HAEMORHEOLOGICAL STUDIES IN THE FETUS, PREGNANT AND NON-PREGNANT WOMAN

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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DECLARATION

I hereby declare that the work described in this thesis was carried out by me in the Department of Obstetrics and Gynaecology, St. James's University Hospital, Leeds, LS9 7TF. The clinical and laboratory work, as described in the chapters, was carried out without technical assistance, except where specifically acknowledged in the text.

Certain parts of this thesis have been published in the following papers in scientific journals, but none of the material has been used for any other degree or qualification.

Buchan, P.C. (1980) Evaluation and modification of whole blood filtration in the measurement of erythrocyte deformability in pregnancy and the newborn. British Journal of Haematology, 44.

Buchan, P.C. (1979) Pathogenesis of neonatal hyperbilirubinaemia after induction of labour with oxytocin. British Medical Journal, 2, 1255-1257.

Buchan, P.C. (1979) Erythrocyte deformability, plasma and whole blood viscosity and plasma fibrinogen in pregnancy and the newborn. I.R.C.S. Medical Science, 7, 474.

Buchan, P.C. (1979) Erythrocyte deformability and plasma constituents in pregnancy and the newborn. I.R.C.S. Medical Science, 7, 484.

Buchan, P.C. & Macdonald, H.N. (1980) Clinical Rheology in Obstetrics and Gynaecology. Chapter in 'Clinical Rheology' edited by Forbes, Lowe & Barbenel. Basel: Springer-Verlag.

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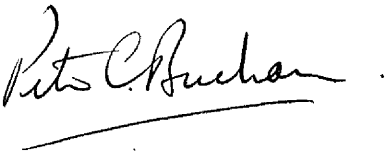

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SUMMARY

The importance of haemorheology in clinical medicine has only recently been widely realised. Haemorheological abnormalities have been found in a variety of pathological conditions but very little has been done in the field of obstetrics and gynaecology either to define normal haemorheological values in the menstrual cycle or in maternal and fetal blood in pregnancy, or to detect haemorheological abnormalities in pathological pregnancy or fetal disease.

Haemorheological measurement techniques are constantly in need of evaluation and improvement. The first original work reported in this thesis was the development of the Deer Rheometer for the measurement of plasma and whole blood viscosity. Sample handling and measurement protocols were developed which gave reliable, fast and reproducible measurement of viscosity in biological fluids. In the initial experiments whole blood filtration was used as the method for measurement of erythrocyte deformability, but factors other than erythrocyte deformability were found to influence the rate of filtration. These factors were identified and eliminated or reduced to a minimum so that a highly sensitive filtration technique for the measurement of erythrocyte deformability was developed.

Having established these two new methodologies and adding standard methods for the measurement of haematocrit and plasma fibrinogen, the normal levels of haemorheological parameters were measured in serial study throughout the menstrual cycle and normal pregnancy and in healthy full term and preterm fetuses.

The cyclical variation in haemorheological parameters seen in the spontaneous menstrual cycle was abolished by ovulation suppression with ethinyloestradiol 30 μg and norgestrel 250 μg and the absolute level of each parameter was altered in such a way as would predispose to thrombosis.

The effects of maternal cigarette smoking on maternal and fetal haemorheology were investigated in serial study. Cigarette smoking caused a reduction in maternal erythrocyte deformability throughout pregnancy, but did not affect other haemorheological factors. In contrast, the fetal blood exhibited an increased haematocrit and whole blood viscosity and reduced erythrocyte deformability. These changes in fetal haemorheology were associated with a significant reduction in birth weight in cigarette smokers and hyperviscosity causing impaired perfusion of the placental villi may be responsible for this fetal growth retardation.

Pre-eclampsia resulted in elevation of maternal haematocrit, plasma fibrinogen, plasma and whole blood viscosity and reduction in erythrocyte deformability. In fetal blood the haematocrit and whole blood viscosity were elevated but plasma fibrinogen and viscosity and erythrocyte deformability were not altered. In the mother pre-eclampsia is characterised by acute hyperviscosity state associated with hypovolaemia and this could lead both to impaired placental perfusion and eventually to disseminated intravascular coagulation.

Essential hypertension of mild or moderate degree had little effect on either maternal or fetal haemorheology and this was in keeping with the good fetal outcome in the cases studied.

Both during labour and in the immediate neonatal period the infant is liable to anoxic acidosis. A series of *in vitro* and *in vivo* experiments investigated the effects of hypoxic acidosis on fetal erythrocyte deformability. Fetal erythrocytes were particularly sensitive to the effects of hypoxic acidosis and a hypothesis was developed to show ways in which haemorheological factors might contribute to the pathogenesis of anoxic intracranial haemorrhage.

The last chapter dealt with the importance of haemorheological factors in the pathogenesis of neonatal hyperbilirubinaemia following induction of labour. A prospective clinical study showed that oxytocin used in the induction of labour caused a decrease in erythrocyte deformability due to osmotic swelling and resulted in increased haemolysis with consequent hyperbilirubinaemia. Bupivicaine, used in epidural anaesthesia, was shown in *in vitro* studies to reduce erythrocyte deformability, but prostaglandin E2, used in the induction of labour, had a variable and clinically insignificant effect on fetal cells.

The work reported in this thesis is largely new and obviously confirmatory work must follow. Haemorheological therapy is already being used in pre-eclampsia and with a better understanding of normal and pathological haemorheology in both mother and fetus a wider application of haemorheological therapies will follow.

CHAPTER 1

INTRODUCTION

Sections

i) HAEMORHEOLOGICAL FACTORS IN THE CONTROL OF THE PERIPHERAL CIRCULATION

ii) THE COMPLEXITY OF HAEMORHEOLOGY

iii) ALTERED HAEMORHEOLOGY IN CLINICAL DISEASE

INTRODUCTION

"It is the heart by whose virtue and pulse the blood is moved, perfected and made apt to nourish and is preserved from corruption and coagulation."

William Harvey (1628) (1)

This quotation from Harvey's classic work contains the essence of haemorheology and of this thesis. Haemorheology is primarily concerned with the movement of blood, and the purpose of this movement, as Harvey pointed out, is threefold. Flow is necessary for the blood to reach and 'nourish' the tissues. In pregnancy the utero-placental circulation is the source of nourishment for the fetus and impairment of this flow by altered haemorheology would be of great importance to the development and health of the fetus. Blood must also flow to preserve itself from 'corruption' or haemolysis. The alteration of erythrocyte deformability in pathological states, as well as by the process of ageing, leads to their haemolysis and destruction and the study of erythrocyte deformability is an important part of this thesis. Thirdly, Harvey stated that stasis led to 'coagulation' and in pregnancy and in patients on oral contraceptives coagulation leading to thrombo-embolism is a major problem. The possible connection between hyperviscosity and thrombo-embolism is investigated. The studies presented in this thesis are self-contained with their own introduction and literature review. This general introduction will deal in broad outline with the role of haemorheological factors in the control of the peripheral circulation, the complexity of haemorheological measurements and, lastly, with those pathological conditions in which it is known that haemorheological factors play a significant part.

i) HAEMORHEOLOGICAL FACTORS IN THE CONTROL OF THE PERIPHERAL CIRCULATION

The control of the peripheral circulation has been the object of study and speculation ever since Harvey described the circulation of the blood (1). Stephen Hales, a minister in Teddington, at the start of the 18th century, first drew together the factors controlling the peripheral circulation when he stated: "The resistance which the blood meets in these capillary passages may be greatly varied, either by different degrees of viscosity or fluidity of the blood or the several degrees of constriction or relaxation of these fine vessels" (2). A century later the French physician Poiseuille, studying the flow of fluids through narrow tubes, related flow rate quantitatively to the driving pressure, the fourth power of the radius of the vessel and inversely to the viscosity of the fluid (3) (Table 1). When considering blood flow through a vascular complex, such as the pregnant uterus, Ohm's law may be used to show the relationship between flow, resistance and driving pressure (Table 1). Combining Poiseuille's and Ohm's formulae, a third formula may be derived (Table 1) to show that the resistance of a vascular bed is directly proportional to the length of the vascular segment and the viscosity of the blood and inversely proportional to the fourth power of the vessel radius.

Under physiological conditions, regulation of the peripheral blood supply is dominated by vasomotor factors altering the vessel radius and viscosity factors play a negligible role. However, in certain pathological conditions and in pregnancy, the situation may be totally different. During pregnancy there is a dilatation affecting both the arterial (4) and venous (5) sides of the circulation and secondly there is a decreased capacity for the uterine vessels to react to constrictor stimuli (4). This loss of vascular reactivity

is also seen in hypoxic states where vasodilator metabolites are produced which paralyse the vascular smooth muscle (6). This may proceed to a point at which the vasomotor reserve is exhausted and then the vasomotor component of flow control is minimal and viscosity factors become dominant. An additional difference between vasomotor control and viscous limitation of perfusion follows the same argument. While vasomotor shutdown of blood vessels is effective in producing a rapid but short lasting arrest in the circulation, metabolic autoregulation will paralyse the constrictors and soon restore flow (7). Any kind of sustained flow retardation or stasis must therefore be caused by an intravascular obstacle. Physiologically or pathologically enhanced hyperviscosity or cellular aggregation therefore may cause such a state of maintained intravscular stagnation. Haemorheological changes are unlikely to initiate slowing or arrest of flow but they are capable of sustaining and worsening them.

ii) THE COMPLEXITY OF HAEMORHEOLOGY

In 1687 Issac Newton wrote in his Principia "the resistance which arises from the lack of slipperiness of the parts of a liquid, other things being equal, is proportional to the velocity with which the parts of a liquid are separated from one another". This "lack of slipperiness" is now called viscosity. Viscosity, as Hales (2) and Poiseuille (3) understood it, was a static fixed property of the fluid under study which did not vary with flow rate (Newtonian behaviour). It is now known, however, that whole blood viscosity is influenced not only by the constituents of blood such as haematocrit (9), erythrocyte deformability (10), erythrocyte aggregation (11), platelet aggregation (12), plasma fibrinogen (13), globulins (14) and fats (15), and the metabolic

state of the blood (16,17) but also by the rate of flow, with a decrease in viscosity accompanying an increase in flow rate (non-Newtonian behaviour) (18), and also by vessel diameter with a decreasing viscosity as blood flows through capillaries of diminishing radius (Fahraeus-Lindquist phenomenon(19)) down to a critical radius below which the viscosity increases sharply (Inversion phenomenon (20)). This complexity of haemorheology results from the complicated interaction between the several constituents of blood. At rest, whole blood has a complicated three-dimensional structure of erythrocytes held in aggregation by the action of plasma proteins interacting at the surface membrane (21-23) and forming macromolecular bridges between erythrocytes (24). To induce flow energy must be applied to break these bridges (25). Initially, as increasing stress is applied to static blood, the matrix of cells undergoes visco-elastic distortion and then once the yield stress is reached, flow begins (26). With the breaking of the erythrocyte matrix and the start of flow, the erythrocytes remain in clumps or aggregates and there is a dynamic state of aggregation and disaggregation which results in the non-Newtonian behaviour of blood. As additional stress is applied, the aggregates and rouleaux become smaller and once they are reduced to individual cells, further increase in stress cannot reduce cell size any more and the shear stress-shear rate relationship becomes almost linear (27). This shear thinning effect is also influenced by the deformability of the red cells. Under stress erythrocytes change from discoid to elongated rod shapes. It has been suggested that this change in shape also relates to the shear stress-flow rate relationship becoming a straight line independent

of aggregation characteristics (28).

iii) ALTERED HAEMORHEOLOGY IN CLINICAL DISEASE

Having reviewed the haemodynamic context of haemorheological factors in the control of peripheral perfusion and the complex and inter-related nature of these factors, the pathological processes that are known to be caused by or to be associated with altered haemorheology will be reviewed.

As there is greater awareness of the importance of haemorheology in the pathogenesis of cardiovascular disease, so more associations are being discovered. Patients with intermittent claudication due to peripheral vascular disease have been shown to have elevated whole blood viscosity (29,30) and reduced erythrocyte deformability (31). These patients experience symptomatic relief when their viscosity is reduced by either reducing the haematocrit by venesection (32) or by reducing the plasma fibrinogen with drug therapy (33). Patients who have had a myocardial infaction or who suffer from angina pectoris also have raised blood viscosity levels (34) and therapy directed towards lowering the viscosity by reducing plasma fibrinogen (35) or by the use of B-adrenergic receptor blockers (36) produces both symptomatic relief and improved long-term survival. In diabetes mellitus, there is hyperviscosity of both whole blood and plasma (37,38) and reduced erythrocyte deformability (39). These changes are largely due to the metabolic abnormalities of diabetes and haemorheological therapy is not yet of proven benefit. Cerebral arteriosclerosis is associated with hyperviscosity and reduction of haematocrit by venesection may significantly improve the cerebral blood flow (40. The haemo-

globinopathies are associated with both an increased whole blood viscosity and decreased erythrocyte deformability (41,42). The paraproteinaemias, with elevated plasma viscosity, are amenable to haemorheological therapy, and removal of the excess protein by plasmaphoresis results in a considerable improvement in peripheral perfusion (43). Raynaud's syndrome is associated with elevated blood viscosity and reduction in viscosity ameliorates the symptoms (44). More recently, hyperviscosity screening has been introduced to select high risk groups in the prediction of venous thrombosis after surgery (45,46) and during oral contraceptive therapy (47,48).

With this wide range of clinical pathology related to haemorheological disturbance, it is interesting that very few investigations have been carried out into the haemorheology of normal or pathological pregnancy or the fetus. What work has been done will be reviewed in the relevant sections of the thesis.

Table 1 Poiseuille's Formula, Ohm's Formula and Peripheral Resistance Formula

Poiseuille's Formula

$$\text{Rate of flow} = \frac{\pi}{8} \times \frac{\triangle P \times r^4}{l \times \eta}$$

where $\triangle P$ = pressure gradient

r = radius of vessel

l = length of vessel

η = viscosity of blood

Ohm's Formula

$$I = \frac{V}{R}$$

where I = flow rate

V = pressure gradient

R = resistance

Peripheral Resistance Formula

$$\text{Peripheral resistance} = \frac{l \times \eta}{r^4}$$

where l = length of vessel

r = radius of vessel

η = viscosity of blood

CHAPTER 2

METHODOLOGY

Sections

i) PATIENT SELECTION

ii) STATISTICAL ANALYSES

iii) DEVELOPMENT OF NEW HAEMORHEOLOGICAL METHODS

- a) The Measurement of Whole Blood and Plasma Viscosity Using the Deer Rheometer
- b) The Development of a Microfiltration Method for the Measurement of Erythrocyte Deformability

iv) STANDARD METHODS USED IN THE STUDIES

- a) Haematocrit
- b) Platelet and White Blood Cell Counts
- c) Plasma Fibrinogen
- d) Plasma Lactate Dehydrogenase
- e) Plasma Bilirubin
- f) Plasma Haptoglobin
- g) Serum Albumin and Total Protein
- h) Plasma Osmolality
- i) Blood pH
- j) Scanning Electron Microscopy
- k) Technical Assistance

METHODOLOGY

i) PATIENT SELECTION

All the women who took part in these studies did so voluntarily after receiving a full explanation of the procedures involved and their purpose. In the case of samples taken from the baby's umbilical cord vein at birth, consent was obtained from the mother before delivery. All the experimental protocols have been scrutinised and approved by the Ethical and Scientific Committee of St. James's University Hospital.

The several different groups of patients who took part in the studies are defined in the relevant sections of the thesis and each group was separately matched with a control group of normal healthy subjects.

ii) STATISTICAL ANALYSES

The mean, standard deviation (SD) and standard error of the mean (SEM) were calculated for all data. Where a test of statistical significance concerned a change with time in the same group of subjects, the calculation was based on the average change in the group giving a pooled variance for obtaining a standard error of the difference between the means. The probability (p) of any difference being due to chance was estimated by use of Student's t-test and the results are expressed in the conventional way. Results with a value of 'p' greater than 0.05 were regarded as not statistically significant (ns); all other results are quoted with the appropriate level of probability. When testing for correlation between different parameters measured in the same group of samples linear regression analysis was carried out to determine the correlation coefficient. When analysing the variation in repeat measurements of a parameter the coefficient

of variation was calculated by expressing the SD as a percentage of the mean.

iii) DEVELOPMENT OF NEW METHODS

a) Measurement of Whole Blood and Plasma Viscosity Using the Deer Rheometer

Early work in viscometry was done using capillary tube viscometers such as the Ostwald or Harkness (49) viscometer. The principle behind all capillary viscometers is the measurement of the time taken for a standard volume of test fluid to flow through a capillary tube of standard length and diameter under a standard force at a standard temperature. These instruments have the advantage of requiring only small volumes of test fluid and show a relatively high degree of accuracy. They have two disadvantages. Firstly, they operate at high rates of shear which precludes their detecting the non-Newtonian influence of cells in whole blood; plasma viscosity measurements are not affected. The second disadvantage arises from the non-uniform flow in the capillary which is slowest at the periphery and fastest at the centre. The rate of shear varies across the tube and mathematical analysis of this phenomenon is complex.

More recently rotational viscometers have been designed where the test fluid is introduced between either a flat plate and a wide angled cone bob or a cup and a suspended cylindrical bob. Viscosity is calculated from the measured torque on the suspending wires when the plate or cup is rotated at a variety of speeds. The shear rate depends on the speed of rotation and such instruments have the advantage that viscosity can be measured over a wide range of shear rates. The Wells-Brookfield

viscometer (50) has been used extensively in whole blood viscosity measurement but is not reliable at shear rates below 23 inverse seconds (sec^{-1}). Recent introduction of the Contraves Low Shear viscometer (51) allows measurement at shear rates as low as 0.07 sec^{-1}.

The Deer Rheometer is a further modification of these rotational viscometers. This instrument, shown in Figure 1, uses a fixed cup and rotating cylinder. The gap between the bottom of the cylinder and the floor of the cup is accurately set by a micrometer and the measurements of the cup and cylinder accurately measured so that the moving area of the sample is known. The measuring geometry is shown diagrammatically in Figure 2. The Deer Rheometer differs from the Wells-Brookfield and Contraves viscometers in that the shear rate is not fixed, but a shear stress of known dimension is applied to the rotating cylinder by means of an induction type electrical motor having performance characteristics in which torsional force is linearly independent of angular velocity over the full operational range of the instrument. All rotating parts are supported upon the effectively friction-free air bearing incorporated in the drive unit, there being no mechanical connection whatsoever between the rotating and the stationary parts of the instrument. The displacement or angular velocity of the rotating part is measured by one of two systems. In the visco-elastic area and at very low shear rates rotational displacement is measured by a non-contacting electronic sensor that measures the gap between itself and the circular ramp, or scroll, that is attached to the rotating spindle (Figure 3). At faster rates of shear the angular velocity is measured

by a tachometer disc attached to the rotating spindle and which has a series of perforations round its perimeter. Passage of the perforations is detected by a photoelectric cell that is fixed to the instrument support bracket (Figure 3). By standard calculations from the measurements of the cup and cylinder and the sample size, the shear rate of the sample under test is calculated and hence the viscosity is determined by dividing the shear stress in dynes/cm^2 by the shear rate in sec^{-1}.

The Deer Rheometer has advantages over the fixed shear rate instruments. Firstly, it can be used to investigate the visco-elastic and yield stress phenomena in haemorheology. It can also measure viscosity over a wide range of shear rates. Its main disadvantage is the sensitivity of the measurement of viscosity at shear stresses just exceeding the yield stress. At this point there is a dynamic and constantly changing aggregation and dis-aggregation of erythrocytes, as described in the section on the complexity of haemorheology. Because of this phenomenon, measurements of viscosity at low shear stresses tend to be erratic and variable, depending on the balances of the aggregation and dis-aggregation. This is a natural phenomenon but it reduces the reproducibility of the method unless it is eliminated. To overcome the variable influence of erythrocyte aggregation, the sample is sheared by a shear stress of 10 dynes/cm^2 for two minutes. This causes complete dis-aggregation of the cells (52) and allows an aggregation-free measurement to be made. The cells start to aggregate again about 30 seconds after the cessation of movement in the sample and so the measurements must be made immediately motion stops following the dis-aggregating

shear. Studies of the visco-elastic and yield stress properties have been omitted from this thesis, as they are difficult to interpret and were far too time consuming to be possible with the number of samples in the studies.

Measurement of Whole Blood Viscosity

Whole blood viscosity was measured in blood samples taken from an antecubital vein without venous occlusion. The blood was anticoagulated with Lithium Heparin and stored at $37^{o}C$ until measurement, which was completed within four hours of sample collection. For five minutes before measurement the sample was agitated in a circular fashion to ensure no separation of cells and plasma and then a 2.5 ml aliquot was drawn up in a clean glass pipette and placed in the measuring cup. The measuring cylinder was lowered into the cup. The whole measuring apparatus was enclosed in a water jacket that was kept at $37^{o}C$ by a circulating water heater. With air flowing through the air-bearing under a pressure of 60 pounds per square inch, the sample was subjected to a shear stress of 10 dynes/cm^{2} for two minutes. At the end of two minutes the shear stress was withdrawn and the required measuring shear stress selected. As soon as the cylinder had stopped, the new shear stress was applied andallowed to stabilise for 30 seconds, following which the angular velocity was measured over a period of a further 30 seconds. From the angular velocity the sample shear rate was calculated and hence the viscosity was worked out. Between each measurement the cup and cylinder were carefully cleaned with distilled water at $37^{o}C$ and dried, great care being taken not to leave any debris or threads on the measuring surfaces as that would profoundly affect the subsequent measurement.

