^N	C	R^{NC}
149	300	0.50	105	96	101
115	259	0.44	92	98	90
86	208	0.41	86	102	88
61	158	0.39	82	104	85
40.4	107	0.38	80	104	83
23.8	52	0.46	96	99	95
12.8	24.5	0.52	109	89	97
8	9.8	0.82	172	83	143
3.9	3.6	1.08	226	83	188
3.0	1.8	1.67	350	83	291
4.3	2.4	1.79	375	83	311
8.6	6.5	1.33	279	83	232
22.6	38	0.60	126	99	125
33	69	0.48	101	99	100
50.7	125	0.41	86	104	89
74	182	0.41	86	104	89
103.7	240	0.43	90	98	88
136.7	309	0.44	92	96	88

Viscosity	Shear Rate (sec $^{-1}$)
5.75	6.7
11.5	5.8
23	5.0
46	4.3
115	4.0
230	3.7

Experiment No.25

Hct = 37

Fibrinogen = 126

Protéin = 5.4

P	Q	R	R^N	C	R^{NC}
96	231	0.42	88	102	90
134.4	303	0.44	92	96	88
90.7	216	0.42	88	102	90
58.5	154	0.38	80	104	83
33.5	94	0.36	75	99	74
17	30.5	0.56	117	89	104
12.7	21.1	0.60	126	89	112
9.1	10.3	0.88	184	83	153
4.3	4.4	0.98	205	83	170
3.8	2	1.9	398	83	330
5.3	2.6	2.04	428	83	355
10	8.1	1.23	258	83	214
18	29	0.63	132	89	117
32	71	0.45	94	99	93
47.7	120	0.40	84	104	87
67.8	171	0.40	84	104	87
94.4	216	0.44	92	102	94
124.5	275	0.45	94	96	90

Viscosity	Shear Rate (sec $^{-1}$)
5.75	6.9
11.5	5.9
23	5.1
46	4.5
115	3.8
230	3.6

Experiment No. 25

Dextran

P	Q	R	R^N
124.7	234	0.53	111
84.7	183	0.46	96
61.8	137	0.45	94
38.3	94	0.41	86
16.7	45	0.37	78
7.9	15	0.54	113
4.3	7.5	0.57	119
2.9	5	0.58	122
4.1	5	0.82	171
13.1	16.7	0.79	166
35	74	0.47	99
55.5	125	0.44	92
80.4	171	0.47	98
107.5	214	0.50	105
140	250	0.56	117

Experiment No. 26
Hct = 43

RBC/Dextran

P	Q	R	R^N	C	R^{NC}
112.5	200	0.56	126	98	123
170	300	0.57	128	96	123
42.5	70	0.61	137	104	142
4.4	4.6	0.96	216	83	179
3.3	2.4	1.38	310	83	257
4.8	3.6	1.33	299	83	248
26.8	44.1	0.61	137	99	136
76	150	0.51	115	104	120
119.3	231	0.52	117	98	115
151	273	0.55	124	96	119
22	30	0.73	164	99	162
21	39	0.54	121	99	120

Experiment No. 26

Hct = 43

Fibrinogen = 148

Protein = 4.8

P	Q	R	R^N	C	R^{NC}
75	214	0.35	79	104	82
134	337	0.40	90	96	86
96	250	0.38	85	102	87
30.3	80	0.38	85	99	84
3.0	3.1	0.97	218	83	181
6.1	8.3	0.73	164	83	136
77	224	0.34	76	104	79
135.5	337	0.40	90	96	86
101	268	0.38	85	98	83

Viscosity	Shear Rate (sec^{-1})
	5.75 7.5
	11.5 6.9
	23 5.7
	46 4.7
	115 3.9
	230 3.5

Experiment No. 26

Dextran

P	Q	R	R^N
151.5	319	0.47	106
99	227	0.44	99
4.8	10	0.48	108
2.6	4.8	0.54	121
30	69	0.43	97
115	259	0.44	99

Experiment No. 27

Hct = 40

Fibrinogen = 150

Protein = 3.7

P	Q	R	R^N	C	R^{NC}
29.5	60	0.49	105	99	104
99	240	0.41	88	102	79
145.5	309	0.47	101	96	97
107	244	0.44	95	98	93
70.5	160	0.44	95	104	99
48.3	107	0.45	97	104	101
34.5	67	0.51	110	104	114
14	22	0.64	137	89	122
10.3	11.5	0.93	200	83	166
17.6	30.5	0.56	120	89	107
26.8	62	0.43	92	99	91
45.4	106	0.43	92	104	96
76	179	0.43	92	104	96
111.7	250	0.45	97	98	95
150	324	0.46	99	96	95
6.5	5.3	1.23	264	83	219