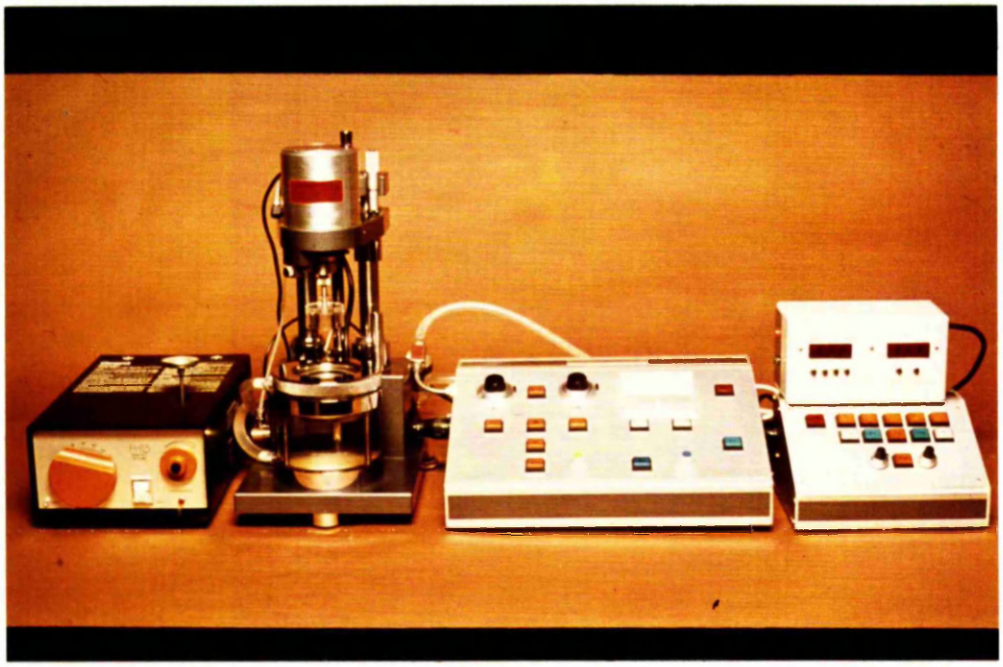

Figure 1 The Deer Rheometer

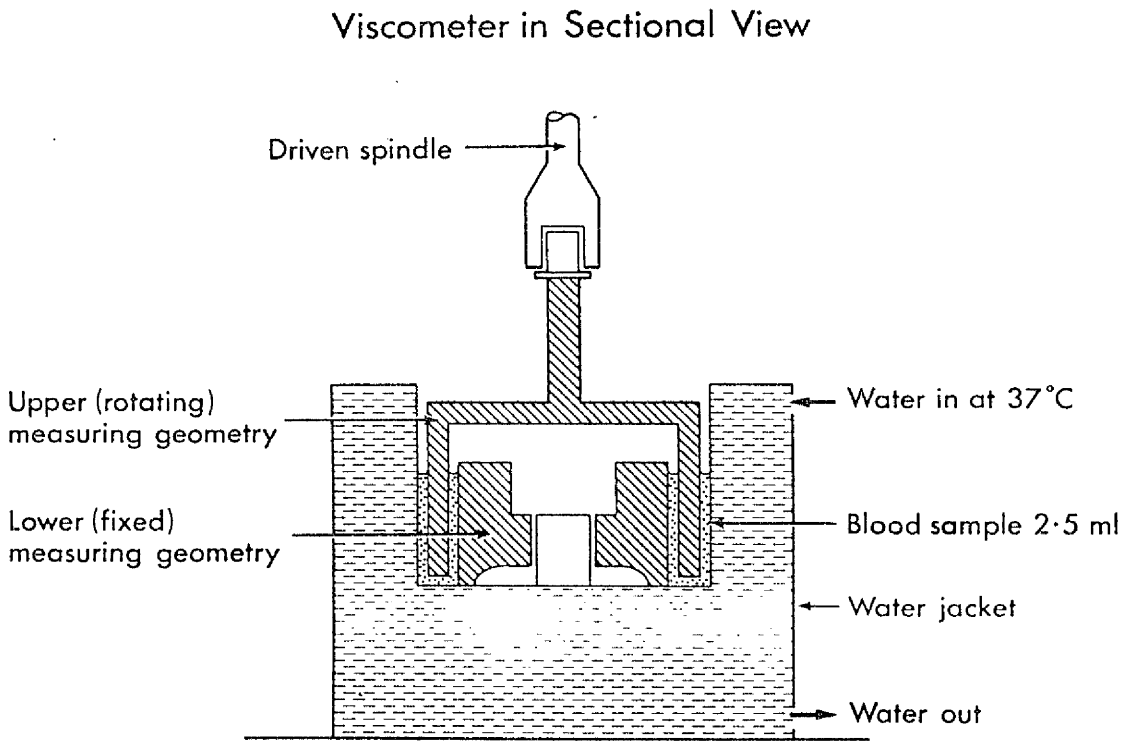

Figure 2 Diagram of the Measuring Geometry of the Deer Rheometer

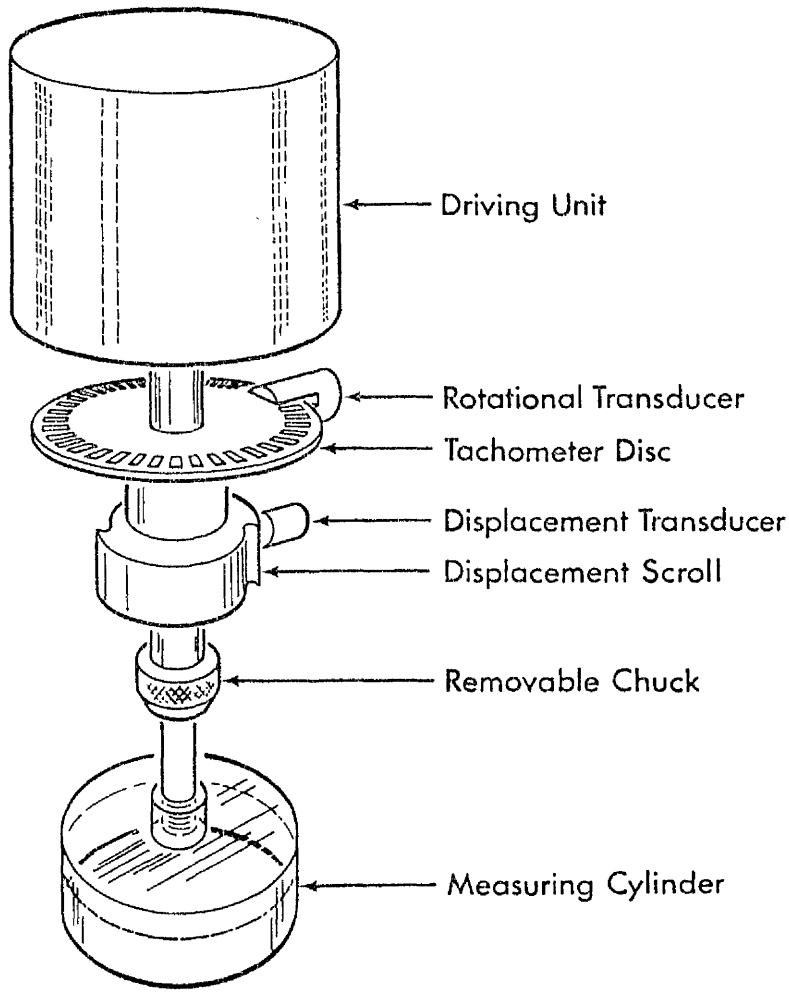

Figure 3 Diagram of the Displacement and Angular Velocity Measuring Systems of the Deer Rheometer

Results

The non-Newtonian behaviour of whole blood was confirmed by plotting the whole blood viscosity measurements made at a series of different shear stress values. The mean and SD of these measurements on 30 aliquots of the same blood sample are shown graphically in Figure 4. At the lowest shear stress at which measurement was made the coefficient of variation was high at 18 per cent, whereas at higher shear stresses the coefficient was significantly lower at between 7.0 and 8.5 per cent.

The effect of haematocrit on whole blood viscosity is shown in Figure 5 where a series of samples of different haematocrits were subjected to shear stress of both 0.574 dynes/cm^2 and 5.739 dynes/cm^2 and their viscosity calculated. Rising haematocrit is associated with increasing blood viscosity. This effect is, however, significantly influenced by the shear stress. As the shear stress increases, so the effect of increased haematocrit is diminished.

Measurement of Plasma Viscosity

Plasma was separated from blood, which had been anticoagulated with Lithium Heparin, by centrifugation at 5,000 G for 15 minutes. Plasma aliquots of 2.5 ml were measured in the Deer Rheometer at 37^{o}C. With plasma there was no need for dis-aggregation shearing and the samples were routinely measured at a shear stress of 0.574 dynes/cm^2.

Results

The Newtonian behaviour of plasma is shown in Figure 6. Increasing shear stress does not alter the viscosity measurement. If the white cells and platelets are not fully removed by centrifugation,

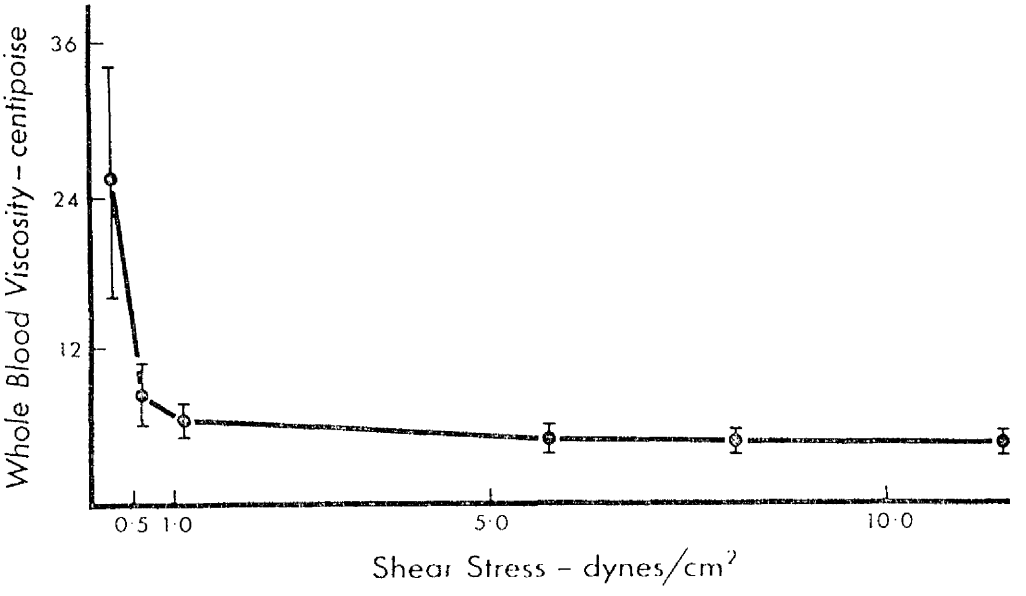

Figure 4 Relationship Between Shear Stress and Whole Blood Viscosity

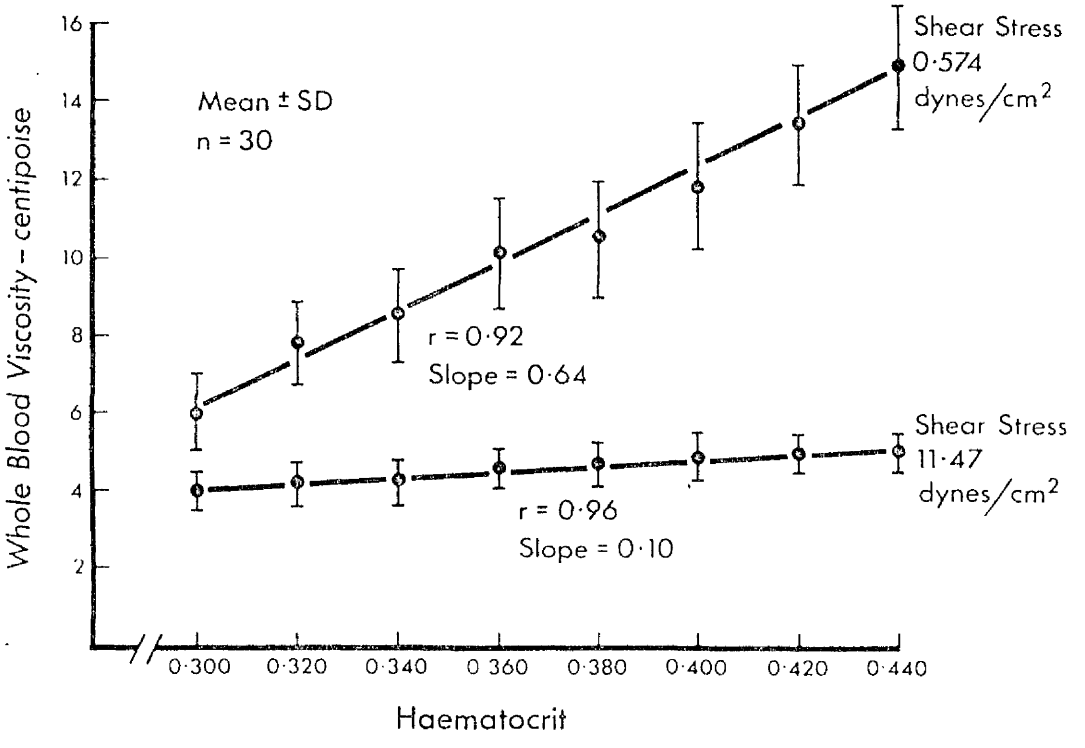

Figure 5 Relationship Between Haematocrit, Whole Blood Viscosity and Shear Stress

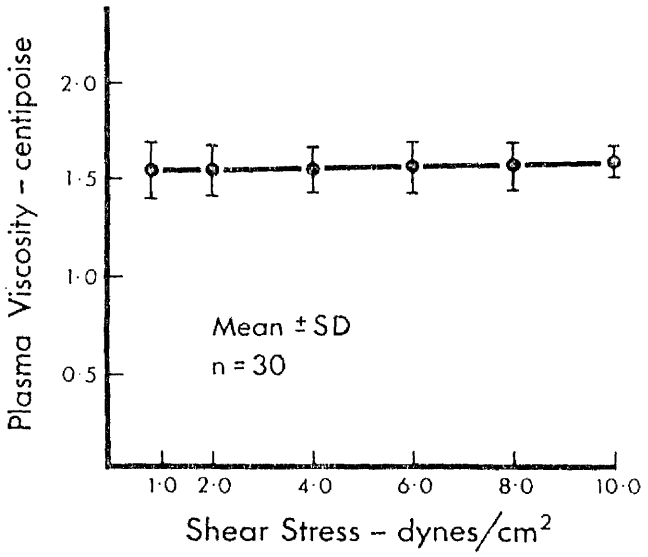

Figure 6 Relationship Between Shear Stress and Plasma Viscosity

then the 'contaminated' plasma will exhibit a modified degree of shear thinning, also if the sample was left in the measuring geometry for more than five minutes drying took place at the sample-air interface and this also gave rise to apparent shear thinning.

Thirty aliquots of the same plasma measured at a shear stress of 0.574 dynes/cm^2 at 37^{o}C gave a mean viscosity of 1.52 centipoise, SD of 0.05 and coefficient of variation of 6.5 per cent.

Discussion

The Deer Rheometer gives rapid and reasonably reproductive measurements of whole blood and plasma viscosity over a wide range of shear stresses. The non-Newtonian behaviour of whole blood found in this study is in agreement with other workers (22,32,53). Aggregation of erythrocytes at low shear rates has long been considered of great importance for the high viscosities encountered under these conditions. Low shear - viscosity relationships in many pathological states have been described (54), but a recent report by Copley et al (1975) (52), using a specially modified rotary viscometer, throws some doubt on these findings, showing, as was seen in my early experience with the Deer Rheometer, that at low shear rates erythrocytes go through aggregation and dis-aggregation in an unpredictable fashion that makes low shear viscometry very difficult to interpret. Even though in the present study every effort was made to eliminate the effects of erythrocyte aggregation, there is no doubt that the variability of measurement is much improved at higher shear stresses.

The influence of haematocrit on whole blood viscosity is the subject of conflicting reports. Using a capillary viscometer, Hess (1911) (55) and others (56,57) described a curvilinear relationship between haematocrit and blood viscosity. Nyggard et al (1935) (58) and others (59), also using capillary viscometers, showed that the logarithm of blood viscosity was related to the haematocrit and this has subsequently been confirmed by several groups using rotational viscometers (32,60).

Plasma viscosity is believed to be Newtonian in behaviour, although doubts were cast (61,62) which were probably due to methodological error (63). Certainly in this present study, both inadequate separation of platelets and white blood cells and sample-air interface drying both resulted in apparent non-Newtonian behaviour.

b) The Measurement of Erythrocyte Deformability

> "An observation of Mr. Leeuwenhoek is very well worth regarding: he took notice, that when he was greatly disordered, the Globules of his Blood appeared hard and rigid, but grew softer and more pliable as his Health returned."
>
> Henry Baker (1743) in
> The Microscope made Easy

Erythrocyte deformability may be defined as those physio-chemical characteristics which permit erythrocytes whose greatest diameter normally exceeds 8 μm, to pass through normal capillaries which range from 3 to 12 μm in diameter (64).

This deformability is important in three situations. Firstly, it is an important determinant of erythrocyte lifespan *in vivo* (65-67). Secondly, it has a significant influence on blood flow in the microcirculation (10,68,69). Thirdly, it is a determinant of whole blood viscosity, particularly at high rates of flow (70), and so it affects peripheral resistance and hence cardiac work.

Erythrocyte deformability can be measured by four types of method: viscometry (71); rate of centrifugal packing (72); deformation of the membrane by micropipettes (73) and lastly, by microfiltration through inert materials of known poricity (74-77).

The method of whole blood filtration described by Reid *et al* (1976) (78) was selected as the simplest to use in the investigation of erythrocyte deformability in this present study, but it was soon found that this method was unreliable and the results were affected by many other factors than erythrocyte deformability. The following study sought to identify these sources of error and to eliminate or minimise them.

Experimental Methods

Patients

Thirty healthy female volunteers aged between 20 and 40 years, none of whom smoked cigarettes, were selected.

Apparatus

The apparatus, seen in Figure 7, experimental method and sample handling were identical to the method of Reid *et al* (1976) (78) in

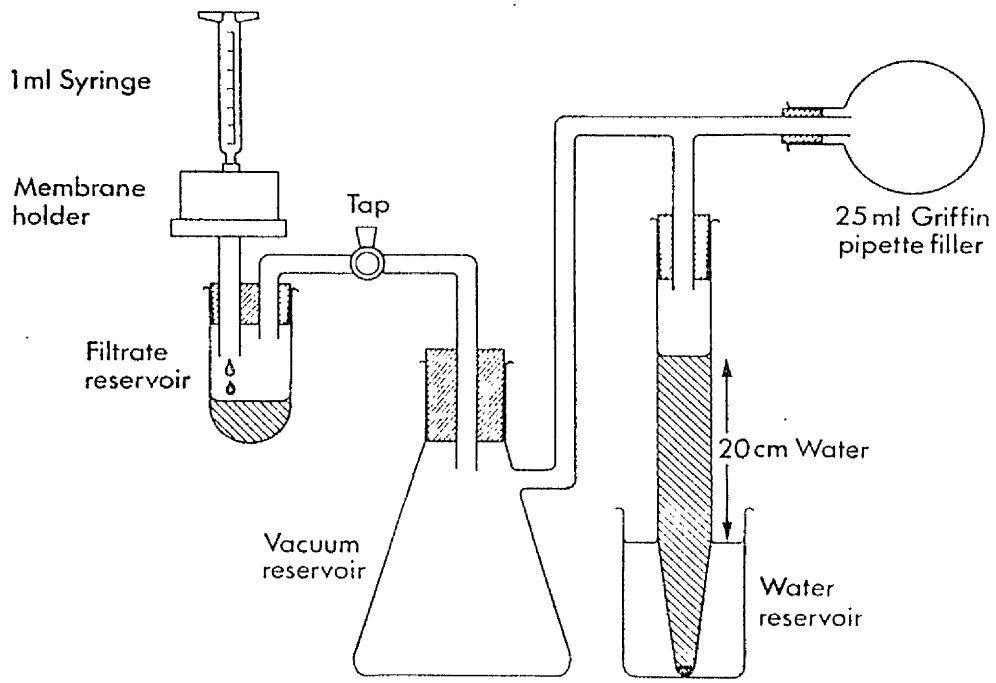

Figure 7 Diagrammatic Representation of the Apparatus for Measuring the Erythrocyte Deformability Index

the evaluation of the whole blood filtration method. Nuclepore 5 μm pore diameter filter membranes were used (Sterilin Ltd., Teddington, Middlesex).

Sample Handling

Venous blood was drawn into clean disposable syringes with minimal venous occlusion. Each sample was anticoagulated with EDTA. In the first section of experiments, whole blood was used and in the second section the samples were centrifuged at 2,000G for 15 minutes then the plasma was separated, the buffy coat aspirated and discarded and the erythrocytes resuspended in their native plasma to give a haematocrit of between 0.15 and 0.20. The sample was stored at room temperature until measurement, which was completed within two hours of venepuncture. Haematocrit and white blood cell counts were made on a Coulter Counter S Plus. Whole blood and plasma viscosity were measured at a shear stress of 5.7 dynes/cm^2 at 37°C using the Deer Rheometer as described in section iii, subsection 'a' of this chapter. Blood pH was measured and controlled as described in section iv, subsection i of this chapter. Plasma fibrinogen was measured by the method of Ratnoff and Menzie (1951) (79).

Erythrocyte filtration rate (EFR) was calculated as the volume of erythrocytes passing through a 5 μm filter in one minute under a perfusion pressure of 30 cm of water. Unless stated otherwise, the sample and apparatus were kept in an incubator at 37°C for 10 minutes prior to measurement in order to standardise the measurement temperature. Erythrocyte Deformability

Index (EDI) was calculated by correcting the Erythrocyte Filtration Rate for variations in plasma viscosity. From Figure 20 it can be seen that the Erythrocyte Filtration Rate has an inverse relationship with the plasma viscosity, the correlation coefficient of this relationship is -0.825, the slope is -0.091 and the standard error of the slope is 0.015. Taking the Erythrocyte Filtration Rate of healthy adult females as the standard and their plasma viscosity of 1.75 centipoise as standard, then the Erythrocyte Deformability Index of a sample 'A' using the following formula derived from the linear regression analysis of Erythrocyte Filtration Rate and plasma viscosity:

$$EDI(A) = EFR(A) - 0.091 \times \boxed{1.75 - \text{Plasma viscosity } (A)}$$

Scanning electron microscopy of the filters was done by the method described in section iv, subsection 'j' of this chapter.

Results

Section 1

Measurement delay had a significant effect on Erythrocyte Filtration Rate (Figure 8). At three and six hours after venepuncture, the mean Erythrocyte Filtration Rate was significantly reduced ($p < 0.001$).

The effect of varying the haematocrit of the erythrocyte suspensions is shown in Figure 9. The most stable measurements of Erythrocyte Filtration Rate were found at haematocrits between 0.10 and 0.30.

Altering plasma viscosity by either evaporation or dilution with normal saline varied the Erythrocyte Filtration Rate (Figure 10). Linear regression analysis on the data in Figure 10 gives a

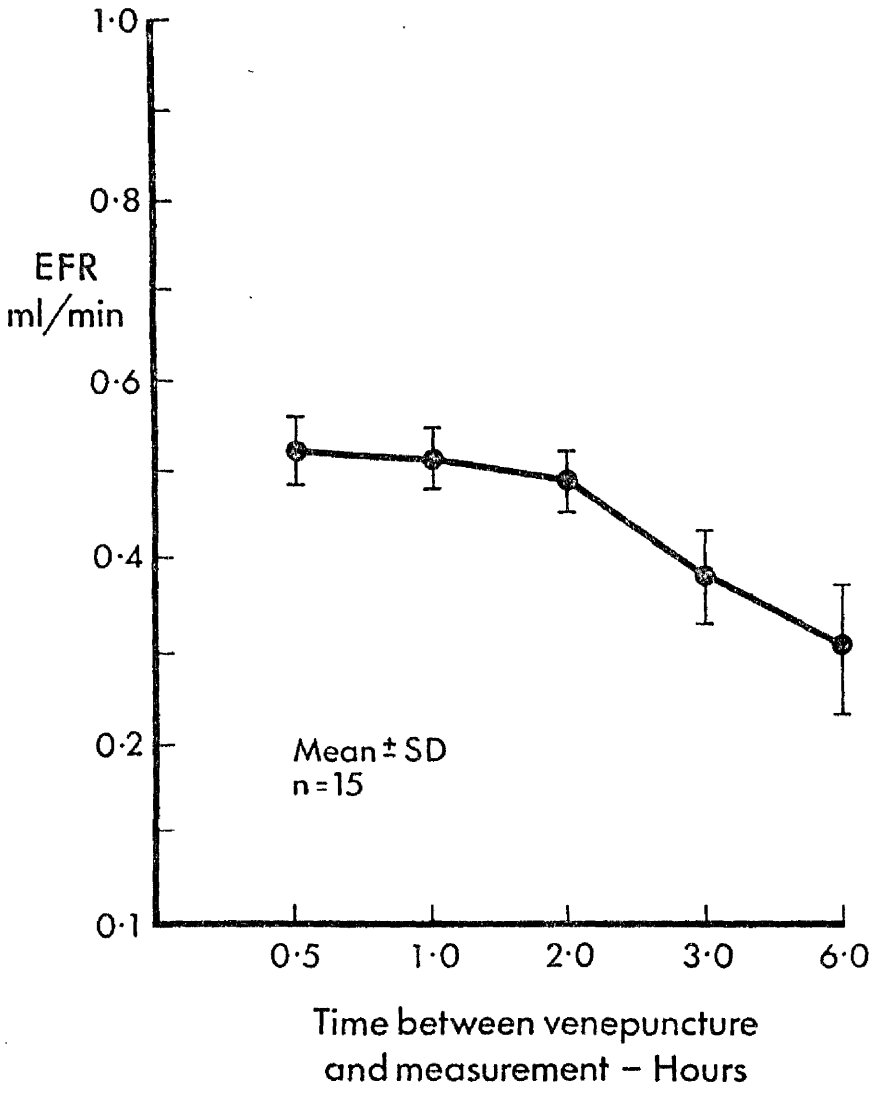

Figure 8 Effect of Measurement Delay on Erythrocyte Filtration Rate (EFR)

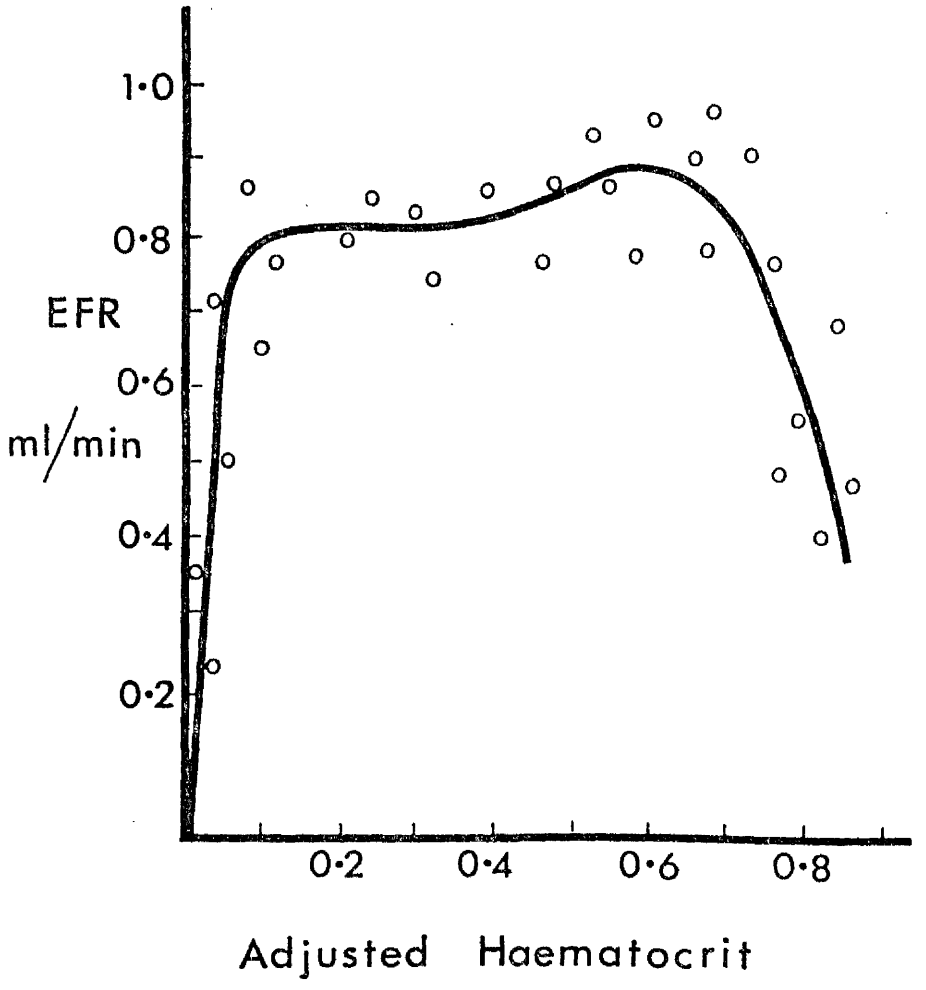

Figure 9 Effect of Variation in Haematocrit on Erythrocyte Filtration Rate (EFR)

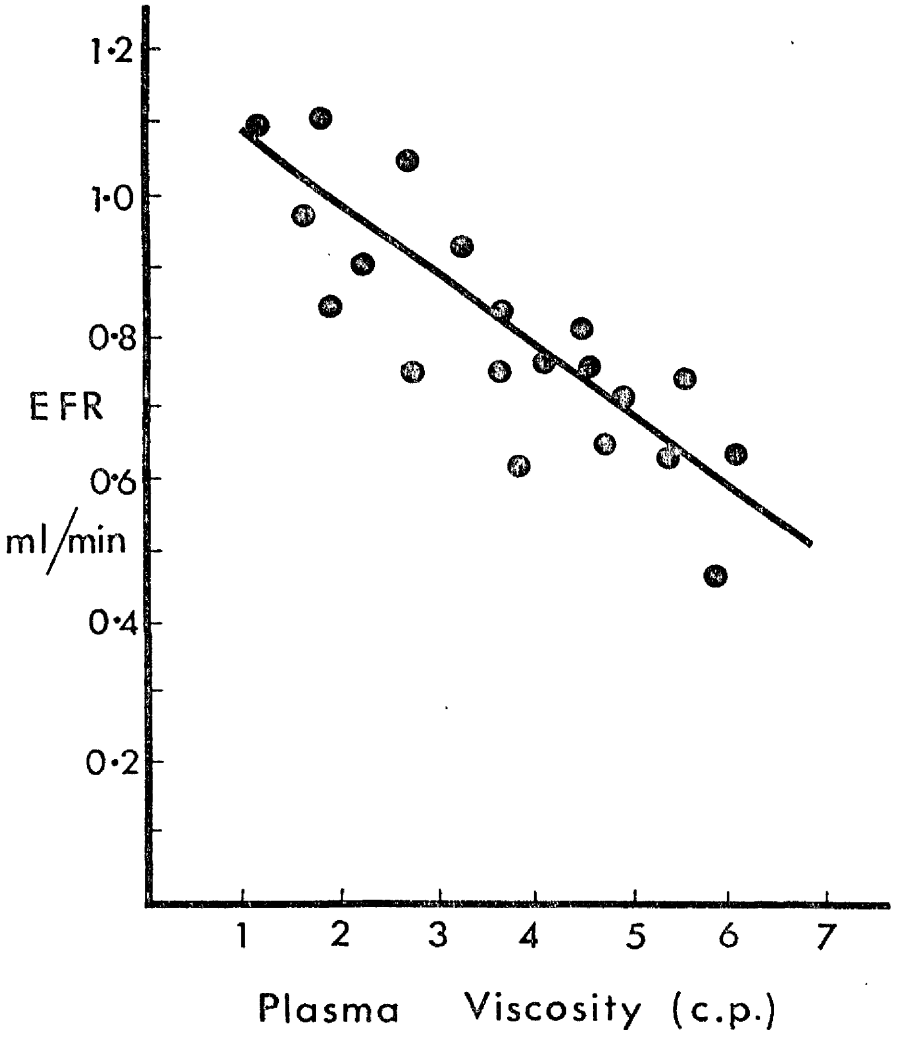

Figure 10 Effect of Variation in Plasma Viscosity on Erythrocyte Filtration Rate (EFR)

correlation coefficient of -0.825, a slope of -0.091 and an intercept of 1.149.

The duration of filtration also affected the Erythrocyte Filtration Rate (Figure 11). Using whole blood the volume of erythrocytes filtered in each successive 20 second period after 40 seconds was significantly lower than that filtered in the first two 20 second periods ($p < 0.001$). In order to investigate the reason for this reduction in Erythrocyte Filtration Rate, specimens of blood were examined before and after filtration. In ten samples the mean haematocrit before filtration was 0.380 and after was 0.379, indicating no significant loss of erythrocytes. The platelet count was not significantly altered by filtration. However, the mean white blood cell count fell from $11.6 \times 10^9/l$ before filtration to $10.2 \times 10^9/l$ after. Using a paired t-test this difference was significant ($p < 0.001$), indicating that white cells were being trapped in the filter and could be blocking the pores.

This was confirmed by examining the filters with a scanning electron microscope after filtration. Filters through which whole blood with a white blood cell count of $12.8 \times 10^9/l$ had been filtered for periods of 20, 40 and 60 seconds are shown in figures 12, 13 and 14 respectively. White cells can be seen progressively blocking more pores as the filtration time is increased. Figure 15 shows a higher power view of one pore blocked by six white cells with two erythrocytes unable to pass through. The effect of removing the white cells from the erythrocyte suspension is seen in Figure 11, with only a slight reduction in Erythrocyte Filtration Rate over prolonged filtration

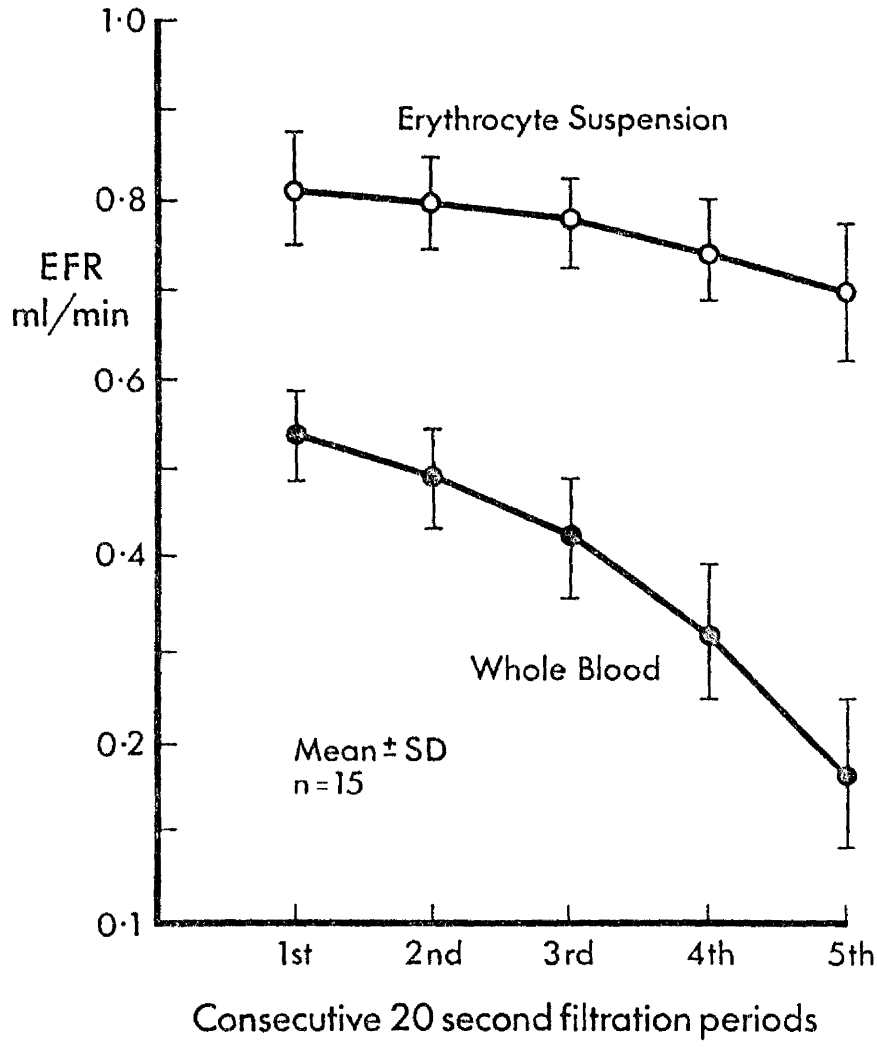

Figure 11 Effect of Variation in Filtration Duration on Erythrocyte Filtration Rate (EFR) of Whole Blood and Erythrocyte Suspension

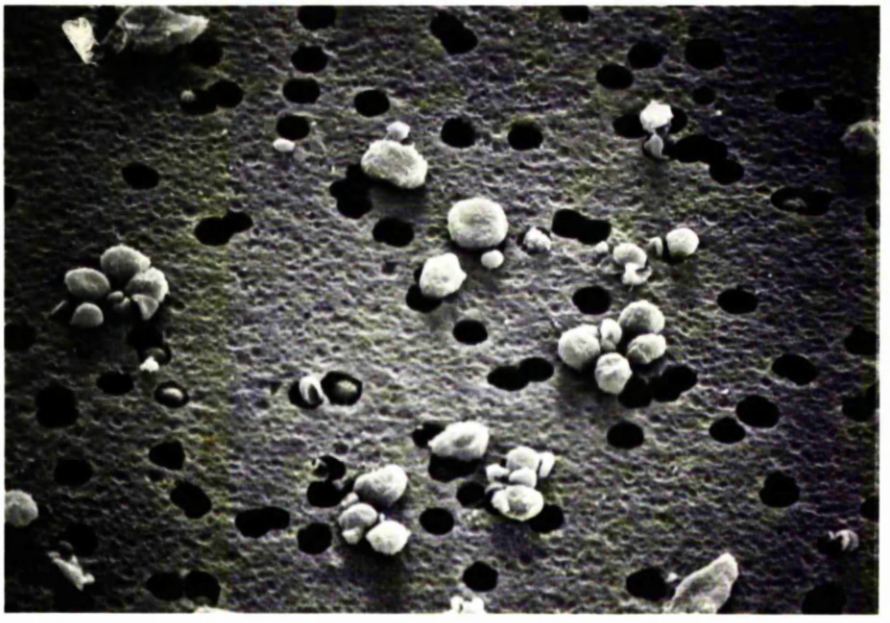

Figure 12 Photoelectronmicrograph x 1,000 of Nuclepore Filter Membrane After 20 Seconds of Whole Blood Filtration

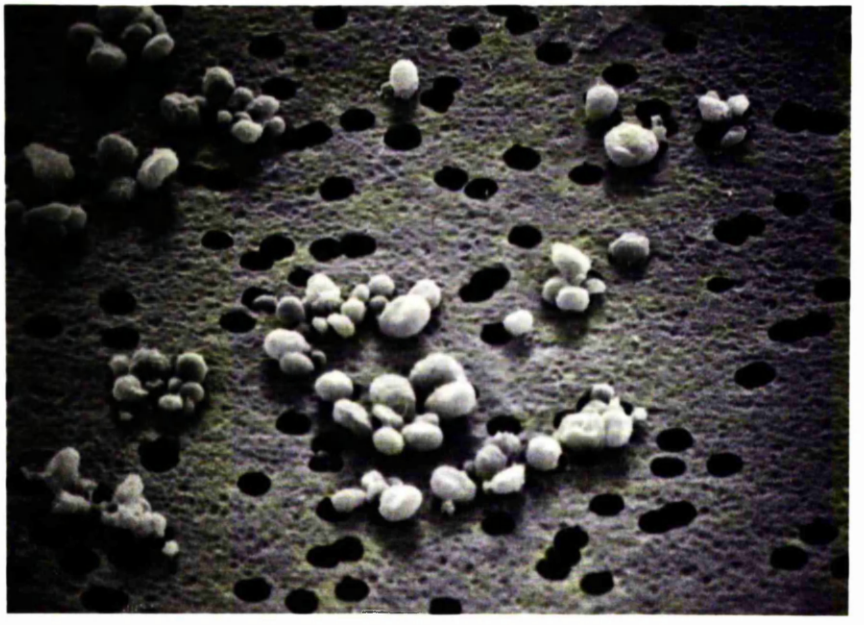

Figure 13 Photoelectronmicrograph x 1,000 of Nuclepore Filter Membrane After 40 Seconds of Whole Blood Filtration

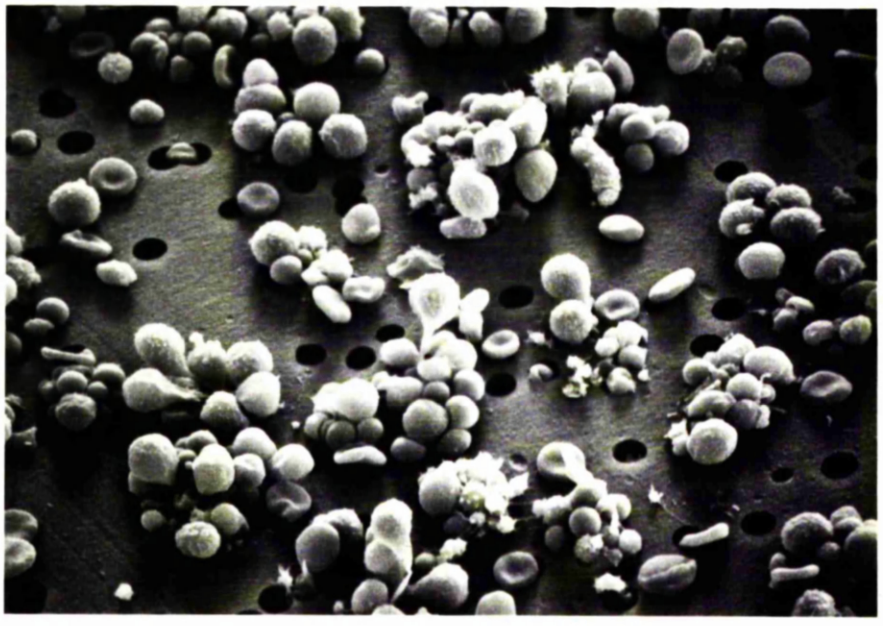

Figure 14 Photoelectronmicrograph x 1,000 of Nuclepore Filter Membrane After 60 Seconds of Whole Blood Filtration

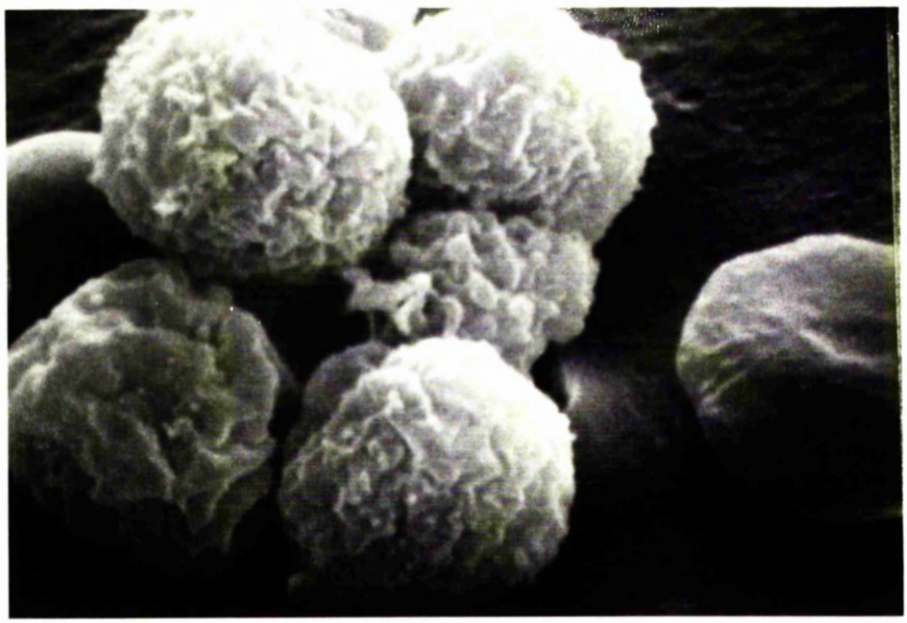

Figure 15 Photoelectronmicrograph x 7,000 of One Pore From Figure 14 Showing White Cells Blocking the Pore

periods. The reduction in white cell blockage of the pores was confirmed by scanning electron microscopic examination of the filters (Figure 16).