Viscosity	Shear Rate (sec $^{-1}$)	
	5.75	8.6
	11.5	7.6
	23	6.6
	46	5.4
	115	4.0
	230	3.8

Experiment No. 27

Hct = 40

RBC/Ringer

Shear Rate (sec^{-1})	
5.75	4.4
11.5	4.4
23	4.4
46	3.8
115	3.2
230	2.8

P	Q	R	R^N	C	R^{NC}
55	112	0.49	105	104	109
166.5	341	0.49	105	96	101
49.5	106	0.47	101	104	105
10.2	14.7	0.69	148	83	123
5	4.4	1.14	245	83	203
4.3	2.7	1.60	344	83	286
54	129	0.42	90	104	94
93	217	0.43	92	102	94
133.7	300	0.43	92	96	88
95	222	0.43	92	102	94
62	150	0.41	88	104	92
39	95	0.41	88	99	87
17.1	33	0.52	112	89	100
9	11.2	0.80	172	83	143
5.7	4.6	1.24	266	83	221
4.3	2.8	1.54	330	83	274
13.4	21.5	0.62	133	89	118
20	41	0.49	105	89	93
34.2	84	0.41	88	99	87
57.5	136	0.42	90	104	94
97.6	231	0.42	90	102	92
136	309	0.44	94	96	90

Experiment No. 27

Dextran

P	Q	R	R^N
127.7	261	0.49	105
81.4	188	0.43	92
50	120	0.42	90
24.5	61	0.40	86
13.1	32	0.42	90
5.3	10	0.53	114
2.7	4.3	0.63	135
6.2	10	0.62	133
31.7	69	0.45	97
61.7	128	0.48	103
89	190	0.47	101
126.2	245	0.52	112

Experiment No. 28

Hct = 38

Fibrinogen = 100

Protein = 4.1

Temperature = 37°C

P	Q	R	R^N	C	R^{NC}
56.3	133	0.42	95	104	99
132	294	0.45	102	96	98
87.5	205	0.43	97	102	99
57.3	143	0.40	91	104	95
28.5	70	0.41	93	99	92
12.4	24	0.52	118	89	105
4	6.4	0.63	143	83	119
2.7	3.6	0.75	170	83	141
3.9	4.2	0.93	211	83	175
11.5	21	0.55	125	89	111
24	62	0.38	86	99	85
53.5	153	0.35	79	104	82
92	231	0.40	91	102	93
126.4	297	0.43	97	96	93

Viscosity	Shear Rate (sec $^{-1}$)	
	5.75	6.2
	11.5	5.6
	23	5.4
	46	4.5
	115	3.8
	230	3.4

Experiment No. 28

Hct = 38

Fibrinogen = 100

Protein =4.1

Temperature = 25°C

P	Q	R	R^N	C	R^{NC}
91.7	207	044	100	102	102
53.5	128	0.42	95	104	99
33.3	66.7	0.50	113	99	112
9.3	13	0.72	163	83	135
5.0	4.9	1.02	231	83	192
3.5	3	1.17	265	83	220
4.5	3.6	1.25	283	83	235
13.5	18	0.75	170	89	151
27.5	60	0.46	104	99	103
51.5	123	0.42	95	104	99
70	176	0.40	91	104	100
105	240	0.44	100	98	98
141.5	308	0.46	104	96	100

Experiment No. 28

Hct =38

Fibrinogen = 100

Protein = 4.1

Temperature = 14°C

P	Q	R	R^N	C	R^{NC}
62	139.5	0.44	100	104	104
138	250	0.55	125	96	120
103	172	0.60	136	98	133
68	107	0.63	143	104	149
30	33	0.91	206	99	204
17.2	11.8	1.45	329	89	293
10.2	4	2.55	578	83	480
6.3	2.3	2.74	621	83	515
8.7	3.3	2.64	598	83	496
15.7	18	0.87	197	89	175
27.3	29.4	0.93	211	99	209
55	113	0.49	111	104	114
107.6	214	0.50	113	98	111
139	273	0.51	116	96	111

Experiment No. 28

Dextran

Temperature = 37°C

P	Q	R	R^N
131.2	273	0.48	109
88.4	207	0.43	97
66.5	140	0.47	106
28	79	0.35	79
12.6	36	0.35	79
5.3	11.7	0.45	102
2.9	6.7	0.43	97
1.4	2.2	0.64	145
5.1	9.2	0.55	124
19.5	40.5	0.48	109
40.3	94	0.43	97
67.5	214	0.32	72
109	231	0.47	106
159.6	323	0.49	111