Section 2

Thirty-six repeat measurements were made on erythrocyte suspensions prepared from 50 ml of blood from a healthy volunteer. The mean Erythrocyte Filtration Rate was 0.82 ml/min, SD 0.06 ml/min and coefficient of variation 7.3 per cent. The mean plasma viscosity was 1.72 centipoise, SD 0.12 and coefficient of variation 7.0 per cent. The mean Erythrocyte Deformability Index was therefore 0.82, SD 0.06 and coefficient of variation 7.3 per cent.

Reducing the temperature at which measurements were made from $37^{o}C$ to $20^{o}C$ resulted in a significant fall in Erythrocyte Filtration Rate from 0.79 ml/min to 0.63 ml/min ($p < 0.01$), and a significant rise ($p < 0.001$) in the plasma viscosity from 2.0 centipoise to 3.8 centipoise (Figure 17). The Erythrocyte Deformability Index, however, showed no significant change with temperature.

The effect of altering the sample pH is shown in Figure 18. With a reduction in pH both the Erythrocyte Filtration Rate and the Erythrocyte Deformability Index decreased significantly. This was not due to changes in the plasma viscosity which was only altered once the fall in pH went beyond 6.9 when the proteins started to be precipitated.

Discussion

As an increasing number of diseases are shown to be associated

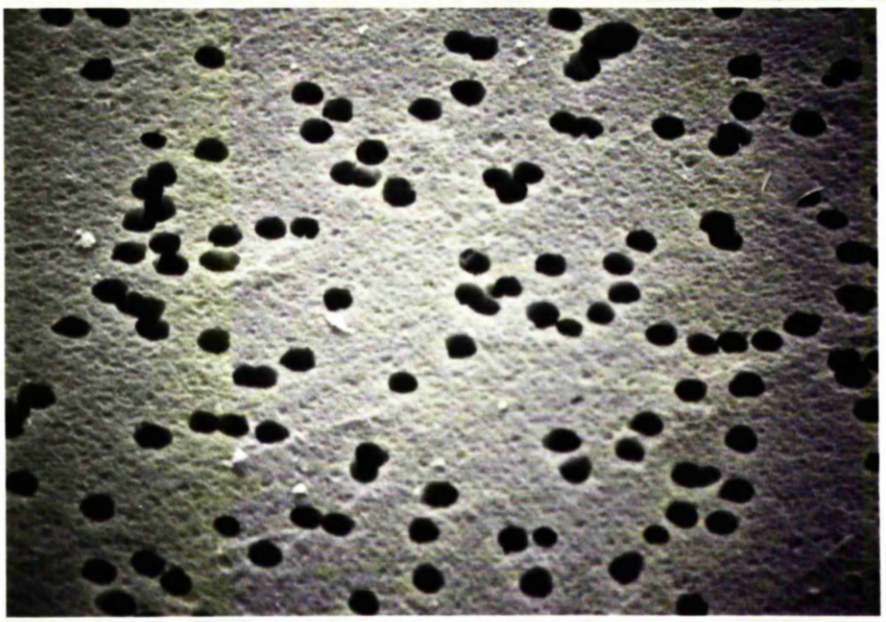

Figure 16 Photoelectronmicrograph x 1,000 of Nuclepore Filter Membrane After 60 Seconds of Erythrocyte Suspension Filtration

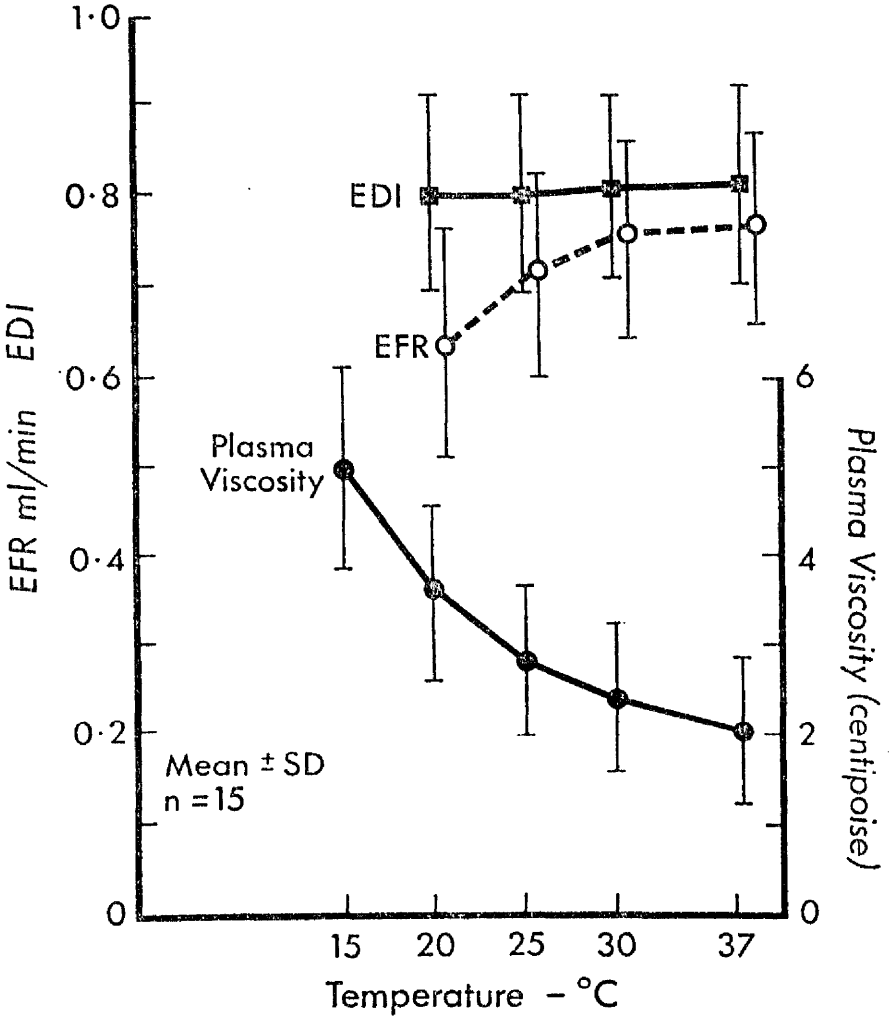

Figure 17 The Effect of Variation of Measurement Temperature on Plasma Viscosity, Erythrocyte Filtration Rate (EFR) and Erythrocyte Deformability Index (EDI)

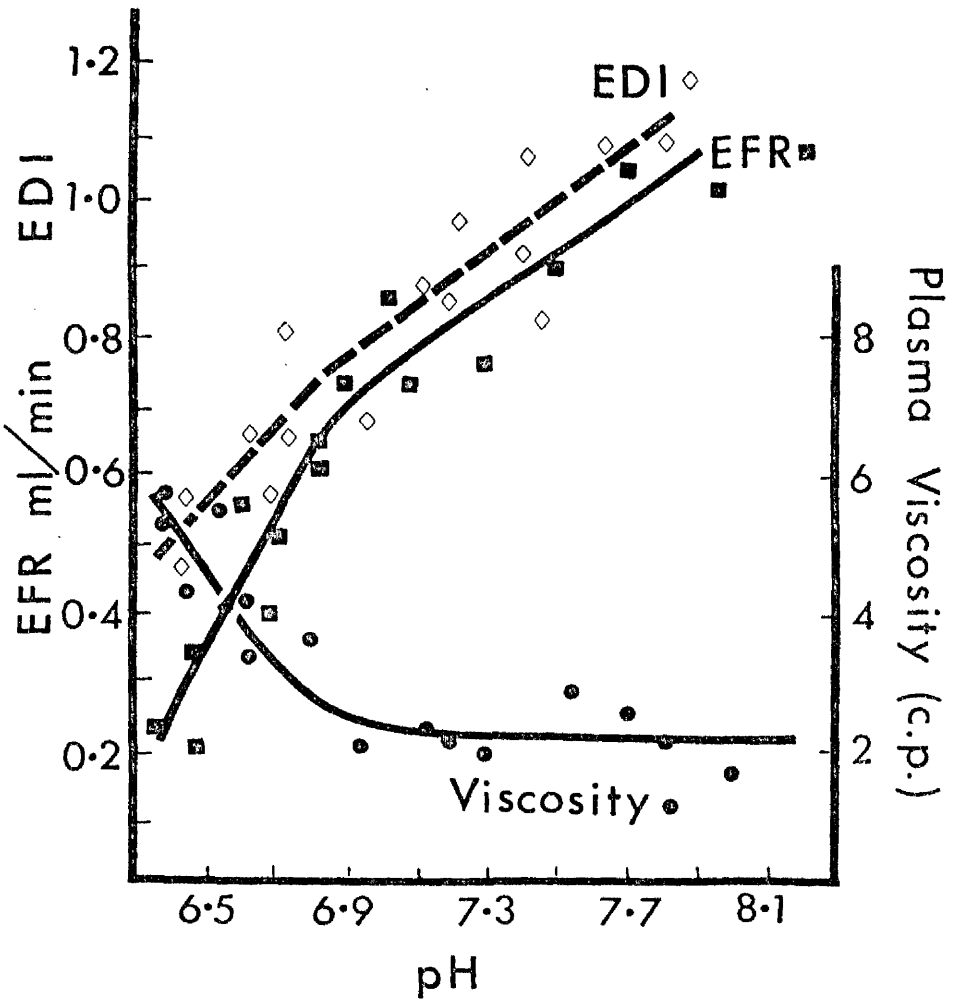

Figure 18 The Effect of Variation in Sample pH Level on Plasma Viscosity, Erythrocyte Filtration Rate (EFR) and Erythrocyte Deformability Index (EDI)

with decreased erythrocyte deformability (80) and this parameter is more frequently measured, so it becomes increasingly important to evaluate the methods of measurement.

In this study, six variables were identified which affect the measurement of erythrocyte deformability using the method of Reid et al (1976) (78). The importance of avoiding measurement delay beyond two hours after venepuncture has been demonstrated and the significant effect of pore blockage by white cells clearly shown. In many pathological states, in pregnancy and in the newborn, the white blood cell count may be considerably elevated and the measurement of erythrocyte deformability by whole blood filtration would give erroneously low deformability values. The importance of adjusting haematocrit to between 0.15 and 0.35 is clearly seen in Figure 9. This has also been found necessary by other workers (75,76), although Reid et al (1976) (78) found their deformability measurement to be independent of haematocrit between 0.20 and 0.70. Using a suspension of erythrocytes, free of white cells, with a haematocrit of between 0.15 and 0.35 gives a perfusate very similar to that found in normal capillaries where, because of the effects of plasma skimming (81), the haematocrit is low. Plasma viscosity affects the filtration rate and must be corrected for when measuring erythrocyte deformability by a filtration technique (75). Reid et al (1976) (31), in the clinical evaluation of their deformability measurement, ignored any effect of differences in plasma viscosity between their groups of patients, and this alone would have accounted for the finding of an apparently low erythrocyte deformability in their pathological group.

The independence of erythrocyte deformability from temperature variation shown in Figure 17 has previously been reported by Schmid-Schonbein et al (1973) (75), who found deformability to be independent of temperature between 4 and 40°C. Although in this study all measurements of erythrocyte deformability were made at 37°C, provided that the Erythrocyte Filtration Rate and the plasma viscosity are measured at the same temperature, there should be no variation in the Erythrocyte Deformability Index result, and this simplifies the technique for measurement of Erythrocyte Filtration Rate which is most easily carried out at room temperature.

The effect of pH on erythrocyte deformability is well documented (16,82) and the results of this study confirm these findings and show the sensitivity of this method in detecting changes in erythrocyte deformability.

The few studies of erythrocyte deformability in pregnancy, in the fetus and in oral contraceptive users, will be reviewed in the appropriate clinical sections of the thesis.

iv) STANDARD METHODS USED IN THE STUDIES

a) Haematocrit

In the studies on pregnant and non-pregnant adult women, haematocrit was measured on samples anticoagulated with ethylenediaminotetra-acetic acid (EDTA) 1.5 mg/ml, using a Coulter Counter S Plus. In the studies on fetal blood haematocrit was measured using a Hawksley Microhaematocrit centrifuge. The samples were spun at 15,000 G for 7 minutes.

b) Platelet and White Blood Cell Counts

Platelet and white blood cell counts were made using a Coulter Counter

S Plus and samples anticoagulated with EDTA.

c) Plasma Fibrinogen

In the studies on non-pregnant women and on fetal blood, the plasma fibrinogen was estimated by the method of Ratnoff and Menzie (1951) (79). In the pregnancy studies the method of Ellis and Stransky (1961) (83) was chosen because it is less time-consuming and the number of samples was considerable.

d) Plasma Lactate Dehydrogenase

Plasma lactate dehydrogenase was measured by the method of Wroblewski and La Due (1955) (84).

e) Plasma Bilirubin

Plasma bilirubin was measured autoanalytically by the method of Gambio and Schreiber (1964) (85).

f) Plasma Haptoglobin

Plasma haptoglobin was measured by radial immunodiffusion using M-Partigen plates supplied by Behring Diagnostics.

g) Serum Albumin and Total Protein

Total protein was measured by the biuret method of Henry, Sobel and Berkman (1957) (86), as was albumin after initial separation by the salt fractionation technique of Wolfson et al (1948) (87).

h) Plasma Osmolality

Plasma osmolality was estimated by freezing point depression with an Advanced Osmometer, using 0.2 ml samples.

i) Blood pH

Blood pH was measured on an AVL Gas Check 937-C (Sandoz). The pH of samples in the in vitro experiments was varied by equilibrating the sample in a stoppered tube with varying

concentrations of carbon dioxide and oxygen.

j) Scanning Electron Microscopy

Scanning electron microscopy of the filters was performed using an ISI Mini-Sem Scanning Electron Microscope, after the filter had been fixed in gluteraldehyde, dehydrated in acetone, critical point dried and gold plated in a Polaron SEM Coating Unit. Photomicrographs were obtained with a Polaroid Camera.

k) Technical Assistance

All measurements of erythrocyte deformability, whole blood and plasma viscosity, plasma osmolality, blood pH, haematocrit using the Hawksley Microhaematocrit and plasma haptoglobin and the scanning electron microscopy were carried out in the Department of Obstetrics and Gynaecology of St. James's University Hospital by me. All the other estimations were made by the technical staff of the Departments of Clinical Chemistry and Haematology in the hospital.

CHAPTER 3

HAEMORHEOLOGICAL PROFILE OF THE NON-PREGNANT ADULT FEMALE AND THE EFFECTS OF THE ORAL CONTRACEPTIVE PILL

Sections

i) MENSTRUAL CYCLE VARIATIONS IN HAEMORHEOLOGICAL PARAMETERS

ii) EFFECT OF THE ORAL CONTRACEPTIVE PILL ON HAEMORHEOLOGICAL PARAMETERS

HAEMORHEOLOGICAL PROFILE OF THE NON-PREGNANT ADULT FEMALE AND THE EFFECT OF THE ORAL CONTRACEPTIVE PILL

i) MENSTRUAL CYCLE VARIATIONS IN HAEMORHEOLOGICAL PARAMETERS

In common with most biological measurements, rheological parameters vary in response to the physiological rhythms of the body. Whole blood viscosity, haematocrit and plasma protein levels show clear circadian variations (88), as do other haematological parameters (89).

The study reported in this section concerns the haemorheological changes throughout the spontaneous menstrual cycle in a series of healthy women.

Patients and Methods

Blood samples were collected from 12 healthy young women aged between 22 and 34 years, none of whom smoked cigarettes or was taking any medication. Blood samples were taken, at the same time each day in order to avoid any circadian variation between samples, on four occasions a week apart. The haemorheological measurements were by the methods described in Chapter 2.

Results

The results in this Chapter are presented figuratively against a diagrammatic representation of the oestrogen-progesterone profile of the menstrual cycle.

The Erythrocyte Deformability Index (Figure 19) remained stable in the follicular phase of the cycle and rose to a peak in the mid luteal phase, falling off towards the onset of menstruation.

Plasma fibrinogen (Figure 20) remained stable throughout the follicular and early luteal phases, rising to a peak just prior

to the onset of menstruation and then falling off during the menses.

Plasma viscosity (Figure 20) fell by an insignificant amount during the follicular and early luteal phases. There was then a significant rise towards the time of menstruation.

Haematocrit (Packed Cell Volume) rose to a peak at ovulation and then remained relatively constant until the onset of menstruation when it fell sharply (Figure 21).

Whole blood viscosity showed a slight, statistically insignificant, peak at ovulation and then a second and significant peak prior to the onset of menstruation with a sharp fall during the menses (Figure 21).

Discussion

In a study on haem catabolism during the menstrual cycle, Mercke and Lundh (1976) (90) reported that erythrocyte filterability was significantly depressed during the luteal phase, compared with the follicular phase of the cycle. Although confirming a decrease in the luteal phase, the present study suggests that this is a reaction from a postovulatory rise rather than a continuation of a fall from higher preovulatory levels. Although both studies estimate erythrocyte deformability by microfiltration, Mercke and Lundh (1976) (90) used erythrocytes washed and suspended in artificial medium, whereas in the present study, deformability was measured with erythrocytes suspended in their native plasma.

The plasma constituents exert a considerable effect on erythrocyte deformability (91), as is shown in Chapter 5, section i, and this may explain the difference in the two studies.

Variation in plasma fibrinogen level, with a peak at ovulation

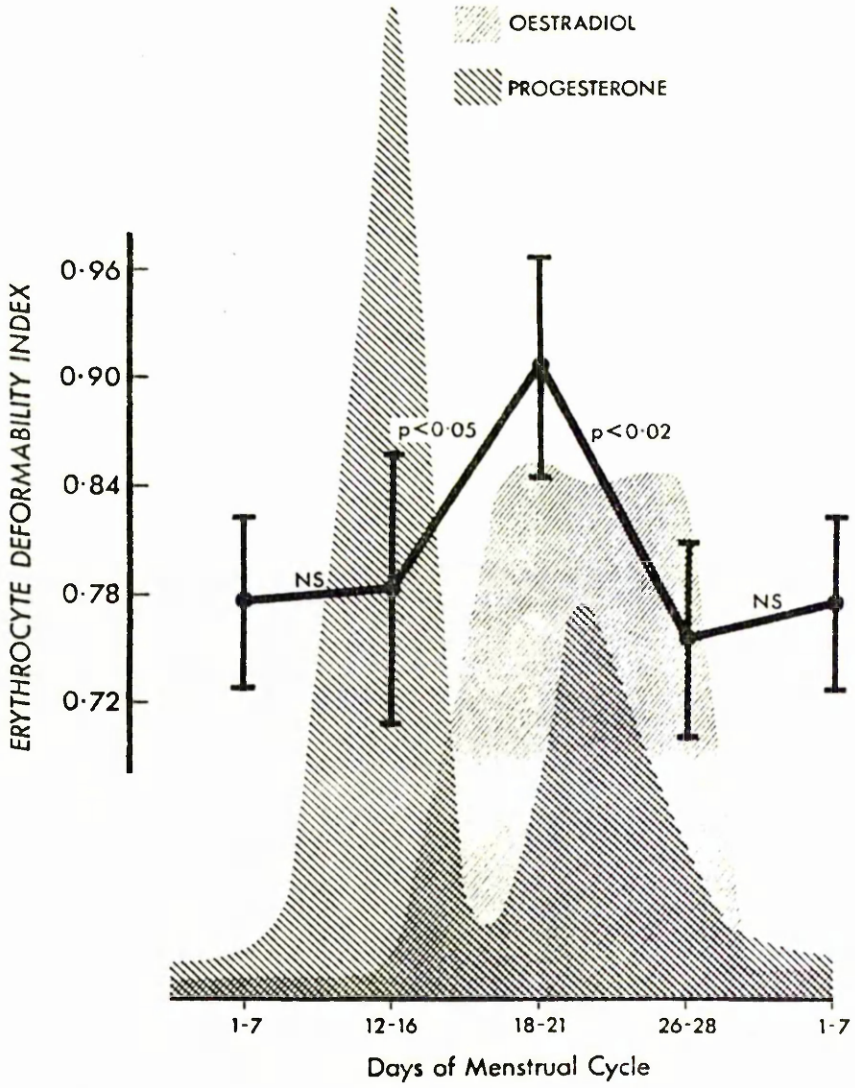

Figure 19 Erythrocyte Deformability Index During the Normal Menstrual Cycle Against a Diagrammatic Background of the Hormone Profile of the Normal Cycle