Experiment No. 29

Hct = 33

Fibrinogen = 100

Protein = 4.8

Temperature = 37°C

P	Q	R	R^N	C	R^{NC}
129	300	0.42	89	96	85
75.3	210	0.36	76	104	79
47.4	120	0.40	84	104	87
22	54	0.41	86	99	85
8.5	23	0.37	78	83	65
1	2.1	0.48	101	83	84
4.2	9	0.47	99	83	82
19	60	0.32	68	89	61
41	120	0.34	72	104	75
65	188	0.35	74	104	77
96.6	242	0.40	84	102	86
124	300	0.41	86	96	83

Viscosity	Shear Rate (sec^{-1})
5.75	5.1
11.5	4.5
23	4.0
46	3.6
115	3.2
230	2.9

Experiment No. 29

Hct = 33

Temperature = 25°C

P	Q	R	R^N	C	R^{NC}
38.2	76	0.51	108	99	107
109	235	0.46	97	98	95
141.3	300	0.47	99	96	95
95.5	207	0.46	97	102	99
61.2	138	0.44	93	104	97
43	86	0.50	105	104	109
24.5	44	0.56	118	99	117
15.8	26	0.61	129	89	115
1.8	2.2	0.82	173	83	144
5.7	9	0.63	133	83	110
23	63	0.36	76	99	75
55.2	139	0.40	84	104	87
113.5	250	0.45	95	98	93
159	319	0.50	105	96	101

Experiment No. 29

Hct = 33

Temperature = 15°C

P	Q	R	R^N	C	R^{NC}
120	242	0.50	105	98	103
89.4	188	0.48	101	102	103
61	133.3	0.46	97	104	101
32.5	74	0.44	93	99	92
15.6	21	0.74	156	89	139
2.8	3	0.93	196	83	163
2.8	2.4	1.17	247	83	205
8.2	9.8	0.84	177	83	147
30	71	0.42	89	99	88
59	135	0.44	93	104	97
98	207	0.47	99	102	101
156	300	0.52	110	96	106
21	42	0.50	105	99	104

Experiment No. 29

Dextran

P	Q	R	R^N
140	273	0.51	108
55	128	0.43	91
23.5	45	0.52	110
1.7	2.2	0.77	162
2.0	3.1	0.65	137
14.6	34	0.43	91
51	120	0.43	91
106.5	217	0.49	103
162	319	0.51	108

Experiment No. 30

Hct = 40

Fibrinogen = 142

Protein = 5.3

P	Q	R	R^N	C	R^{NC}
71.5	153	0.47	104	104	108
99	221	0.45	100	102	102
66.7	154	0.43	96	104	100
34.5	75	0.46	102	99	101
8.5	11	0.77	171	83	142
5.7	6	0.95	211	83	175
11.7	19	0.62	138	89	123
24.2	60	0.41	91	99	90
43	112	0.38	84	104	87
60.5	162	0.37	82	104	85
91	214	0.42	93	104	95
124	273	0.45	100	96	96

Viscosity	Shear Rate (sec^{-1})
5.75	6.0
11.5	5.3
23	4.7
46	4.1
115	3.6
230	3.3

Experiment No. 30

Hct = 40

RBC/Ringer

P	Q	R	R^N	C	R^{NC}
73.5	183	0.40	89	104	93
115.6	270	0.43	96	98	94
141.3	300	0.47	104	96	100
95.4	240	0.40	0.89	102	91
69.9	186	0.38	84	104	87
34.3	52	0.65	144	99	143
13.4	22	0.61	136	89	121
4.6	5.6	0.82	182	83	151
4.5	4.6	0.98	218	83	181
7.2	8.4	0.86	191	83	159
16.3	34	0.47	104	89	93
27.6	67	0.41	91	99	90
56	140	40	88	104	92
100	250	40	88	102	90
136	315	0.43	96	96	92

Experiment No. 30

Hct = 52

Fibrinogen = 118

Protein = 4.7

P	Q	R	R^N	C	R^{NC}
106	214	0.49	109	98	107
76.6	167	0.46	102	104	106
55.8	115	0.48	107	104	111
33.2	68	0.49	109	99	108
9.2	16.2	0.57	127	83	105
6	6.4	0.94	209	83	173
13.2	16.4	0.81	180	89	160
23	47	0.49	109	99	108
42.3	98	0.43	96	104	100
74.6	160	0.47	104	104	108
115.4	234	0.49	109	98	107
154.5	300	0.51	113	96	108
20	27	0.74	164	89	146
7.5	6.1	1.23	273	83	227

Viscosity	Shear Rate (sec^{-1})
5.75	11.8
11.5	9.4
23	7.5
46	6.1
115	4.8
230	4.3

Experiment No. 30

Hct = 55

RBC/Ringer

P	Q	R	R^N	C	R^{NC}
44.3	115	0.38	84	104	87
92.4	227	0.41	91	102	93
128.5	283	0.45	100	96	96
92.5	216	0.43	96	102	98
62	169	0.37	82	104	85
39.7	109	0.36	80	99	79
16.6	29	0.57	127	89	113
19.5	33	0.59	131	89	117
6.9	7.7	0.90	200	83	166
16.7	35	0.48	107	89	95
33.2	80	0.42	93	99	92
54.8	132	0.42	93	104	97
81	188	0.43	96	102	98
108	234	0.46	102	98	100
144.5	300	0.48	107	96	103
12.5	18	0.69	153	89	136
5.5	5.4	102	227	83	188

Experiment No. 30

Dextran

P	Q	R	R^N
98	195	0.50	111
64.5	136	0.47	104
33.5	77	0.44	98
22	50	0.44	98
9	18	0.50	111
6.5	9.5	0.68	151
15	32	0.47	104
41	95	0.43	96
83	202	0.41	91

REFERENCES

Atencis, A.C., Burdick, D.C., and Reeve, E.B.(1965)
An accurate isotope dilution method for measuring plasma fibrinogen.
J.Lab.Clin.Med., 66, 137-145.

Azuma, T. (1964)
The flow of blood through blood vessels.
Biorheology, 2, 159-61.

Ballantine, D., Personal communication.

Barbee, J.H. (1973)
The effect of temperature on the relative viscosity of human blood.
Biorheology, 10, 1-5.

Barrie, W.W., Wood, E.H., Crumlish, P., Forbes, C.D., Prentice, C.R.M. (1974)
Low dosage ancrod for prevention of thrombotic complications after surgery for fractured neck of femur.
Brit.Med.J., 130-133.

Barrie, W.W., and Schenk, W.G. (1976)
Improvement in blood flow through a critical arterial stenosis by defibrination with Ancrod.
Arch.Surg., 111, 561-563.

Barrie, W.W., and Schenk, W.G. (1977)
Isolated limb perfusion: A canine model for the study of vascular resistance.
J.Surg. Res., 22, 69-71.

Bayliss, L.E. (1952)
Rheology of blood and lymph. in Deformation and flow in biological systems, ed. Frey-Wissling, A., North Holland Publ. Co., Amsterdam, pp. 354-418.

Bayliss, L.E. (1963)
The rheology of blood, Handbook of Physiology, Circulation, Vol. 2., section 2., American Physiological Society.

Benis, A.M., Usami, S., and Chien, S. (1970)
Effect of hematocrit and inertial losses on pressure-flow relations in the isolated hindpaw of the dog.
Circ.Res. 27, 1047-67.

Benis, A.M., Chien, S., Usami, S., and Jan, K.M. (1973)
Inertial pressure losses in perfused hindlimb: a re-interpretation of the results of Whittaker and Winton.
J.Appl. Physiol., 34, 383-389.

Bronte-Stewart, B. (1965)
Epidemiology and dietary factors in occlusive vascular disease.
Ann. Roy.Coll.Surg.Eng., 36, 206-14.

Casson, N. (1959)
A flow equation for pigment-oil suspensions of the printing ink type in Rheology of Disperse systems. ed. Mill, C.C.
Pergamon, London, pp. 84-102.

Castle, J.R.(1964)
The rheology of abnormal human blood.
(Sc. D. Thesis) Cambridge, Mass. M.I.T.

Charm, S., and Kurland, G. (1965)
Viscometry of human blood for shear rates of 0-100,000 sec^{-1}.
Nature, 206, 617-8.

Chien, S., Usami, S., Taylor, H.H., et al(1966)
Effects of haematocrit and plasma proteins on human blood rheology at low shear rates.
J.Appl. Physiol., 21, 81-7.

Chien, S., Usami, S., Dellenback, and Gregersen, M.I. (1971)
Influence of fibrinogen and globulins on blood rheology at low shear rates: Comparison among elephant, dog and man, in Theoretical and Clinical Hemorheology.
Ed. Hartert, H.H., and Copley, A.L.,
Springer-Verlag, Berlin, p. 144-153.

Cokelet, G.R. (1963)
The rheology of human blood (Sc.D.Thesis)
Cambridge, Mass, M.I.T.

Collins, G.M., and Ludbrook, J. (1967)
Behaviour of vascular beds in the human upper limb at low perfusion pressure.
Circ. Res. 21, 319-325.

Copley, A.L., (1960)
in Flow Properties of Blood. Ed. Copley, A.L., and Stainsby, G. p. 110, Pergamon Press, New York.

Copley, A.L. (1973a)
On biorheology
Biorheology, 10: 87-105.

Copley, A.L., Huang, C.R., and King, R.C. (1973b)
Rheogoniometric studies of whole human blood at shear rates from 1000 to 0.0009 sec^{-1} 1. Experimental findings
Biorheology, 10, 17-22.