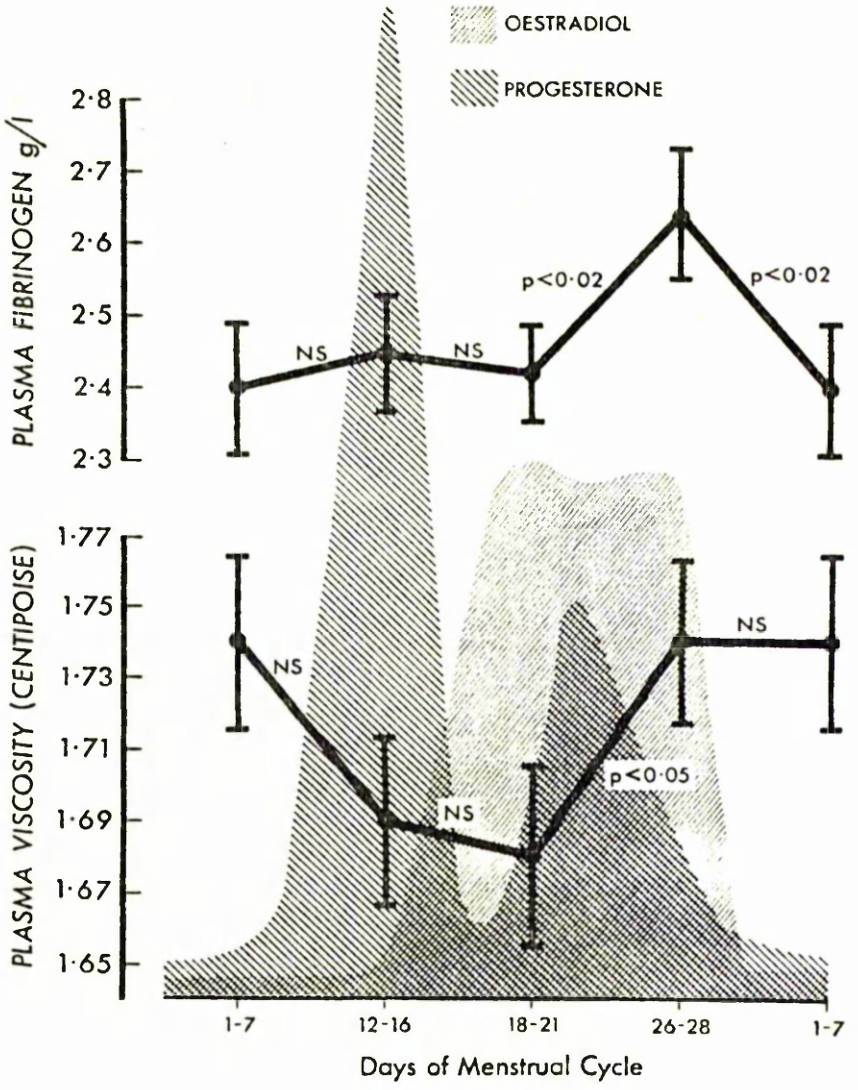

Figure 20 Plasma Fibrinogen Level and Plasma Viscosity During the Normal Menstrual Cycle Against a Diagrammatic Background of the Hormone Profile of the Normal Cycle

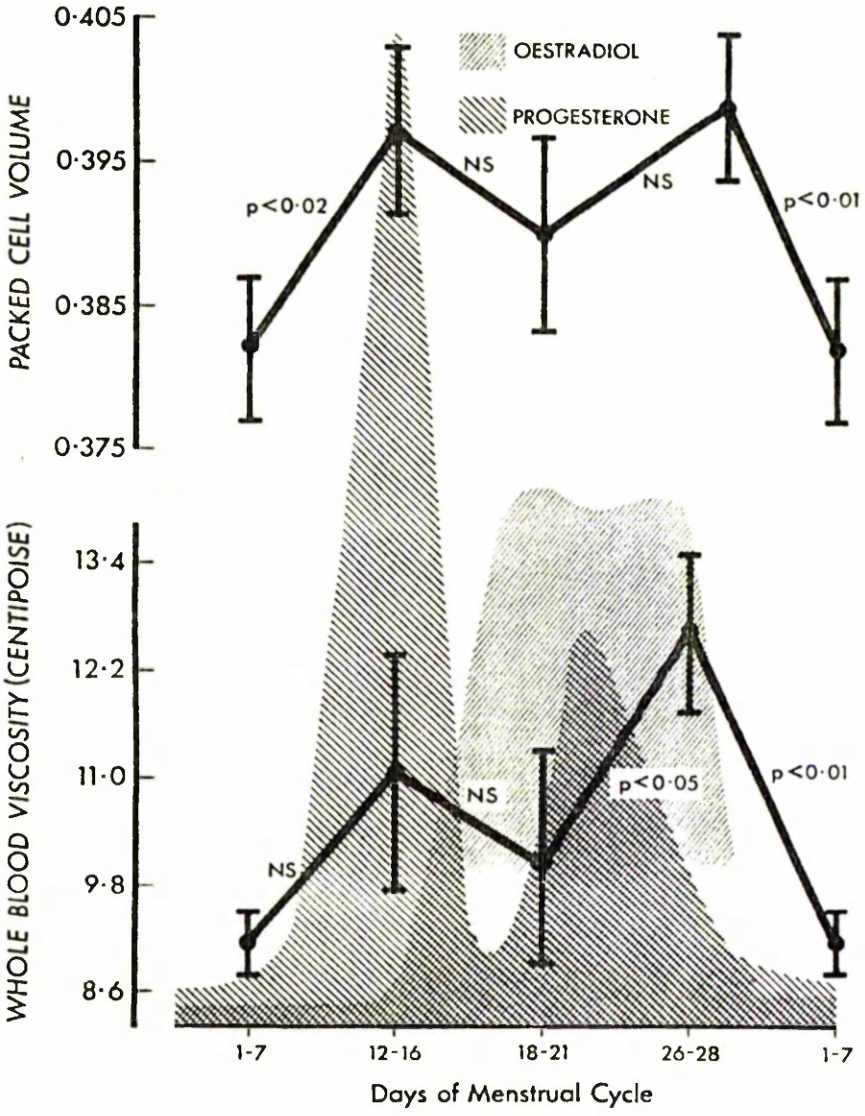

Figure 21 Haematocrit (Packed Cell Volume) and Whole Blood Viscosity During the Normal Menstrual Cycle Against a Diagrammatic Background of the Hormone Profile of the Normal Cycle

and a secondary rise prior to menstruation, has been reported by some (92), but this finding has not been confirmed by others (93,94). The trend in the present study was in agreement with that described by Howie et al (1970) (93), although their pre-menstrual rise failed to reach statistical significance. Other individual coagulation factors do not appear to vary with the menstrual cycle (93,94,95).

There is no published study of plasma viscosity throughout the menstrual cycle, so these findings require confirmation, however, the trend in plasma viscosity does follow the general trend of the plasma fibrinogen, its main determinant, in the premenstrual rise, but does not show the postmenstrual decline in plasma fibrinogen. The factors responsible for the maintenance of the plasma viscosity in the postmenstrual phase is not known.

There is agreement in the literature that haematocrit falls during menstruation and then rises to a peak after ovulation (96,97). The variations in haematocrit are mirrored by the whole blood viscosity.

The two other published studies of whole blood viscosity in the menstrual cycle, both by Dintenfass (98,99), described a pre-menstrual rise which was reversed during menstruation with little subsequent change, similar to the present study. The trend in whole blood viscosity matches that of both plasma fibrinogen and haematocrit, which are the two main determinants of whole blood viscosity (9), and reciprocates the changes in erythrocyte deformability with the lowest Erythrocyte Deformability Index coinciding with the highest whole blood viscosity.

ii) EFFECT OF THE ORAL CONTRACEPTIVE PILL ON HAEMORHEOLOGICAL

PARAMETERS

Because of the strong connection between arterio-venous thrombo-embolism and oral contraception (100), much interest has been focussed on the alterations in rheological parameters caused by hormonal ovulation suppression (47,48,101-103). In very few studies has any attention been paid to the physiological back-ground against which oral contraceptives exert their effect and in the following studies comparison will be made with the changes in the normal ovulatory menstrual cycle described in the previous section.

Patients and Methods

Two groups of healthy women aged between 22 and 34, none of whom smoked cigarettes or took medication other than an oral contraceptive preparation containing 30 μg ethinyloestradiol and 250 μg norgestrel. All subjects had been taking this preparation for at least six months prior to the study. The first group of eight subjects donated blood at the same time of day on four occasions a week apart during their cycle. The second group of 30 volunteers donated one blood sample between the seventh and twenty-first day of their cycle. All haemorheological measurements were made as described in Chapter 2.

Results

The results in this section are portrayed graphically in Figures 22-24 against a diagrammatic representation of the oestrogen-progestagen profile of the 'Pill cycle'.

Erythrocyte deformability (Figure 22) fell over the first few days of contraceptive steroid therapy and then rose to a peak at the onset of menstruation.

Plasma fibrinogen remained stable throughout the period on

treatment and then fell by a small but significant amount during the time off therapy (Figure 23).

Plasma viscosity mirrored the changes in fibrinogen but none of the variations were statistically significant (Figure 23).

Haematocrit (Packed Cell Volume) did not alter significantly throughout the ovulation suppressed cycles (Figure 24), nor did the whole blood viscosity (Figure 24).

In the cross-sectional study, comparison is made in Table 2 between the 30 contraceptive pill 'Users' and the different stages of the menstrual cycle in the 'Non-users'.

The level of erythrocyte deformability found in the pill 'Users' was significantly lower than the luteal phase peak noted in the normal menstrual cycle, but was not different from the levels at other stages.

Plasma fibrinogen was significantly higher in pill 'Users' than at each stage of the normal menstrual cycle except the premenstrual phase when the levels were not significantly different.

Plasma viscosity was significantly elevated in 'Users' when compared with the pre- and postovulatory phases of the normal cycle. At both the pre- and postmenstrual phases there was no significant difference in pill 'Users'.

The haematocrit was significantly higher in pill 'Users' than in the normal cycle at every stage examined.

The whole blood viscosity was not significantly different when compared with the ovulatory or premenstrual stages, but was significantly elevated when compared with the postmenstrual and luteal stages.

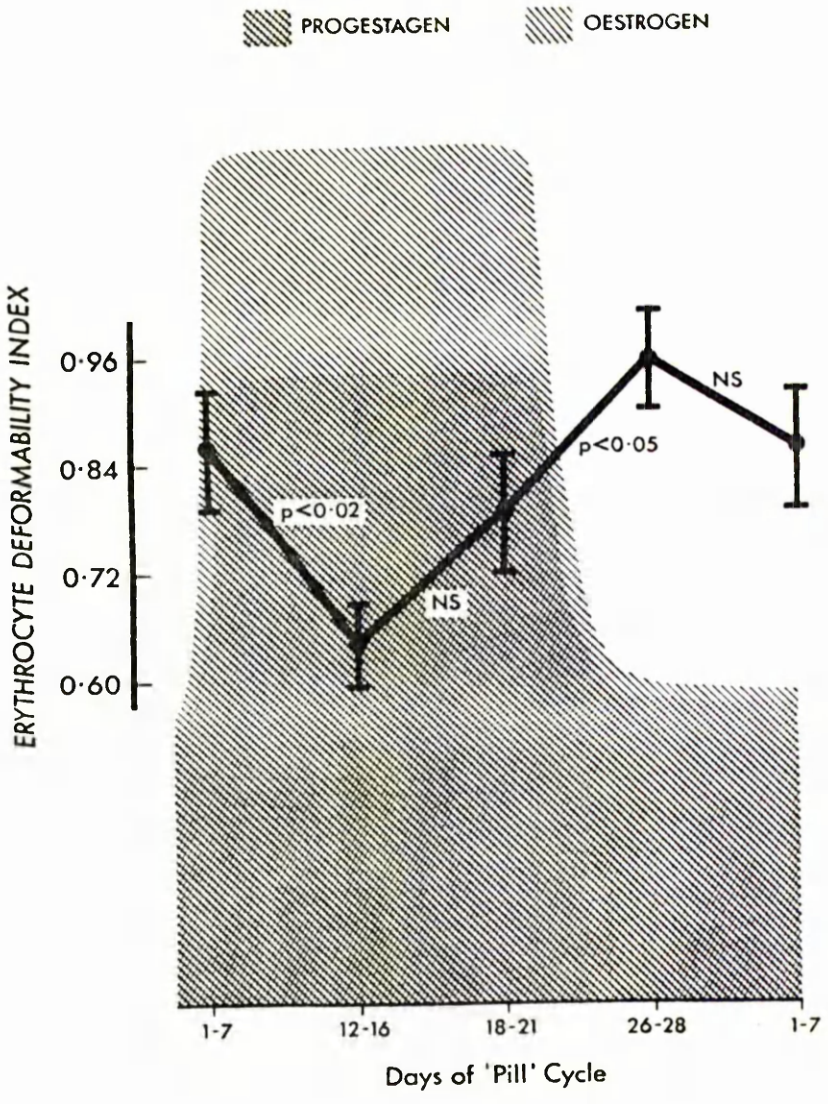

Figure 22 Erythrocyte Deformability Index During the Contraceptive Pill Cycle Against a Diagrammatic Background of the Hormonal Profile of the Pill Cycle

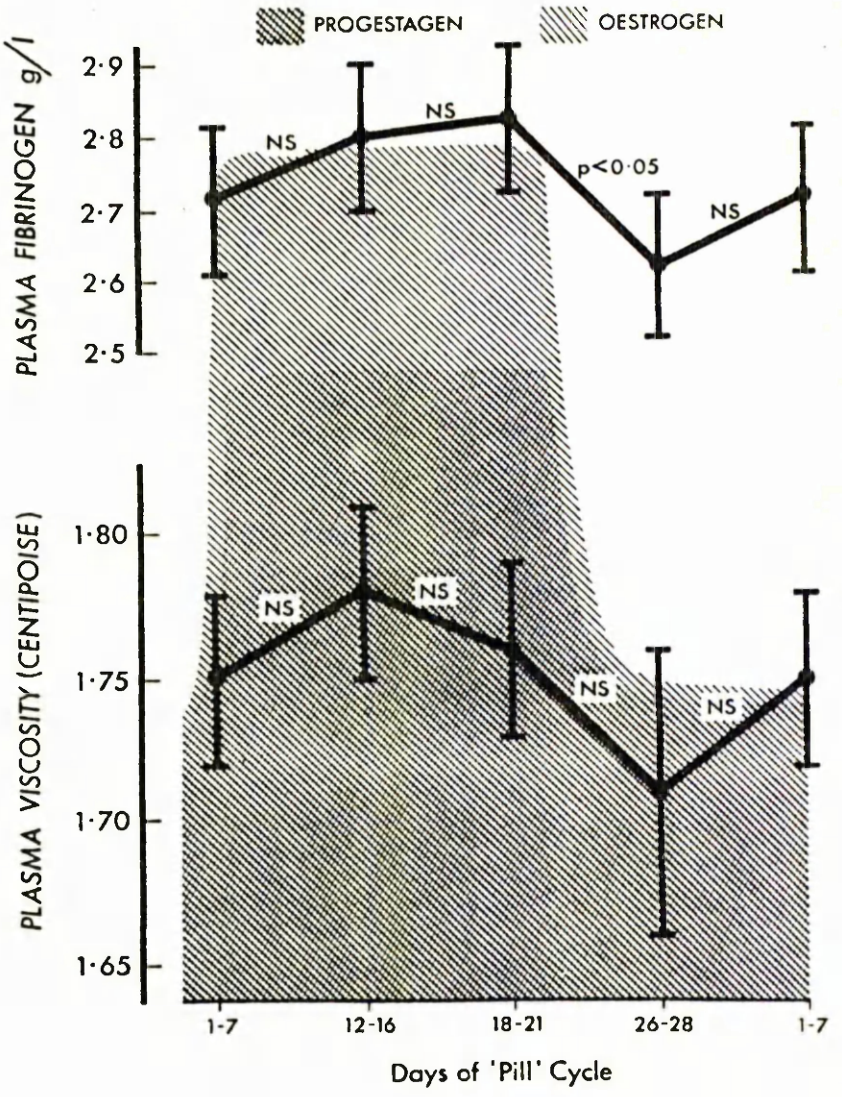

Figure 23 Plasma Fibrinogen Level and Plasma Viscosity During the Contraceptive Pill Cycle Against a Diagrammatic Background of the Hormonal Profile of the Pill Cycle

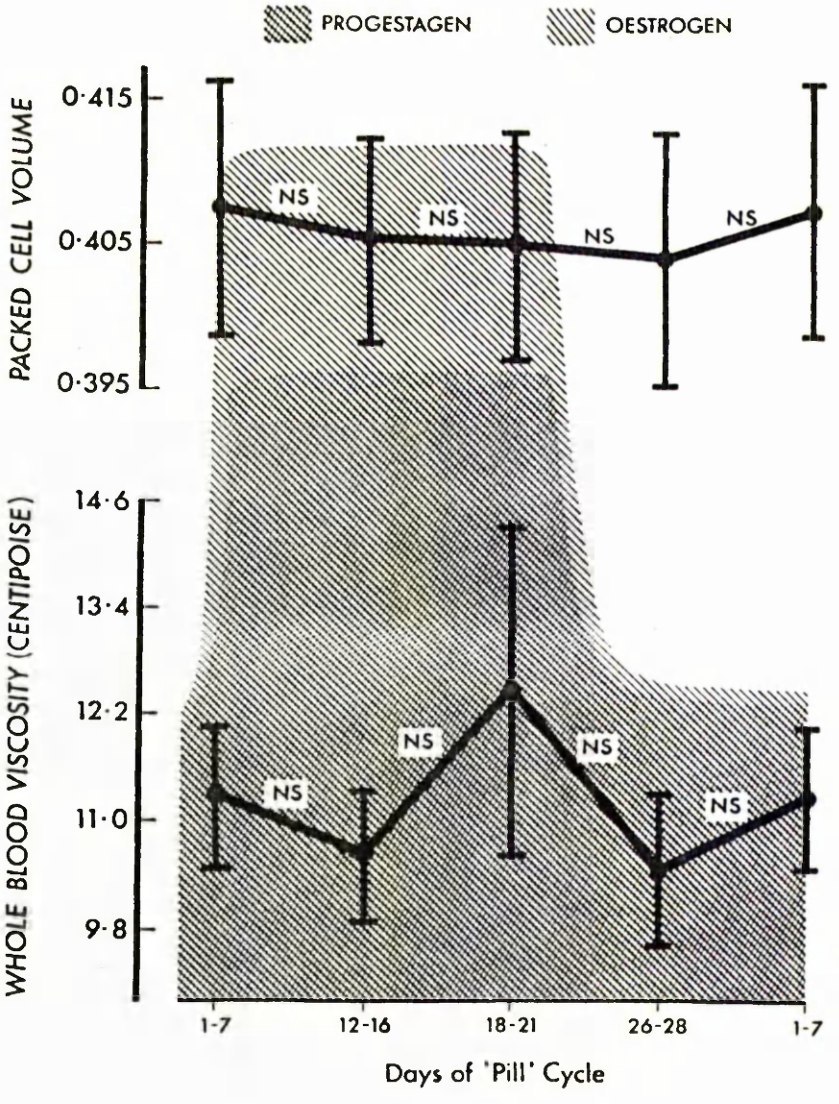

Figure 24 Haematocrit (Packed Cell Volume) and Whole Blood Viscosity During the Contraceptive Pill Cycle Against a Diagrammatic Background of the Hormonal Profile of the Pill Cycle

Table 2 Mean Values of Haemorheological Parameters in 'Users' and 'Non-users' of Oral Contraceptives

	12 'Non-users'				30 'Users'
Days of Cycle	1 - 7	12 - 16	18 - 21	26 - 28	7 - 21
Erythrocyte Deformability Index	0.776^{ns}	0.783^{ns}	0.907^{*}	0.756^{ns}	0.772
Standard Error	0.047	0.075	0.007	0.054	0.092
Plasma Fibrinogen (g/l)	2.40^{*}	2.45^{**}	2.35^{**}	2.64^{ns}	2.79
Standard Error	0.09	0.08	0.08	0.10	0.17
Plasma Viscosity (centipoise)	1.74^{ns}	1.69^{*}	1.68^{*}	1.74^{ns}	1.77
Standard Error	0.024	0.023	0.025	0.023	0.022
Haematocrit	0.382^{**}	0.397^{*}	0.390^{**}	0.399^{*}	0.417
Standard Error	0.005	0.006	0.007	0.005	0.008
Whole Blood Viscosity (centipoise)	9.13^{**}	11.05^{ns}	10.01^{*}	12.63^{ns}	11.90
Standard Error	0.375	1.23	1.17	0.88	1.210

Comparison of 30 'Users' with the 'Non-users' at each stage of cycle using Student's t-test. ns = $p > 0.05$ * = $p < 0.05$ ** = $p < 0.01$

Discussion

Erythrocyte deformability has been reported as being reduced in oral contraceptive users (104), although two other studies found no change (102,105). The present study found erythrocyte deformability to be reduced in pill users when compared with the luteal phase of the spontaneous cycle, but in the serial study of pill users (Figure 22), it is seen that the erythrocyte deformability falls during the first ten days on therapy and then remains stable until the contraceptive therapy is stopped when it rises to a peak prior to the onset of menstruation. This is, therefore, in agreement with the work of Oski et al (1972) (104) and the reasons for the contention in the other studies (102,105) may be due to irregularities in sample timing in controls.

Several groups have reported an elevation of plasma fibrinogen levels associated with oral contraceptives (106,107,108) and a similar effect is apparent in the present study when compared with all stages of the normal cycle, except the immediately pre-menstrual stage. Low oestrogen preparations (30 μg ethinyloestradiol) are reported as having less effect of clotting factors than higher doses (109). Progestagen only preparations have been thought by some to have little or no effect on blood clotting factors (110), but others (109) have suggested that when combined with oestrogen, progestagens may cause some potentiation of the oestrogen effect. In the menstrual study, Figure 20, the plasma fibrinogen only rose in the luteal phase with the introduction of progesterone, thus giving support to this latter suggestion.

Oral contraceptives abolished the menstrual variation in plasma viscosity and raised the absolute level in pill users when

compared with the pre- and postovulatory stages. This is due to the elevated plasma fibrinogen at these stages. Two other studies (102,103) of plasma viscosity during oral contraception found no change, although no details of control sample timing were given.

Haematocrit showed no variation during the pill cycle and the level in contraceptive users was higher than at all stages of the normal cycle, in agreement with other studies (101,103). This elevation of haematocrit in oral contraceptive users is due to both a reduced menstrual loss and a stimulatory effect on haemopoiesis, as is seen in the spontaneous menstrual cycle in the luteal phase (Figure 21) and in pregnancy.