Cranley, J.J., Fogarty, T.J., Krause, R.J., Strasser, E.S., and Hafner, C.D.(1963)
Phlebotomy for moderate erythrocythemia
JAMA, 186, 206-210.

Crowell, J.W., Ford, R.G., and Lewis, V.M. (1958)
Oxygen transport in hemorrhagic shock as a function of low haematocrit ratio.
Am.J.Physiol. 196, 1033-1038.

De Bakey, M.E., Burch, G., Ray, T., and Ochsner, A.(1947)
The 'borrowing-lending' hemodyamic phenomenon (hemometakinesia) and its therapeutic application in peripheral vascular disturbances.
Ann.Surg., 126, 850- 865.

Denning, A., and Watson, J.M.(1906)
The viscosity of the blood..
Proc.Roy.Soc., 78, 328-358.

Di Perri, T., Forconi, S., Guerrini, M., et al (1977)
Action of Cinnarizine on the hyperviscosity of blood in patients with peripheral obliterative arterial disease.
Proc.Roy.Soc.Med., 70, Suppl.8, 25-28.

Dintenfass, L. (1962)
Thixotropy of blood and proneness to thrombus formation.
Circ.Res., 11, 233-239.

Dintenfass, L., Julian, D.G., and Miller, G.E.(1966)
Viscosity of blood in normal subjects and in patients suffering from coronary occlusion and arterial thrombosis.
Am.Heart. J., 71, 587-600.

Dintenfass, L. (1971)
The rheology of blood in vascular disease.
J.R.Coll.Phys. Lond., 5, 231-240.

Dintenfass, L. (1974)
Blood viscosity in healthy men, measured in rhombospheroid viscometer on EDTA blood.
Biorheology, 11, 397-403.

Djojosugito, A.M., Folkow, B., Oberg, B., and White, S. (1970)
A comparison of blood viscosity measured in vitro and in a vascular bed.
Acta. Physiol.Scand. 78, 70-84.

Dormandy, J.A. (1970)
Clinical significance of blood viscosity.
Ann.Roy.Coll.Surg. Engl. 47, 211-28.

Dormandy, J.A., (1971)
Influence of blood viscosity on blood flow and the effect of low molecular weight dextran.
Brit. Med. J., 4, 716-719.

Dormandy, J.A., Moore, E., Colley, J., Arrowsmith, D.E., and Dormandy, T.L. (1973a)
Clinical, haemodynamic, rheological and biochemical findings in 126 patients with intermittent claudication.
Br.Med.J. 4, 576-581.

Dormandy, J.A., Hoare, E., Khattab, A.H., Arrowsmith, D.E., and Dormandy, T.L. (1973b)
Prognostic significance of rheological and biochemical findings in patients with intermittent claudication.
Br. Med. J. 4, 581-3

Dormandy, J.A., Edelman, J.B., (1973c)
High blood viscosity: an aetiological factor in venous thrombosis.
Br. J. Surg. 60, 187-190.

Dormandy, J.A., Gutteridge, J.M.C., Hoare, E., and Dormandy, T.L. (1974)
Effects of clofibrate on blood viscosity in intermittent claudication.
Brit.Med.J., 4, 259-262.

Dormandy, J.A., Goyle, K.B., and Reid, H.L. (1977)
Treatment of severe intermittent claudication by controlled defibrination.
Lancet, 1, 625-26.

Dormandy, J.A., (1978)
Letter to Lancet, Jan. 7, 1978, p.41.

Eastcott, H.H.G., (1974)
Arterial Surgery, 2nd Edn., p. 2. Pitman, London.

Eastcott, H.H.G., (1974)
Arterial Surgery, 2nd Edn., p. 113, Pitman, London.

Editorial (1977)
Lancet i, 961-962.

Ehringer, H., Dudczak, R., Lechner, K. (1972) (1972)
Therapeutic defibrination with Arvin in arterial circulatory disturbances.
German Association for Internal Medicine Conference, 1972.

Ehringer, H., Dudczak, R., Lechner, K. (1974)
A new approach in the treatment of peripheral arterial occlusions : defibrination with Arvin.
Angiology, 25, 279-289.

Ehrly, A.M. (1973)
Influence of Arvin on the flow properties of blood.
Biorheology, 10, 453-456.

Ellis, F., and Hyams, D.E. (1977)
Vascular response with cinnarizine to standard exercise in patients with intermittent claudication.
Proc.Roy.Soc.Med. 70, supple. 8, 13-16.

Fahraeus, R., and Lindqvist, T. (1931)
The viscosity of blood in narrow capillary tubes.
Am. J. Physiol. 96, 562-568.