The combined action of the elevated haematocrit, fibrinogen and plasma viscosity combined with the reduced erythrocyte deformability results in the elevation of the whole blood viscosity in contraceptive users. Other workers (101,102,103) have reported this increased whole blood viscosity and related it to an increased incidence of venous thrombosis in oral contraceptive users. Certainly hyperviscosity predisposes to postoperative thrombo-embolism (45,46) and cigarette smoking, which increases the risk of thrombo-embolism in oral contraceptive users also increases haematocrit and other viscosity factors (111,112).

It is interesting to note, however, that with the exception of haematocrit, none of the haemorheological factors examined in this study were elevated out with the range found in the normal menstrual cycle. The most striking effect of oral contraceptives is the abolition of the normal menstrual variability and this loss of variation may be important in the aetiology of thrombo-embolism.

CHAPTER 4

THE HAEMORHEOLOGICAL PROFILE OF NORMAL PREGNANCY

Sections

i) INTRODUCTION

ii) SERIAL STUDY THROUGHOUT PREGNANCY

iii) COMPARISON OF PRIMIGRAVIDAE AND MULTIPARAE

iv) DISCUSSION

HAEMORHEOLOGICAL PROFILE OF NORMAL PREGNANCY

i) INTRODUCTION

Pregnancy presents a unique challenge to maternal cardiovascular physiology and the changes which take place during pregnancy are among the most extreme alterations occurring in non-pathological states. Not only is the blood flow to many, if not all, maternal organs increased, but a virtually new vascular bed is developed which demands, over an extended period, up to 15 per cent of the cardiac output. In the non-pregnant state the uterine blood flow is in the order of 20 ml per minute (113). The pregnant uterus at term has a blood flow of between 500 and 750 ml per minute (113). To cope with the increased demands of pregnancy the cardiac output rises from the non-pregnant level of around five litres per minute to between 6.1 and 6.8 litres a minute by 12 weeks, this level being maintained until term (114,115) and the blood volume increases by 30 per cent above non-pregnant levels (116). This rise in cardiac output might be expected to lead to an increase in mean arterial pressure but, in fact, the mean arterial pressure falls in the first trimester of pregnancy below non-pregnant levels and then it rises in the third trimester towards term (117,118). This fall in mean arterial pressure in the face of increased cardiac output indicates a significant fall in the total peripheral resistance. Bader et al (1955) (119) showed, in catheterisation studies, that the total peripheral resistance was 1,250 dyne sec cm $^{-5}$ in the non-pregnant state and this fell to 986 dyne sec cm $^{-5}$ by 14 to 24 weeks and then rose progressively towards a normal non-pregnant figure of 1,250 dyne sec cm $^{-5}$ at term. These findings have been confirmed by other workers (114).

As was explained in the section in Chapter 1 dealing with the control

of the peripheral circulation, the peripheral resistance in pregnancy can be lowered in two ways. Firstly, there is clear evidence of vascular dilatation in arterioles and capillaries (4,120,121) and in the venous side of the circulation (5,122). These changes are present by 12 weeks and are maintained until term, returning quickly to non-pregnant levels in the puerperium. Secondly, peripheral resistance may be lowered by reduction in whole blood viscosity and other haemorheological parameters (2,3,32). The following study deals with these changes in haemorheological parameters and reviews the relevant literature.

ii) SERIAL STUDY THROUGHOUT NORMAL PREGNANCY

Patients and Methods

Fifty patients were selected at the antenatal booking clinic. All were less than 12 weeks pregnant, 30 were primigravidae and 20 were multigravidae, none had any significant illness or took any regular medication. Blood was taken at monthly intervals until 28 weeks and then fortnightly until delivery. Patients were not given haematinics unless their haemoglobin concentration fell below 11.5 g per litre, when they were given ferrous sulphate 400 mg twice daily and folic acid 5 mg daily. All blood samples were collected between 9 and 11 a.m. at the patient's routine clinic attendance. All the haemorheological measurements were made in the manner described in Chapter 2.

Results

One of the difficulties besetting obstetric research is that the patient is rarely available for measurement before she becomes pregnant and so non-pregnant levels are not available. In the pregnancy studies reported here, the measurements made at six weeks post partum are taken as the non-pregnant control levels,

although it is realised that these may not correspond exactly to the pre-pregnancy levels. The levels of all the parameters measured are shown in Table 3. The statistical comparisons are between the level mentioned and the 'non-pregnant' level.

Haematocrit had fallen significantly by 12 weeks ($p < 0.05$) and continued to do so, reaching its nadir at 30 weeks ($p < 0.001$), following which it rose slightly, remaining significantly below the 'non-pregnant' value at term ($p < 0.001$).

Plasma fibrinogen showed a steady rise during pregnancy, with the level at 12 weeks already significantly elevated ($p < 0.001$) and reaching its zenith at 36 weeks and then falling insignificantly towards term.

Total serum protein was not significantly lowered by 12 weeks, but was so by 16 weeks ($p < 0.05$) and increasingly so until term ($p < 0.001$).

Plasma viscosity was significantly elevated by 12 weeks ($p < 0.001$), but then there was a trend of decline throughout pregnancy with the term level staying only just significantly above the 'non-pregnant' level ($p < 0.05$).

Whole blood viscosity was not significantly lowered until 24 weeks ($p < 0.001$). It remained significantly lower than the 'non-pregnant' level until 36 weeks when it rose towards non-pregnant levels and remained there until term.

Erythrocyte deformability was just significantly raised by 12 weeks ($p < 0.05$) but by 16 weeks it was increasingly raised ($p < 0.001$), this trend continuing until 32 weeks ($p < 0.001$), following which there was a slight decline in deformability, with the level at term still significantly above the 'non-pregnant' level.

Table 3 Haemorheological Parameters Throughout Pregnancy - all Patients - Serial Study

Gestation in Weeks		12	16	20	24	28	30	32	34	36	38	40	41	6 weeks Post Partum
Number of Subjects		50	50	50	50	50	50	50	48	44	36	30	15	50
Haematocrit	Mean	0.373	0.365	0.353	0.348	0.336	0.335	0.344	0.354	0.349	0.361	0.362	0.368	0.394
	SD	0.023	0.028	0.021	0.020	0.019	0.017	0.017	0.019	0.020	0.021	0.018	0.020	0.018
Plasma Fibrinogen g/l	Mean	3.20	3.05	3.37	3.46	3.39	3.42	3.50	3.75	3.82	3.35	3.75	3.76	2.45
	SD	0.32	0.31	0.32	0.34	0.34	0.32	0.29	0.30	0.31	0.34	0.35	0.29	0.20
Total Serum Protein g/l	Mean	68.6	66.2	65.71	64.0	62.9	62.5	61.3	61.0	61.8	61.9	60.5	62.0	69.5
	SD	3.4	3.5	3.3	3.5	3.2	3.1	2.9	2.3	2.9	3.4	3.2	3.3	3.4
Plasma Viscosity centipoise	Mean	2.26	2.16	2.12	2.13	1.97	1.95	1.94	1.95	1.85	1.98	1.87	1.96	1.71
	SD	0.17	0.13	0.15	0.16	0.14	0.13	0.11	0.12	0.12	0.14	0.15	0.16	0.09
Whole Blood Viscosity centipoise	Mean	10.13	9.74	9.10	7.49	7.23	7.01	7.82	7.56	8.64	9.84	9.91	10.42	10.82
	SD	1.68	1.72	1.64	1.58	1.49	1.52	1.61	1.57	1.62	1.72	1.73	1.74	1.58
Erythrocyte Deformability Index	Mean	0.98	1.04	1.08	1.09	1.13	1.15	1.18	1.12	1.09	1.04	1.04	0.97	0.83
	SD	0.12	0.12	0.13	0.13	0.14	0.14	0.13	0.14	0.14	0.14	0.11	0.10	0.11

Table 4 Haemorheological Parameters Throughout Pregnancy - Primigravidae - Serial Study

Gestation in Weeks		12	16	20	24	28	30	32	34	36	38	40	41	6 weeks Post Partum
Number of Subjects		30	30	30	30	30	30	30	30	26	22	18	11	30
Haematocrit	Mean	0.368	0.364	0.344	0.338	0.331	0.326	0.346	0.351	0.350	0.358	0.360	0.368	0.403
	SD	0.025	0.022	0.022	0.021	0.017	0.019	0.018	0.020	0.020	0.021	0.019	0.019	0.020
Plasma Fibrinogen g/l	Mean	3.23	3.10	3.22	3.33	3.38	3.25	3.52	3.63	3.75	3.64	3.51	3.56	2.33
	SD	0.32	0.31	0.32	0.33	0.34	0.31	0.30	0131	0.32	0.35	0.34	0.30	0.19
Total Serum Protein g/l	Mean	68.6	64.5	64.5	63.7	62.9	62.3	61.3	62.7	62.3	65.1	61.3	62.5	70.2
	SD	3.4	3.3	3.2	3.4	3.0	2.8	2.7	2.8	2.7	3.2	3.2	3.1	3.3
Plasma Viscosity centipoise	Mean	2.16	2.0	2.12	2.01	1.95	1.91	1.86	1.90	1.91	1.93	1.89	1.94	1.73
	SD	0.17	0.14	0.14	0.15	0.13	0.11	0.13	0.10	0.12	0.12	0.15	0.16	0.10
Whole Blood Viscosity centipoise	Mean	9.25	8.15	8.93	7.16	7.55	6.60	8.87	7.69	9.14	9.56	9.23	9.35	10.97
	SD	1.65	1.71	1.66	1.52	1.47	1.47	1.51	1.63	1.57	1.62	1.73	1.75	1.52
Erythrocyte Deformability Index	Mean	0.97	1.03	1.06	1.09	1.12	1.14	1.16	1.15	1.17	1.12	1.05	1.00	0.85
	SD	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.15	0.14	0.12	0.11	0.10	0.10

Table 5 Haemorheological Parameters Throughout Pregnancy - Multigravidae - Serial Study

Gestation in Weeks		12	16	20	24	28	30	32	34	36	38	40	41	6 weeks Post Partum
Number of Subjects		20	20	20	20	20	20	20	20	18	14	12	4	20
Haematocrit	Mean	0.375	0.367	0.360	0.354	0.342	0.344	0.342	0.356	0.348	0.363	0.365	0.368	0.380
	SD	0.020	0.025	0.019	0.019	0.021	0.015	0.016	0.017	0.020	0.021	0.017	0.021	0.020
Plasma Fibrinogen g/l	Mean	3.00	3.52	3.59	3.40	3.60	3.48	3.85	3.96	3.95	3.95	3.89	3.87	2.58
	SD	0.32	0.31	0.30	0.35	0.34	0.33	0.20	0.29	0.30	0.33	0.34	0.28	0.24
Total Serum Protein g/l	Mean	68.5	67.9	67.0	64.2	62.9	62.6	61.5	60.71	61.5	61.5	60.0	61.8	68.9
	SD	3.5	3.6	3.4	3.5	3.3	3.2	3.0	3.0	3.0	3.1	3.5	3.2	3.5
Plasma Viscosity centipoise	Mean	2.36	2.32	2.12	2.24	2.00	1.95	1.98	2.00	1.79	2.03	1.84	1.98	1.68
	SD	0.17	0.14	0.15	0.16	0.15	0.14	0.12	0.13	0.13	0.15	0.16	0.16	0.08
Whole Blood Viscosity centipoise	Mean	11.25	9.24	7.75	6.95	7.38	6.68	7.15	8.57	10.13	10.58	10.62	11.38	10.60
	SD	1.72	1.63	1.52	1.51	1.53	1.45	1.53	1.62	1.74	1.73	1.74	1.75	1.62
Erythrocyte Deformability Index	Mean	0.99	1.06	1.10	1.12	1.14	1.17	1.19	1.15	1.11	1.07	1.07	1.00	0.87
	SD	0.12	0.13	0.13	0.15	0.15	0.14	0.13	0.13	0.15	0.12	0.10	0.10	0.12

iii) COMPARISON OF PRIMIGRAVIDAE AND MULTIGRAVIDAE

The results in these two groups have been separated and are shown in Tables 4 and 5 respectively. There were no striking, and few statistically significant differences between the two groups. Some points, however, are worthy of mention. In the first half of pregnancy the plasma fibrinogen was lower in multiparous patients, but in the second half it was higher in multipara, suggesting a greater increase in fibrinogen production in multigravidae as pregnancy advances. The total serum protein was generally lower in multigravid subjects and this was reflected in a somewhat lower plasma viscosity in parous patients. From 36 weeks onwards, parous patients had a higher whole blood viscosity and past term this increase in whole blood viscosity was even more marked in multigravidae, reaching statistical significance at 41 weeks ($p < 0.05$). Neither haematocrit nor Erythrocyte Deformability Index were different in the two groups.

iv) DISCUSSION

The 'physiological anaemia' of pregnancy has been reported in many studies (123-126) and appears in this study as a steady decline in haematocrit (Table 6). This study also confirms the finding in some studies (123-126) that the haematocrit rises towards term.

There is general agreement in the literature (Table 7) (127-130), that plasma fibrinogen levels increase during pregnancy and the present study confirms this. The increased fibrinogen production in parous patients seen in this study has not been previously recorded and its significance is not clear.

Despite a variety of different methodologies, the published papers on total serum protein are in broad accord with the present study (Table 8) (131-133). The finding that multigravid subjects had a somewhat lower

Table 6 Haematocrit Throughout Pregnancy - Review of the Literature

Mean Haematocrit

Author		Six weeks Post Partum	Weeks of Pregnancy										
			12	16	20	24	20	32	34	36	38	40	41
Rath et al, 1950			0.388	0.372	0.370	0.367	0.372	0.337		0.370		0.379	
Lund, 1951			0.400	0.390	0.370	0.355	0.352	0.342		0.344		0.357	
Benstead and Theobald, 1952				0.398	0.383	0.371	0.355	0.346		0.355		0.368	
Edgar and Rice, 1956				0.378	0.362	0.344	0.342	0.330		0.330		0.363	
	Mean	0.394	0.373	0.365	0.353	0.348	0.336	0.344	0.354	0.349	0.361	0.362	0.368
Present Study	SD	0.018	0.023	0.023	0.020	0.020	0.019	0.017	0.019	0.020	0.021	0.018	0.020
	n	50	50	50	50	50	50	50	48	44	36	30	15

Table 7 Plasma Fibrinogen Levels Throughout Pregnancy - Review of the Literature

Mean Plasma Fibrinogen (g/l)

Author		Six weeks Post Partum	Weeks of Pregnancy											
			12	16	20	24	28	30	32	34	36	38	40	41
Phillips and Skrodelis, 1958					3.20	3.50	3.30		3.60		3.80		4.00	
Talbert and Langdell, 1964			5.00		4.50		4.40			4.90	5.10		5.50	
Bonnar et al, 1969		2.41	3.60		3.80	3.90		4.25			4.45		4.50	
Condie and Ogston, 1976		3.85	3.38		3.41			4.24				4.63		
	Mean	2.45	3.20	3.05	3.37	3.46	3.39	3.42	3.50	3.75	3.82	3.35	3.75	3.76
Present Study	SD	0.20	0.32	0.31	0.32	0.34	0.34	0.32	0.29	0.30	0.31	0.34	0.35	0.29
	n	50	50	50	50	50	50	50	50	48	44	36	30	15

Table 8 Total Serum Protein Throughout Pregnancy - Review of the Literature

Author		Mean Total Serum Protein (g/l)												
		Six weeks Post Partum	Weeks of Pregnancy											
			12	16	20	24	28	30	32	34	36	38	40	40+
Von Studnitz, 1955		71	70	68	66	66	65		66		66		68	
De Alvarez et al, 1961		71.8	66.1	56.2	55.9	57.4	59.6		60.1		57.4		59.0	
Reboud et al, 1967		70.3	67.3	66.3	61.4	63.3	59.4		62.7		61.3		61.3	
Present Study	Mean	69.5	68.6	66.2	65.7	64.0	62.9	62.5	61.3	61.0	61.8	61.9	60.5	62.0
	SD	3.4	3.4	3.5	3.3	3.5	3.2	3.1	2.9	2.8	2.9	3.4	3.2	3.3
	n	50	50	50	50	50	50	50	50	48	44	36	30	15

Table 9 Plasma Viscosity Levels Throughout Pregnancy - Review of the Literature

Author		Mean Plasma Viscosity (centipoise)										
		Non-pregnant	Weeks of Pregnancy									
			6-9	10-13	14-17	18-21	22-25	26-29	30-33	34-37	38-40	
Hamilton, 1950												
		1.74	1.59	1.61	1.62	1.58	1.63	1.63	1.66	1.69	1.68	
				9-19			20-32			33-40		
Eastham, 1965												
		1.60		1.66			1.64			1.66		
		Six weeks Post Partum	12	16	20	24	28	32	34	36	38	40	41
	Mean	1.71	2.26	2.16	2.12	2.13	1.97	1.94	1.95	1.85	1.98	1.87	1.96
Present Study	SD	0.09	0.17	0.13	0.15	0.16	0.14	0.11	0.12	0.12	0.14	0.15	0.16
	n	50	50	50	50	50	50	50	48	44	36	30	15

Table 10 Whole Blood Viscosity Levels Throughout Pregnancy - Review of the Literature

Whole Blood Viscosity (centipoise)

Author	Non-pregnant	Weeks of Pregnancy								
		9–12	13–16	17–20	21–24	25–28	29–32	33–36	37–40
Cohen and Thomson, 1936									
	4.80	4.68	4.46	4.33	4.31	4.26	4.21	4.20	4.42
		6–9	10–13	14–17	18–21	22–25	26–29	30–33	34–37	38–40
Hamilton, 1950										
	4.61	4.20	4.19	4.11	3.92	3.84	3.95	4.32	4.16	4.49

		Six weeks Post Partum	12	16	20	24	28	32	34	36	38	40	41
	Mean	10.82	10.13	9.74	9.10	7.49	7.23	7.82	7.56	8.84	9.84	9.91	10.42
Present Study	SD	1.53	1.68	1.72	1.64	1.58	1.49	1.61	1.57	1.62	1.72	1.73	1.72
	n	50	50	50	50	50	50	50	48	44	36	30	15

serum protein level throughout pregnancy is in keeping with the observation that multigravid patients expand their blood volume significantly more during pregnancy than do primigravid patients (134).

The two studies on plasma viscosity in pregnancy (Table 9) (135, 136) present contradictory data. Hamilton (1950) (135), using an Ostwald viscometer, found plasma viscosity consistently reduced during pregnancy, in contrast to Eastham (1964) (136), whose measurements with a Harkness viscometer suggested an increase. The present study is in agreement with the latter study. The plasma viscosity represents a balance between the rising fibrinogen and the falling total serum protein.

The literature is confused as regards the effect of pregnancy on (105) erythrocyte deformability, for there are reports of both a decrease and no change (137) and in the present study an increase in deformability in pregnancy was found. All these investigators used microfiltration techniques for deformability measurement, but both the other studies used washed erythrocytes in artificial media, thus removing the physiological action of fibrinogen (91), which markedly increases erythrocyte deformability, and this may account for the increased deformability found in the present study when erythrocytes were measured in their native, fibrinogen rich plasma.

The literature with regard to whole blood viscosity requires careful attention to the methods of measurement and the calculations by which viscosity is derived. By and large the British and American workers have reported straightforward whole blood viscosity measurements as in the present study, whereas the German investigators (139,140) report either 'relative viscosity', i.e. the ratio of whole blood to plasma viscosity, or whole blood viscosity corrected to a standard

haematocrit. In the present study the plasma viscosity, as well as the whole blood viscosity is known and 'relative viscosity' calculation is of no additional help. The correction of whole blood viscosity for haematocrit is not only unnecessary but may be clinically unhelpful, as in many of the hyperviscosity states reviewed in Chapter 1, section iv, it is the elevated haematocrit that is of greatest clinical importance.

Pillisier (1912) (141), using a Hess capillary viscometer, showed that whole blood viscosity was lowered by the later months of pregnancy and Esiaschwili (1933)(142), by an undisclosed technique, found a fall in whole blood viscosity during pregnancy. The two most comprehensive studies throughout pregnancy are those of Cohen and Thomson (1936) (138), who derived whole blood viscosity by a calculation based on the haematocrit (58) and Hamilton (1950) (135) who used an Ostwald viscometer. Their results are contrasted with the present study in Table 10, and all three studies show a gradual fall in whole blood viscosity to a nadir between 30 and 34 weeks, followed by a rise towards term. Leonhardt et al (1975) (143), using a Wells-Brookfield viscometer, and correcting the whole blood viscosity to a standard haematocrit, reported a significantly raised 'corrected whole blood viscosity' in pregnancy, but in fact this merely reflects the raised fibrinogen of pregnancy and its effect on plasma viscosity and erythrocyte aggregation. Schmid-Schonbein and his colleagues (140) reported the results of 11 pregnant women whom they investigated for various haemorheological parameters, using a modified Wells-Brookfield viscometer. They found a rise in 'relative viscosity' of blood and increased erythrocyte aggregation.

As stated in Chapter 1, section iii, whole blood viscosity is a complex parameter which is influenced by many other haemorheological factors.

Pregnancy presents us with a haemorheological workshop where the cellular and plasma constituents of blood are physiologically varied, so their interaction may be observed. The whole blood viscosity profile in pregnancy follows that of haematocrit most closely, but in the first half of pregnancy the whole blood viscosity is maintained by the rapid rise in plasma fibrinogen. As the plasma fibrinogen levels off and the total serum protein falls along with the haematocrit the whole blood viscosity falls after the 24th week. The increase in erythrocyte deformability seen from early pregnancy will also assist in the lowering of whole blood viscosity. In the last four weeks of pregnancy the whole blood viscosity rises towards term. This rise is compounded of an increasing haematocrit and a decreasing erythrocyte deformability offset by a fall in the plasma viscosity due to the slight reduction in plasma fibrinogen in the last four weeks of pregnancy and the continuing fall in total serum protein (Table 3).

The haemorheological changes described in this chapter are of obvious advantage in the hyperdynamic circulation of pregnancy described in the introduction and help to explain the alterations in peripheral resistance found in pregnancy. Bader et al (1955) (119) and Pyorola (1966) (114) both showed a reduction in total peripheral resistance in early pregnancy that remained static until the last trimester, when it rose to non-pregnant levels. This mirrors the described changes in whole blood viscosity and reciprocates the changes in erythrocyte deformability. The part played by haemorheological factors in the peripheral resistance may be considerable during pregnancy, with the degree of arterio-venous dilatation (120-122) and arteriolar insensitivity to pressor stimuli (4) that are present (Chapter 1, section ii). Exaggeration of the haemorheological changes of pregnancy could obviously

affect blood flow in the sensitive placental circulation and the haemorheological profiles of pathological pregnancy are reviewed in Chapters 6, 7 and 8.

Attempts were made to study the changes in haemorheological factors during labour and the puerperium, but because of the high incidence of intravenous fluid therapy during labour, no standard measurements could be gathered in sufficient numbers for analysis. Similarly in the puerperium, with most patients being discharged from hospital 48 hours after delivery, and those whose stay in hospital was longer had often had operative delivery or post partum complication that rendered them unsuitable for physiological measurements to be made, insufficient normal puerperal cases have been available for useful description and analysis.

All patients in the study were seen and had measurements made at the post natal clinic six weeks after delivery. The results of these measurements (Table 3), when compared with those measurements made in non-pregnant women who were not on the oral contraceptive pill (Table 2), showed no difference in any of the haemorheological parameters, suggesting that the return to the normal non-pregnant state was virtually complete.

None of the patients in the study suffered any thrombo-embolic complication of pregnancy, either ante- or post natally. It is well established that pregnancy is associated with an increased risk of venous thrombosis (144). Comparing the levels of haemorheological change induced by oral contraceptives and pregnancy reveals two very different pictures, both of which may give an increased risk of thrombosis. In oral contraceptive users (Chapter 3, Table 2) all the haemorheological parameters are altered very slightly in

a direction likely to predispose to thrombosis. In pregnancy, the plasma fibrinogen is markedly increased and the plasma viscosity moderately increased, whereas the erythrocyte deformability is significantly increased, the haematocrit reduced and the whole blood viscosity also reduced. The only common factor between oral contraception and pregnancy is the loss of the rapid and pronounced swings in all haemorheological parameters that take place in the normal spontaneous menstrual cycle (Figures 19,20 and 21).

CHAPTER 5

HAEMORHEOLOGICAL PROFILE OF THE NORMAL FETUS

Sections

i) INTRODUCTION

ii) COMPARISON OF THE HAEMORHEOLOGICAL PROFILES OF FULL TERM FETUSES, ADULT FEMALES AND PREGNANT WOMEN

iii) CHANGES IN FETAL HAEMORHEOLOGY WITH GESTATIONAL AGE

iv) DISCUSSION

HAEMORHEOLOGICAL PROFILE OF THE NORMAL FETUS

i) INTRODUCTION

The delivery of oxygen and nutrients to and the removal of waste products and hormones from the feto-placental unit may be affected by alterations in the blood flow rate on either the maternal or fetal side of the placenta. Maternal haemorheology in normal and pathological pregnancy, which may alter intervillous blood flow in the placenta, is discussed in Chapters 4, 6, 7 and 8.

A great deal of attention has been paid to the haemorheological status of the newborn infant during the first few days of life. Hyperviscosity states have been described causing neonatal plethora, lethargy, jitteriness, convulsions, respiratory distress, cardiomegaly, cardiac failure and cyanosis (145-149). The haematocrit, and hence the whole blood viscosity, in the early neonatal period, is influenced by the timing of cord clamping and by plasma volume changes in the first hours of life (150), and for this reason haemorheological studies in the neonatal period cannot be related to those in the fetus in utero. However, if hyperviscosity states cause such severe disturbances in the neonatal period, it seems reasonable to suggest that hyperviscosity of fetal blood may affect intrauterine growth and development. The fetal side of the placental circulation is particularly vulnerable to haemorheological alterations, with the extensive microcirculation of the villi being susceptible to changes in erythrocyte deformability, plasma and whole blood viscosity. However, little investigation has been conducted into fetal haemorheology in either normal or pathological pregnancy. The literature contains only a few superficial, and at times, contradictory, accounts of normal fetal haemorheology.

This chapter deals with the haemorheology of healthy full term fetuses and with the alterations that occur in haemorheological parameters with variation in gestational age.

ii) COMPARISON OF THE HAEMORHEOLOGICAL PROFILES OF FULL TERM FETUSES, ADULT FEMALES AND PREGNANT WOMEN

Having already described the normal haemorheological profile in adult women and in normal pregnancy, the fetal profile will be presented in contrast with them and the differences between them investigated and discussed.

Patients and Methods

Firstly, 20 ml of blood was collected, without venous occlusion, from an antecubital vein in 20 healthy pregnant women at between 30 and 40 weeks' gestation, from 20 healthy adult female volunteers, aged between 22 and 34 years, and from the umbilical cord vein of 20 healthy full term fetuses at birth. Fetal cord blood was collected as soon as the baby was delivered and before the placenta had separated. Haemorheological parameters were measured, as described in Chapter 2.

Secondly, in a further group of samples from similar patients, the plasma was separated and the erythrocytes washed three times with normal saline at $37^{o}C$. The erythrocytes were then suspended in plasma to give a haematocrit of 0.15 to 0.20. Preparations were made of erythrocytes from each group with the plasma of each group, the suspensions were incubated for one hour and then the Erythrocyte Deformability Index was measured. Samples from each group were assayed for plasma fibrinogen, albumin, total protein and osmolality.

Thirdly, fetal blood and blood from non-pregnant adult women was measured for Erythrocyte Deformability Index at a variety of blood pH levels as described in Chapter 2.

RESULTS

The contrasting haemorheological profiles of the three groups are shown in Table 11. For statistical comparison, the fetal and pregnancy values of each parameter were compared with those of healthy non-pregnant adult females.

The deformability of fetal erythrocytes was less than that of erythrocytes from adult females ($p<0.001$) and they, in turn, were less deformable than those from pregnant women ($p<0.001$).

The haematocrit of the three groups were also significantly different from each other ($p<0.001$), with fetal blood having the highest and pregnant women the lowest values.

The plasma fibrinogen also showed significant differences ($p<0.001$) with pregnant women having the highest and fetal blood the lowest levels.

Fetal plasma had a lower viscosity than adult female's ($p<0.05$) and pregnant women's plasma had a higher viscosity ($p<0.001$).

Whole blood viscosity was significantly different in all three groups, with fetal blood the highest ($p<0.001$) and pregnant women's the lowest ($p<0.001$).

Analysing the relationship between Erythrocyte Deformability Index and plasma fibrinogen in the three groups (Figure 25) gave a correlation coefficient of +0.733 with a slope of +0.167 and an intercept of 0.552.

The second set of experiments further investigated the relationship between the Erythrocyte Deformability Index and plasma constituents in the three groups. Table 12 shows the Erythrocyte Deformability Index in each of the nine erythrocyte-plasma combinations. When

Table 11 Haemorheological Measurements in Adult Females, Pregnant Women and Full Term Fetuses

	Pregnant Women n = 20 (SD)	Full Term Infants n = 20 (SD)	Adult Women n = 20 (SD)
Mean Whole Blood Viscosity (centipoise)	9.8 (2.8)**	16.8 (2.4)**	12.7 (3.1)
Mean Plasma Viscosity (centipoise)	2.25 (0.46)**	1.46 (0.33)*	1.75 (0.40)
Mean Haematocrit	0.373 (0.028)**	0.492 (0.037)*	0.447 (0.033)
Mean Erythrocyte Deformability Index	1.13 (0.14)**	0.72 (0.10)*	0.81 (0.11)
Mean Plasma Fibrinogen (g/l)	4.32 (0.81)**	1.76 (0.73)*	2.80 (0.69)

Comparison of measurements with those from Adult Women using Student's t-test *$p<0.05$ **$p<0.001$

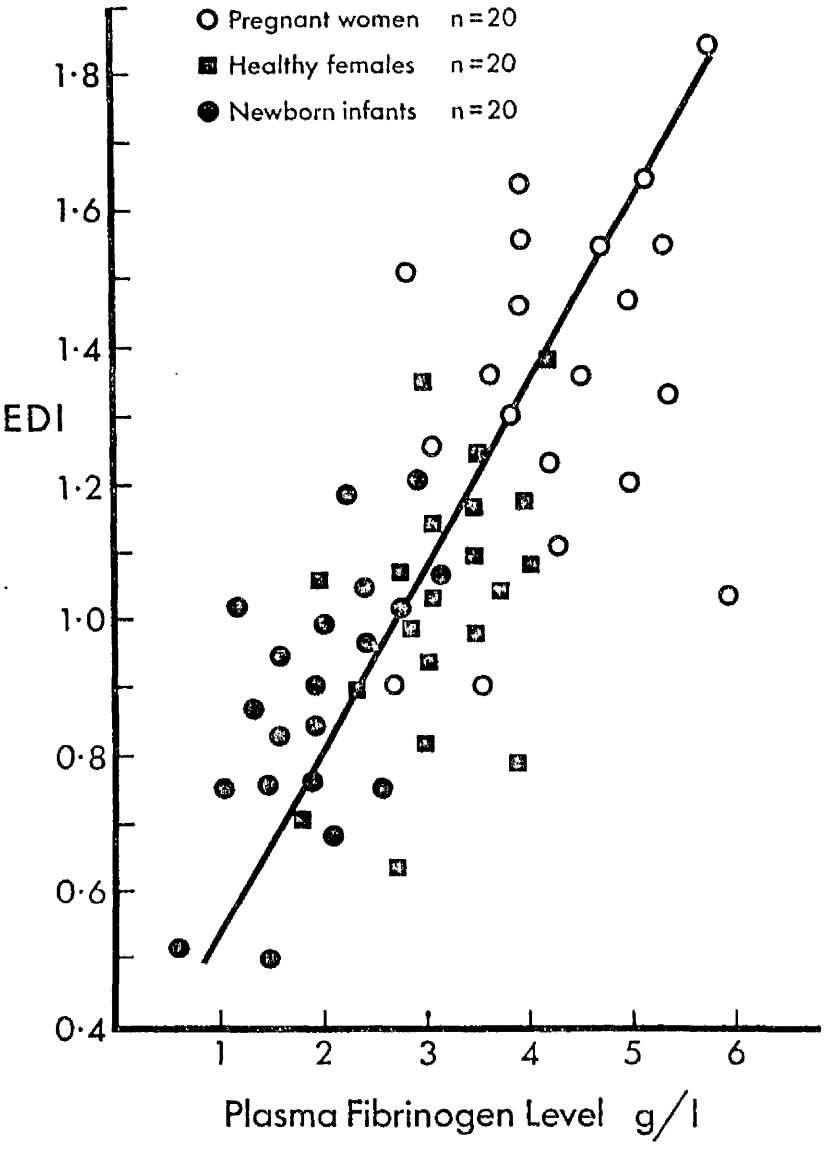

Figure 25 The Relationship Between Plasma Fibrinogen Concentration and Erythrocyte Deformability Index (EDI) in Samples From Pregnant Women, Adult Females and Full Term Fetuses

Table 12 Erythrocyte Deformability Index (Mean ± SD) in Ten Aliquots of Each Erythrocyte/Plasma Combination

	Fetal Plasma	Adult Female's Plasma	Pregnant Female's Plasma
Fetal Erythrocytes	0.73 ± 0.10	0.81 ± 0.10	1.08 ± 0.12
Adult Female's Erythrocytes	0.76 ± 0.11	0.84 ± 0.11	1.11 ± 0.12
Pregnant Female's Erythrocytes	0.80 ± 0.14	0.90 ± 0.12	1.15 ± 0.13

measured in their native plasma fetal cells were less deformable than adult cells measured in their native plasma ($p < 0.05$), which were, in turn, less deformable than erythrocytes from pregnant women measured in their native plasma ($p < 0.001$). When the erythrocytes were incubated with plasma from the other groups, these differences were abolished and irrespective of the source of the erythrocytes, the Erythrocyte Deformability Index was lowest when measurement was made in fetal plasma ($p < 0.05$) and highest when made in plasma from pregnant women ($p < 0.001$).

The analysis of plasma constituents in the three groups is shown in Table 13. Plasma albumin concentration was similar in the fetus and pregnant women, but significantly higher in adult women ($p < 0.001$). Adult women also had a higher total protein level than the other two groups ($p < 0.001$). Pregnant women had a higher plasma fibrinogen than adult females ($p < 0.001$) and fetuses a significantly lower level than adult females ($p < 0.05$). The plasma osmolality of fetuses and pregnant women were similar and were significantly lower than that of adult females ($p < 0.001$).

Analysing the relationship between the Erythrocyte Deformability Index and the several plasma constituents revealed a positive correlation coefficient of +0.754 and a slope of +0.168. No other significant correlation with any other of the plasma constituents was found.

The third set of experiments investigated the alteration in the Erythrocyte Deformability Index in response to alterations in pH in fetal and adult female's blood. These alterations are shown in Figure 26. Decrease in pH caused a significant reduction in deformability of both fetal and adult erythrocytes, but below pH 6.9 the reduction in deformability was greater in fetal than in adult erythrocytes, suggesting

Table 13 Albumin, Total Protein, Fibrinogen and Osmolality (Mean ± SD) in Plasma From Full Term Fetuses, Adult Females and Pregnant Women

	Albumin g/l	Total Protein g/l	Fibrinogen g/l	Osmolality m osmol/kg
Full Term Fetuses n = 10	37.5 ± 3.8	60.8 ± 7.1	1.73 ± 0.64	278 ± 3.4
Adult Females n = 10	44.5 ± 4.2	68.2 ± 8.3	2.78 ± 0.75	285 ± 3.1
Pregnant Females n = 10	36.9 ± 4.1	62.7 ± 6.9	4.50 ± 0.80	274 ± 4.8

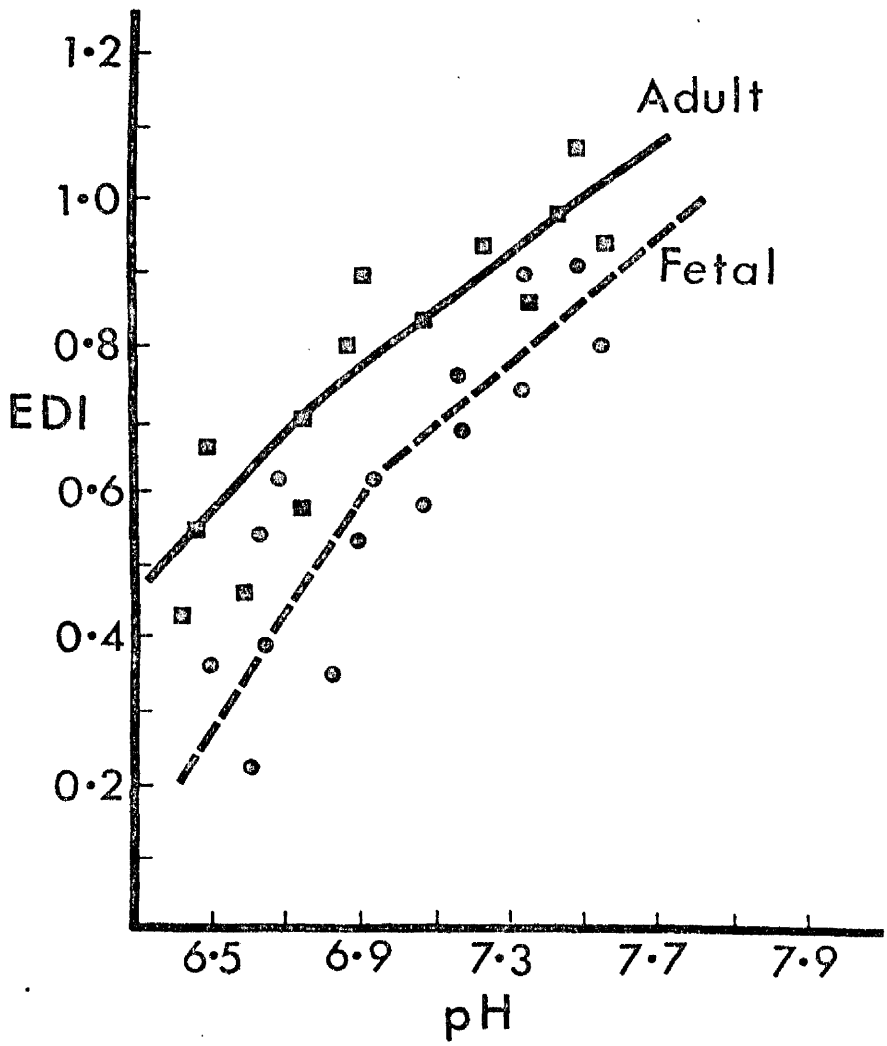

Figure 26 The Relationship Between pH Level and Erythrocyte Deformability Index (EDI) in Fetal and Adult Blood

an increased sensitivity to anoxic acidosis in fetal cells.

iii) CHANGES IN FETAL HAEMORHEOLOGY WITH GESTATIONAL AGE

Apart from a number of studies describing the alterations in cord blood haemoglobin and haematocrit with increasing gestation (151, 152), no work has been done on haemorheology in the preterm infant. This study seeks to investigate the way in which the haemorheological profile is altered by variation in gestational age.

Patients and Methods

Cord vein blood was collected from 190 infants at the moment of birth. These infants were born at a variety of gestations, but cases were excluded where a clearly identifiable pathological cause was found for the preterm labour. The parameters were measured as described in Chapter 2.

Results

The changing pattern of the four parameters measured with advancing fetal age is shown in Table 14. The statistical analysis in the table is a comparison of the measurement marked with the measurement in the preceding gestational period.

The haematocrit showed a progressive rise from 34 weeks past term to 42+ weeks with significant increases in haematocrit between each of the gestational periods measured.

Plasma viscosity also showed a gradual increase from 34 to 42 weeks. The rise from 34 to 40 weeks was significant ($p < 0.05$), as was the rise from 40 to 42+ weeks ($p < 0.05$).

Whole blood viscosity followed the trend of haematocrit and plasma viscosity, with a significant rise between 34 and 36 weeks ($p < 0.05$) and another significant increase between 40 and 42+ weeks ($p < 0.05$).

Table 14 Variations in the Fetal Haemorheological Profile with Gestational Age - Cross-sectional Study

Gestational Age in Weeks		34–35	36–37	38–39	40–41	42+
Number of Subjects		15	25	45	75	30
Haematocrit	Mean	0.421	0.459*	0.488*	0.512*	0.564***
	SD	0.032	0.031	0.033	0.032	0.037
Plasma Viscosity (centipoise)	Mean	1.24	1.38^{ns}	1.43^{ns}	1.46^{ns}	1.69*
	SD	0.25	0.29	0.26	0.33	0.35
Whole Blood Viscosity (centipoise)	Mean	12.5	14.6*	15.5^{ns}	16.8^{ns}	19.7**
	SD	1.8	2.1	2.3	2.3	3.5
Erythrocyte Deformability	Mean	0.65	0.69^{ns}	0.72^{ns}	0.72^{ns}	0.64**
	SD	0.11	0.08	0.09	0.09	0.11

Statistical significance of difference between result marked and preceding result using Student's t-test

ns $p > 0.05$ *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

The Erythrocyte Deformability Index rose slightly, but not statistically significantly, between 34 and 38 weeks and then levelled off only to fall significantly between 40 and 42+ weeks ($P < 0.01$).

iv) DISCUSSION

The fetus *in utero* has, until very recently, been inaccessible for direct observation and measurement. However, blood taken from the umbilical vessels at birth gives the investigator a sample of fetal blood which is representative of the fetus at the end of labour. What changes come about during labour is not known, and just as maternal haemorheology is altered by the intravenous and other therapies of labour (Chapter 4, section iv), so the fetal haemorheology may be altered. However, all the fetal samples collected in these studies followed very similar types of labour, with no medication that was known to have specific or profound affects on the parameters measured. Once more reliable and safe methods of fetal blood sampling *in utero* and micro-haemorheological measurement techniques have been developed, these objections will be overcome.

Compared with his pregnant mother, the fetus is poorly situated to cope with an increase in any of his haemorheological parameters. With an elevated haematocrit and reduced erythrocyte deformability combining to give an elevated whole blood viscosity, the fetus's only advantage lies in the low plasma viscosity which is due to reduced levels of total protein and fibrinogen. Previous studies (153,154) have shown low levels of fibrinogen in cord blood. Immunoglobulin levels, particularly IgM, are exceedingly low unless the fetus has been exposed to an intrauterine infection (155). The study of Foley, Isherwood and McNicol (1978) (153) reported values of cord plasma viscosity, proteins and fibrinogen very similar to the present study. The main area of difference between these two studies was in the values of whole blood viscosity. Foley,

Isherwood and McNicol (1978) (153) found no difference in whole blood viscosity between bloods from pregnant women and their newborn infants, despite the marked difference in haematocrit. They accounted for this lack of difference by the fact that the plasma viscosity in the fetus was low. Certainly the whole blood viscosity in the fetus was moderated by the low plasma viscosity in the present study, but the difference between the two studies exists and confirmatory investigation is required. It may be that the Deer Rheometer with its variable shear rate characteristics (Chapter 2, section iii) is more sensitive to the aggregation of erythrocytes than the fixed shear rate Contraves instrument used by Foley, Isherwood and McNicol (1978) (153). Fetal erythrocyte deformability has been the object of study by two groups of workers (82,156), both used microfiltration methods for deformability measurement. Gross and Hathaway (1972) (82) reported that fetal erythrocytes were less deformable than adult cells and also that fetal cells were more sensitive to anoxic acidosis. These findings were confirmed in both aspects by the present study. Bergqvist, Bygdeman and Rylander (1977) (156), however, found no difference in the deformability of fetal erythrocytes. The main difference in methodology between the present study and that of Bergqvist, Bygdeman and Rylander (1977) (156) is that they measured washed erythrocytes suspended in artificial medium and therefore eliminated any affect of the native plasma fibrinogen, that has been shown to have such a sensitive affect on erythrocyte deformability (Chapter 5, sections ii and iii) (76,91).