Folkow, B., and Lofving, B. (1956)
The distensibility of the systemic resistance blood vessels.
Acta. Physiol. Scand. 38, 37-52.

Ford, T.F., Berent, A., Speed, K., Rose, M.S., and Dormandy, J.A. (1978).
Symptomatic and objective effects of venesection on patients with intermittent claudication.
Brit.Med.J., 1, 1189.

Fowler, N.O., and Holmes, J.C. (1975)
Blood viscosity and cardiac output in acute experimental anaemia.
J. Appl. Physiol. 39, 453-456.

Frasher, W.G. (1967a)
Blood sampling by a chronic artificial external arteriovenous shunt in dogs.
J. Appl. Physiol. 22, 348-351.

Frasher, W.G., Wayland, H., and Meiselman, H.J. (1967b)
Outflow viscometry in native blood.
Bibl. Anat. 9, 266-271.

Frasher, W.G., Wayland, H., and Meiselman, H.J. (1968)
Viscometry of circulating blood in dogs.
i. Heparin injection. ii. platelet removal.
J. Appl. Physiol. 25, 751-760.

Frasher, W.G., Meiselman, H.J., and Wayland, H. (1971)
A variable shear rate capillary viscometer for outflow viscometry in dogs.
Proceedings of the Second International Conference on Hemorheology.
Heidelberg, 1969, (A.L.Copley, Ed.),
Pergamon Press, New York.

Fulton, G.P., and Lutz, B.R.(1957)
Cinemicrography of living blood vessels.
Med. Biol. Illus. 7, 26-32.

Gillespie, J.A. (1959)
The case against vasodilator drugs in occlusive vascular disease of the legs.
Lancet, ii, 995-97.

Goldsmith, H.L., Mason, S.G. (1965)
Further comments on the radial migration of spheres in Poiseuille flow.
Biorheology, 3, 33-6.

Green, H.D., Lewis, R.N., Nickerson, N.D., and Heller, A.L. (1944)
Blood flow, peripheral resistance and vascular tonus with observations on the relationships between blood flow and cutaneous temperature.
Am. J.Physiol., 141, 518-536.

Gregersen, M.I., Perić, B., Chien, S., et al (1965)
Viscosity of blood at low shear rates.
Proc. Fourth Int. Congr. on Rheol., 4, Symp on Biorheol, ed. Copley, A.C., Interscience, New York, pp. 613-28.

Hagen, G., (1839)
Uber die Bewegung des Wassers in engen zylindrischen Rohren.Pogendorff's Ann Physik Chemie, 46, 423-442.

Halmagyi, D.F.J., and Goodman, A.H.(1977)
Vascular resistance and oxygen transport as functions of haematocrit.
Resuscitation, 3, 165-170.

Haynes, R.H.(1960)
Physical basis of the dependence of blood viscosity on tube radius.
Am.J.Physiol. 198, 1193-1200.

Hess, W.R. (1907)
Ein neuer apparat zur Bestimmung der Viskosität des blutes.
Munch. med. Wschr. 32, 1590.

Horstman, D.H., Gleser, M., Wolfe, D., Tryon, T., and Delehunt, J. (1974)
Effects of hemoglobin reduction on Vo_2 max and related hemodynamics in exercising dogs.
J. Appl. Physiol. 37, 97-102.

Humphreys, W.V., Walker, A., Cave, F.D., and Charlesworth, D. (1976)
The effect of an infusion of low molecular weight dextran on peripheral resistance in patients with arteriosclerosis.
Br. J. Surg. 63, 691-3.

Humphreys, W.V., Walker, A., and Charlesworth, D. (1977)
A report on the use of Arvin in patients with pre-gangrene of the lower limb.
Br. J. Surg. 64, 31-33.

Jeyasingham, K., Althaus, U., Berg, E., and Albrechtsen, O. (1972)
Alterations in the plasma proteins and lipids of human and canine blood in bubble and disc oxygenators.
Scand. J. Thor. Cardiovasc. Surg. 6. 172-177.

Kilman, J.W., Waldhausen, J.A., Shumaker, H.S. (1967)
Effects of low molecular weight dextran on peripheral blood flow with controlled cardiac output.
Ann. Surg. 166, 190-194.

Kingsley, G.R. (1942)
The direct biuret method for the determination of serum proteins as applied to photo electric and visual colorimetry.
J.Lab.Clin.Med. 27, 840-1.

Kowalski, E., Kopec, M., Wegrzynowicz, Z. (1964)
Influence of fibrinogen degradation products (FDP) on platelet aggregation, adhesiveness and viscous metamorphosis.
Thromb. Diath. Haemorrh. 10 (suppl). 406-423.

Larrieu, M.S., Marder, V.J., Inceman, S. (1966)
Effects of fibrinogen degradation products on platelets and coagulation.
Thromb. Diath. Haemorrh. 20 (suppl) 215-223.

Levy, M.N., and Share, L. (1953)
The influence of erythrocyte concentration upon the pressure-flow relationships in the dog's hindlimb.
Circ. Res. 1.247-255.

McDonald, D.A.(1974)
Blood flow in arteries, ed. McDonald, D.A., 2nd Edn.
Arnold, London.

McMillan, D.E., Utterback, N.G., and La Puma, J. (1978)
Reduced erythrocyte deformability in diabetes.
Diabetes, 27, 895-901.

Mashiah, A.,Patel, P., Schraibman,I.G., and Charlesworth, D. (1978)
Drug therapy in intermittent claudication : an objective assessment of the effects of three drugs on patients with intermittent claudication.
Br. J. Surg. 65, 342-345.

Mayer, G.A., and Kiss, O. (1965)
Blood viscosity and in vitro anticoagulants.
Am. J. Physiol. 208, 795-797.

Meiselman, H.J., Frasher, W.G., and Wayland, H. (1971)
Variable shear-rate viscometry of native dog blood; effect of heparin injection.
Biorheology, 8, 91-101.

Meiselman, H.J., Frasher, W.G., and Wayland, H. (1972)
The effects of fibrination on the in vivo rheology of dog blood.
Microvascular Research, 4, 26-41.

Merrill,E.W., Cokelet, G.C., Britten, A., and Wells, R.E. (1963a)
Non Newtonian rheology of human blood - effect of fibrinogen deduced by "subtraction".
Circ. Res. 13, 48-55.

Merrill, E.W., Gilliland, E.R., Cokelet, G., Shin, M., Britten, A., and Wells, R.E. (1963b)
Rheology of blood and flow in the microcirculation.
J.Appl.Physiol. 18, 255 -60.

Merrill, E.W., Gilliland, E.R., Margetts, W.G., and Hatch, F.T., (1964)
Rheology of human blood and hyperlipaemia
J. Appl. Physiol. 19, 494-496.

Merrill, E.W. (1969)
Rheology of blood.
Physiol. Rev., 49, 863-888.

Messmer, K. (1975)
Hemodilution.
Surg. Clin. N. Amer. 55, 3, 659-678.

Morris, T., Bouhoutsos, J., Charatzas, D., and Martin, P. (1975)
The influence of haemoglobin concentration and platelet counts on the operative and early post-operative complications of arterial surgery.
J. Cardiovasc. Surg., 16, 152-155.

Nash, C.B., Davis, F., Woodbury, R.A. (1956)
Cardiovascular effects of anesthetic doses of pentobarbital sodium.
Am. J. Physiol. 185, 107-112.

Nicolaides, A.V., Bowers, R., Horbourne, T., Kidner, P.M., and Besterman, E.M. (1977)
Blood viscosity, red cell flexibility, haematocrit, and plasma fibrinogen in patients with angina.
Lancet, 5th Nov. 1977, 943-945.

Pappenheimer, J.R., and Maes, J.P. (1942)
A quantitative measure of the vasomotor tone in the hindlimb muscles of the dog.
Am. J. Physiol. 137, 187-199

Peric, B., (1963)
Viscosity of the blood at low shear rates. Israel.
J. Exp. Med., 11, 139-142

Poiseuille, J.L.M., (1847).
Recherches experimentales sur le mouvement des liquides de nature differente dans les tubes de tres petit diametre.
Ann. Chim. Phys. Serie 3, T21, 76-110.

Postlethwaite, J.C., Goyle, K.B., Dormandy, J.A., et al (1977)
Improvement in experimental vascular graft patency by controlled defibrinogenation.
Br. J. Surg. 64(1) 28-30.

Prentice, C.R.M., Hassaneim, A.A., Turpie, A.G.G. (1969)
Changes in platelet behaviour during Arvin therapy.
Lancet 1, 644-647, 1969.

Pringle, R., Walder, D.N., Weaver, J.P.A. (1965)
Blood viscosity and Raynaud's disease.
Lancet 1, 1086-88.

Prothero, J., and Burton, A.C. (1962)
The physics of capillary flow. 11 the capillary resistance flow.
Biophys. J., 2, 199-211.

Rand, P.W., Lacombe, E., Hunt, H.E., and Austin, W.H. (1964)
Viscosity of normal human blood under normothermic and hypothermic conditions.
J. Appl. Physiol. 19, 117-22.

Reid, H.A., and Chan, K.E. (1968)
The paradox in therapeutic defibrination.
Lancet 1, 485-486.

Reid, H.L., Dormandy, J.A., Barnes, A.J., Lock, P.T., and Dormandy, T.L. (1976a)
Impaired red cell deformability in peripheral vascular disease.
Lancet 1, 666-668.

Reid, H.L., Barnes, A.J., Lock, P.J., Dormandy, J.A., and Dormandy, T.L.(1976b)
A simple method of measuring erythrocyte deformability.
J. Clin. Path. 29, 855-858.

Reid, W., Watt, J.K., and Gray, T.G. (1970)
Phenol injection of the sympathetic chain.
Brit. J. Surg. 57, 45-50.

Replogle, R.L., Meiselman, H.J., and Merrill, E.W. (1967)
Clinical implications of blood rheology studies.
Circulation, 36, 148-160.

Ruckley, C.V., Callam, M.J., Ferrington, C.M., and Prescott, R.J. (1978)
Naftidrofuryl for intermittent claudication : a double-blind controlled trial.
Brit.Med.J. 1, 622.

Saffman, P.G. (1956)
On the motion of small spheroidal particles in a viscous liquid.
J. Fluid Mech. 1, 540-553.

Sirs, J.A.(1968)
The measurement of the haematocrit and flexibility of erythrocytes with a centrifuge.
Biorheology, 5, 1-14.

Skovborg, F., Neilsen, A.V., Schlichtkrull, J., and Ditzel, J.(1966)
Blood viscosity in diabetic patients.
Lancet, i, 129-31.

Smith, E.E., and Shepherd, A.P. (1974)
Pressure-flow relationships in the isolated altitude-acclimatized hindlimb.
Am. J. Physiol. 226(5), 1204-8.

Staessen, A.J. (1977)
Treatment of peripheral circulatory disturbances with Cinnarizine. A multicentre, double-blind, placebo-controlled evaluation.
Proc.Roy. Soc. Med. 70, Suppl. 8, 12-20.

Strand, L. (1969)
Lumbar sympathectomy in the treatment of peripheral arterial disease.
Acta. Chir. Scand. 135, 597-600.

Strandness, D.E., and Sumner, D.S.(1975)
Hemodynamics for surgeons.
New York, Grune and Stratton, p.325.

Sunder-Plassman, L., Kessler, M., Jesch, F., Dieterle, R., and Messmer, K., (1975)
Acute normovolemic hemodilution. Changes in tissue oxygen supply and hemoglobin-oxygen affinity.
'Intentional Hemodilution', Bibl. Haemat. 41, Ed. K. Messmer, and H. Schmid-Schonbein, pp. 44-53. Karger, Basel).

Swank, R.L., and Cullen, C.F. (1953)
Circulatory changes in the hamster's cheek pouch associated with alimentary lipemia.
Proc. Soc. Exp. Biol. Med., 82, 381-384.

Thomas, D.J., Di Boulay, G.H., Marshall, J., et al (1977)
Effect of haematocrit on cerebral blood-flow in man.
Lancet, Nov. 5th 1977, pp 941-943.

Tietjen, G.W., Chien, S., Leroy, E.C., Gavras, I., Gavras, H., Gump, F.E. (1975)
Blood viscosity, plasma proteins and Raynaud Syndrome.
Arch. Surg. 110. 1343-1346.

Von Restorff, W., Hofling, B., Holtz, J., and Bassenge, E. (1975a)
Effect of increased blood fluidity through hemodilution on coronary circulation at rest and during exercise in dogs.
Pflugers Arch. 357, 15-24.

Von Restorff, W., Hofling, B., Holtz, J., and Bassenge, E. (1975b)
Effect of increased blood fluidity through hemodilation on general circulation at rest and during exercise in dogs.
Pflugers Arch. 357, 25-34.

Watt, J.K., Gillespie, G., Pollock, J.G., and Reid W. (1974)
Arterial surgery in intermittent claudication.
Brit. Med. J., 1, 23-26.

Wayland, H. (1967)
Rheology and the microcirculation
Gastroenterology, 52, 342-355.

Wells, R.E. (1971)
Yield stress in absence of fibrinogen. in Theoretical and Clinical Hemorheology, ed. Hartert and Copley, Springer-Verlag, Berlin.

Whitmore, R.L. (1968)
Rheology of the circulation.
Pergamon Press, Oxford, pp 64-69.

Whitmore, R.L. (1968)
Rheology of the circulation.
Pergamon Press, Oxford, p 96.

Whittaker, S.R.F., and Winton, F.R. (1933)
The apparent viscosity of blood flowing in the isolated hindlimb of the dog, and its variation with corpuscular concentration.
J. Physiol. 78, 339-369.

Wiederhielm, G.A., and Billig, L. (1967)
Effects of erythrocyte orientation and concentration on light transmission through blood flowing through microscopic blood vessels.
Biorheol. 4, 99-100.