Even apart from the affect of the low fibrinogen level in fetal plasma, there are other features of fetal erythrocytes that would tend to make them less deformable. Availability of adenosine triphosphate (ATP) is a key factor in the maintenance of cell shape and deformability (157-159). In fetal erythrocytes, stability of ATP is decreased (160), ouabain-sensitive ATPase is low for cell age (161) and phosphate

incorporation is diminished (162). Membrane lipids and their interaction with plasma proteins are important for membrane function (163) and it is known that fetal erythrocytes have a diminished ability to handle oxidant stress and exhibit other membrane lipid alterations (164,165). Structural differences in the fetal erythrocyte, such as increased Heinz body formation related to low glutathione stability (160,162), pitting of the erythrocyte surface (166), and increased pyknocyte formation (160, 167) would correlate with a decreased deformability. Erythrocyte deformability is a major determinant of erythrocyte lifespan *in vivo* (65-67) and it is clearly established that fetal erythrocytes have a lifespan of around 80 days, compared with 120 days in the adult (168).

Plasma factors, as well as cellular factors, have been suggested as being responsible for fetal erythrocyte differences (160,167,169). Oski and Naiman (1965) (160), showed that incubating adult cells in premature infant's plasma resulted in pyknosis. Both increased bilirubin and decreased tocopherol affect erythrocyte shape and possibly deformability (169,170).

Turning to the changes in haemorheological parameters with gestational age. The rising haematocrit found in this study agrees with the published works (151,152), as does the level of plasma fibrinogen and protein (153,154). Walker and Turnbull (1953) (151) suggest that during intrauterine life, there are two separate mechanisms at work in the development of the blood picture seen in the fetus at birth. Firstly, there is an increase in haemoglobin and erythrocytes as part of the normal growth and maturation of the fetus. Secondly, an increased production of haemoglobin and erythrocytes may be forced at any stage of the pregnancy by a fall in the oxygen supply to the

fetus, and the affect of this abnormal stimulation may be superimposed on the normal growth pattern. They went on to present evidence to show that fetal haemoglobin rises above adult levels in response to diminished oxygen diffusion across the placenta after about 36 weeks' gestation. Although Marks et al (1955) (171) and Rooth and Sjosted (1957) (172) did not find elevated red cell counts in postmature infants, and therefore doubted the occurrence of 'placental insufficiency', careful studies by Bratteby (1968) (152) confirmed the relationship between gestational age and cord haemoglobin and haematocrit values. This investigator studied infants with gestational ages between 32 and 43 weeks, and clearly demonstrated a positive association between increasing gestational age and higher red cell volumes.

A similar situation may well pertain with the whole range of haemorheological parameters. Hepatic and immunological maturation result in gradually rising concentrations of plasma fibrinogen, albumin and globulins; maturation of the haemopoietic system results in a rising haematocrit and consequent upon these changes, the plasma and whole blood viscosity rise. Stress situations, such as those dealt with in the following three chapters, may cause an acceleration in production of these parameters and then a vicious circle is set up, with hyperviscosity leading to impaired placental perfusion and further hypoxia adding renewed stimulus to erythropoiesis. It is known that postmaturity of the fetus is associated with an increased risk of fetal distress in labour and with an increased perinatal mortality and morbidity (173,174). In Table 14, it is evident that there is a significant and rapid increase in haematocrit ($p < 0.001$), plasma viscosity ($p < 0.05$) and whole blood viscosity ($p < 0.01$) between 40 and 42+ weeks, and an associated decrease in Erythrocyte Deformability Index ($p < 0.01$). The cumulative affect of these changes must be a

significant reduction in villous perfusion on the fetal side of the placenta, as well as an increased strain of the fetal heart because of the increase in peripheral resistance. Once again, the situation is open for a vicious circle of hypoxia increasing viscosity factors, thus resulting in impaired perfusion and leading to further hypoxia. This situation could well make a significant contribution to the aetiology of fetal distress in labour in postmature fetuses where any intrapartum anoxia will further exacerbate the already compromised haemorheological state of the placental blood flow.

CHAPTER 6

THE EFFECTS OF CIGARETTE SMOKING ON MATERNAL AND FETAL HAEMORHEOLOGY

Sections

i) INTRODUCTION

ii) THE EFFECTS OF CIGARETTE SMOKING ON MATERNAL AND FETAL HAEMORHEOLOGY

iii) DISCUSSION

THE EFFECTS OF CIGARETTE SMOKING ON MATERNAL AND FETAL HAEMORHEOLOGY

"A custome lothsome to the eye, hateful to the nose,
harmful to the braine, dangerous to the lungs, and
the blacke stinking fume thereof, neerest resembling
the horrible Stigian smoke of the pit that is bottomless."

James I of Great Britain (1566 - 1625)
in a Counter-Blaste to Tobacco, Chapter 4.

i) INTRODUCTION

King James I may be excused for omitting from his highly accurate list of the evils of tobacco smoking the consequences for the fetus of maternal smoking during pregnancy. It was not until 1935 that Sontag and Wallace (1935) (175) showed that cigarette smoking caused acceleration of the fetal heart. Campbell in 1935 (176) and 1936 (177) warned that smoking was prejudicial to efficient childbearing, but little interest was shown in his observations. In 1957 a report (178) was published showing that the incidence of neonates weighing less than 2,500 g was twice as high in smokers as in non-smokers. Since then a large volume of evidence has accumulated implicating cigarette smoking with low birth weight (179-181), preterm delivery (182-184), increased fetal wastage (179,180) and long term impairment of mental and physical growth (185,186). Smokers also show an increased incidence of abruptio placentae (187,188), placenta praevia and other causes of ante-partum haemorrhage (180,188) and premature rupture of the membranes (184). The only apparent advantage from cigarette smoking in pregnancy is a lower incidence of hypertensive disorders of pregnancy (180,182,184,189), although the perinatal risk in

smokers who do develop hypertensive disorders is much increased when compared with non-smokers with the same problem (190).

The chemistry and pharmacology of tobacco is complex and not fully understood. The mechanisms whereby cigarette smoking affects fetal growth is not clear. Nicotine crosses the placenta and in animal experiments injection of nicotine into the pregnant mother results in reduction of fetal birthweight (191,192). Suzuki et al (1971) (193) concluded that nicotine caused vaso-constriction of uterine vessels with consequent reduction in intervillous blood flow and hence fetal asphyxia. Carbon monoxide appears in much higher levels in the blood of smokers and crosses the placental barrier (194). Astrup et al (1972) (195) exposed pregnant rabbits to different levels of ambient carbon monoxide and found a pronounced effect on average litter weight, fetal development and neonatal death rates.

The effect of cigarette smoking on haemorheological parameters has been studied in non-pregnant subjects. Chmiel et al (1973) (196) observed an increase in blood viscosity in smokers, this increase being much more marked immediately after smoking. Dintenfass (1975) carried out a comparison of male smokers and matched controls. He reported that smokers had an increase in haematocrit, plasma fibrinogen, plasma and whole blood viscosity and erythrocyte aggregation. All these findings have been confirmed in a recent study by Leonhardt et al (1978) (102).

ii) EFFECTS OF CIGARETTE SMOKING ON MATERNAL AND FETAL HAEMORHEOLOGY

The following study sought to investigate the haemorheological profile throughout pregnancy in women who smoked regularly and to investigate the weight and haemorheology of the newborn infants

of smoking mothers.

Patients and Methods

Firstly, twenty women of mixed parity who smoked 20 or more cigarettes a day were identified at the antenatal clinic. Initially, a larger number of heavy smokers were questioned and advised about the dangers of smoking to the health of their unborn child. The 20 selected declined to make any attempt to reduce their smoking but agreed to enter the study so that a close watch could be kept on their fetal growth. Blood was taken at regular intervals throughout pregnancy and haemorheological measurements made. A control group of age, social class and parity matched controls was selected.

Secondly, blood was collected from the umbilical cord vein, at birth, from 40 infants born to mothers who smoked more than 20 cigarettes per day and from 40 infants born to mothers who did not smoke. Haemorheological measurements were made on all samples as described in Chapter 2.

Results

The haemorheological parameters throughout pregnancy in the groups of smokers and non-smokers are shown in Tables 15 and 16 respectively. The changes in the six haemorheological parameters measured in the non-smokers were not in any way different from the serial study of patients shown in Table 3 (Chapter 4, section ii). Comparing the results in Tables 15 and 16, using a Student's t-test, it was found that in smokers the pattern of change throughout pregnancy in haematocrit, plasma fibrinogen, total serum protein, plasma and whole blood viscosity was not significantly different from non-smokers. The Erythrocyte Deformability Index, however, was decreased in cigarette smokers at all stages of pregnancy ($p < 0.05$),

this difference being most marked from 30 weeks onwards ($p<0.01$). When the six weeks post partum measurements were compared between the two groups, the smokers had a significantly higher haematocrit ($p<0.001$), and elevated plasma fibrinogen ($p<0.05$), a higher total serum protein concentration ($p<0.05$), a higher whole blood viscosity ($p<0.001$) and a reduced Erythrocyte Deformability Index ($p<0.05$). The plasma viscosity was not different in the two groups.

The haemorheological measurements in the infants born to smoking and non-smoking mothers, with their gestational age and birth weights are set out in Table 17. The statistical comparison was made using Student's t-test. There was no statistical difference between the gestational ages in the two groups, but the birth weight was significantly less in the smoking group ($p<0.01$). The haematocrit was elevated significantly in the smokers ($p<0.001$), as was the whole blood viscosity ($p<0.02$). The plasma viscosity was the same in both groups but the Erythrocyte Deformability Index was significantly lower in the smoking group ($p<0.02$).

iii) DISCUSSION

It is probable that cigarette smoking affects fetal growth by several routes. Nicotine is said to cause a reduction in intervillous blood flow (193), carbon monoxide certainly decreases oxygen availability for the fetus (194) and there is clear evidence, at least in the fetus, of a haemorheological effect that would further reduce placental perfusion and lead to increased fetal hypoxia. The only clear difference in the maternal haemorheology of pregnancy, between smokers and non-smokers, was the reduced Erythrocyte Deformability Index throughout pregnancy in smokers. This is probably due to carboxyhaemoglobin altering the internal

viscosity of the erythrocyte as well as affecting the oxygen availability for the cell's metabolism, and so reducing deformability. Clear differences did exist in all parameters, except plasma viscosity when measurement was made at the postnatal visit. These findings are in keeping with the published effects of smoking in non-pregnant subjects (196,197).

The alteration in haemorheology produced by smoking was much more pronounced in the fetus. The whole blood viscosity was elevated because of the raised haematocrit and reduced erythrocyte deformability. Chronic fetal hypoxia has been suggested as a stimulus for erythrocyte production (151,198) and as with postmature infants (Chapter 5, section iii), it is easy to see how a vicious circle could be set up with carbon monoxide causing fetal hypoxia leading to stimulation of erythropoiesis and therefore raised haematocrit, causing increased viscosity of blood and hence reduced placental perfusion leading to further fetal hypoxia. In support of this hypothesis, elevated erythropoietin levels (199) and elevated haematocrit have been reported in cord blood from babies whose mothers smoked during pregnancy and in the present study increased whole blood viscosity and decreased erythrocyte deformability complete the circle.

In conclusion, yet another way in which smoking may affect fetal oxygenation and nutrition has been demonstrated. Haemorheological factors operate in both the maternal and fetal sides of the placental circulation and combined with the primary toxic effects of nicotine and carbon monoxide help explain the poor reproductive performance of cigarette smokers.

Table 15 Maternal Haemorheological Parameters Throughout Pregnancy - Cigarette Smokers - Serial Study

Gestation in Weeks		12	16	20	24	28	30	32	34	36	38	40	41	6 weeks Post Partum
Number of Subjects		20	20	20	20	20	20	20	18	16	11	8	5	20
Haematocrit	Mean	0.367	0.367	0.342	0.341	0.342	0.336	0.341	0.351	0.340	0.357	0.365	0.377	0.411
	SD	0.019	0.022	0.017	0.029	0.017	0.020	0.017	0.017	0.018	0.016	0.021	0.023	0.020
Plasma Fibrinogen g/l	Mean	3.14	2.97	3.19	3.28	3.49	3.28	3.33	3.41	3.73	3.65	3.56	3.64	2.54
	SD	0.35	0.20	0.33	0.27	0.38	0.38	0.26	0.42	0.30	0.33	0.24	0.21	0.21
Total Serum Protein g/l	Mean	68.5	67.0	64.4	63.7	62.5	63.7	62.0	63.4	62.2	65.6	60.3	60.4	69.2
	SD	3.5	3.2	3.0	1.8	2.9	3.0	3.8	2.9	2.2	2.7	2.9	2.8	3.3
Plasma Viscosity (centipoise)	Mean	2.17	2.08	2.09	2.04	1.96	1.95	1.95	1.92	1.96	1.93	1.87	1.99	1.76
	SD	0.17	0.09	0.11	0.13	0.14	0.14	0.16	0.12	0.19	0.11	0.13	0.15	0.08
Whole Blood Viscosity (centipoise)	Mean	9.64	9.18	9.35	7.24	6.99	6.91	7.53	7.57	7.57	9.76	8.24	8.95	12.20
	SD	1.76	1.93	1.69	1.09	1.51	1.54	1.34	1.33	1.04	1.25	1.09	2.01	1.62
Erythrocyte Deformability Index	Mean	0.91	0.99	1.01	1.03	1.08	1.10	1.12	0.99	0.93	0.91	0.92	0.90	0.76
	SD	0.12	0.12	0.14	0.13	0.12	0.11	0.13	0.11	0.10	0.12	0.13	0.11	0.12

Table 16 Maternal Haemorheological Parameters Throughout Pregnancy - Non-smokers - Serial Study

Gestation in Weeks		12	16	20	24	28	30	32	34	36	38	40	41	6 weeks Post Partum
Number of Subjects		30	30	30	30	30	30	30	30	28	22	10	10	30
Haematocrit	Mean	0.363	0.356	0.345	0.341	0.345	0.347	0.350	0.351	0.355	0.358	0.362	0.368	0.382
	SD	0.014	0.016	0.019	0.019	0.019	0.015	0.015	0.016	0.017	0.016	0.019	0.017	0.017
Plasma Fibrinogen g/l	Mean	3.32	3.24	3.38	3.54	3.46	3.57	3.65	3.82	3.95	3.82	3.72	3.33	2.32
	SD	0.32	0.25	0.34	0.32	0.31	0.24	0.31	0.22	0.25	0.31	0.32	0.28	0.24
Total Serum Protein g/l	Mean	68.2	66.5	64.9	61.5	60.0	62.0	61.8	62.0	62.1	61.7	61.5	62.4	65.8
	SD	2.4	2.6	2.8	3.5	2.9	2.1	2.5	2.9	3.6	3.1	3.7	3.5	3.4
Plasma Viscosity (centipoise)	Mean	2.26	2.17	1.98	2.04	1.88	1.97	1.94	1.91	1.96	1.91	1.92	1.95	1.69
	SD	0.23	0.18	0.23	0.13	0.12	0.15	0.13	0.14	0.12	1.13	0.12	0.14	0.10
Whole Blood Viscosity (centipoise)	Mean	9.56	9.21	8.90	7.54	7.25	6.81	7.77	7.78	9.54	9.48	9.52	9.69	9.96
	SD	1.82	1.60	1.53	1.87	1.10	1.47	1.34	1.45	1.92	1.87	1.93	1.89	1.55
Erythrocyte Deformability Index	Mean	0.99	1.05	1.09	1.11	1.15	1.18	1.21	1.19	0.99	1.08	1.07	1.06	0.89
	SD	0.12	0.13	0.14	0.13	0.12	0.11	0.12	0.14	0.13	0.11	0.10	0.11	0.10

Table 17 Haemorheological Profile of Infants Born to Cigarette Smoking and Non-smoking Mothers

	Non-smokers	Smokers	Statistical Comparison
Number of Subjects	40	40	
Gestational age (weeks) Mean ± SD	39.4 ± 0.6	38.7 ± 0.8	ns
Birth Weight (g) Mean ± SD	3215 ± 210	2897 ± 252	$p < 0.01$
Haematocrit Mean ± SD	0.496 ± 0.031	0.558 ± 0.041	$p < 0.001$
Plasma Viscosity (centipoise) Mean ± SD	1.47 ± 0.34	1.49 ± 0.35	ns
Whole Blood Viscosity (centipoise) Mean ± SD	16.3 ± 2.2	18.7 ± 2.8	$p < 0.02$
Erythrocyte Deformability Index Mean ± SD	0.79 ± 0.10	0.65 ± 0.11	$p < 0.02$

Statistical Comparison using Student's t-test

CHAPTER 7

HAEMORHEOLOGICAL CHANGES IN PREGNANCY COMPLICATED BY PRE-ECLAMPSIA

Sections

i) INTRODUCTION

ii) MATERNAL AND FETAL HAEMORHEOLOGY IN PRE-ECLAMPSIA

iii) DISCUSSION

HAEMORHEOLOGICAL CHANGES IN PREGNANCY COMPLICATED BY PRE-ECLAMPSIA

i) INTRODUCTION

The clinical association between pre-eclampsia and increased perinatal morbidity and mortality is well documented (200,201), but the exact aetiology of pre-eclampsia is complex, with several different pathological processes interacting and combining to produce the widespread changes seen in the mother, placenta and fetus. Abnormalities of the immunological relationship between the maternal host and the feto-placental graft have been described (202), as have breakdown of the normal haemostatic mechanisms (203), abnormalities of the renin-angiotensin system (204) and morphological changes in the utero-placental vasculature (205,206). Although the order and importance of these abnormalities in the pathogenesis of pre-eclampsia is not clear, the end result, from the fetal point of view, is seen in the blood supply to the microvasculature of the placenta.

The association between pre-eclampsia and reduced intervillous blood flow is well established by clinical studies (207-209). McClure Browne and Veal (1953) (207), demonstrated a 33 per cent reduction in intervillous blood flow in patients with toxaemia. Morris et al (1955) (208) and others (209) indicated that the severity of pre-eclampsia was proportional to the degree of reduction in blood flow. It is not clear from these studies if the pre-eclampsia or the reduced placental blood flow comes first.

Animal experiments have shown that artificial uterine ischaemia, induced by partial occlusion of the uterine artery in pregnant animals, produces a situation with maternal and fetal effects almost identical to pre-eclampsia (210,211). Utero-placental ischaemia, as

a basic aetiological factor in the development of pre-eclampsia, has been postulated for many years (212-214).

ii) MATERNAL AND FETAL HAEMORHEOLOGY IN PRE-ECLAMPSIA

Whether the reduction in utero-placental blood flow is primary or secondary, this study was directed to investigate the possible involvement of haemorheological factors affecting both fetal and maternal sides of the placental circulation.

Patients and Methods

Firstly, patients diagnosed as having pre-eclampsia were identified in the antenatal clinics. The diagnosis was made on the basis of a rise in diastolic blood pressure of greater than 15 mm of mercury above the level recorded in the first trimester of pregnancy and the presence of proteinuria or oedema, in patients with no known history of renal or hypertensive disease. Blood was taken for analysis before any specific treatment was started. Control subjects were matched for parity, approximate weight, smoking habits and gestation.

Secondly, cord blood was collected from 30 newborn infants delivered to mothers who had pre-eclampsia and from 30 gestation matched controls. All measurements were carried out as described in Chapter 2.

Results

The haemorheological measurements of 120 patients who had pre-eclampsia and 150 normotensive controls in cross-sectional study are shown in Table 18. Although the patients were all classified as having pre-eclampsia, the definition used gave a lower limit only and there was a greater incidence of more severe hypertension and proteinuria in the patients at the earlier gestations. Twelve of the patients at 32 weeks and 12 of those at 34 weeks had proteinuria, whereas only 7 of those at 38 weeks and 2 of those at

40 weeks had proteinuria.

At 32 weeks, patients with pre-eclampsia had elevated haematocrit ($p<0.01$), plasma fibrinogen ($p<0.05$), plasma and whole blood viscosity ($p<0.01$) and reduced Erythrocyte Deformability Index ($p<0.05$).

At 34 weeks there was still elevated haematocrit ($p<0.05$), plasma fibrinogen ($p<0.05$), plasma and whole blood viscosity ($p<0.01$) and reduced Erythrocyte Deformability Index ($p<0.05$).

At 36 weeks the only parameters to be significantly affected were plasma viscosity ($p<0.05$) and whole blood viscosity ($p<0.01$), both of which were elevated.

At 38 and 40 weeks only the whole blood viscosity was still raised ($p<0.05$) in the pre-eclamptic subjects.

The fetal blood measurements are set out in Table 19. The infants born to pre-eclamptic mothers had a reduced mean birth weight ($p<0.05$), elevated haematocrit ($p<0.01$) and raised whole blood viscosity ($p<0.01$). Neither plasma viscosity nor Erythrocyte Deformability Index was significantly different.

iii) DISCUSSION

Fahraeus (1962) (215) described eclampsia as 'a disease of the checked microcirculation'. Certainly the results of this study would indicate that, in pre-eclampsia, haemorheological factors are altered in such a way as to increase peripheral resistance, elevate blood pressure and reduce blood flow in the microcirculation. Independent of any vascular constriction, the increased whole blood and plasma viscosity and reduced erythrocyte deformability seen in maternal blood in pre-eclampsia would reduce intervillous blood flow. A 10 per cent increase in whole blood viscosity causes a

20 per cent reduction in peripheral blood flow (32).

The elevation in maternal haematocrit in pre-eclampsia has been previously reported (151), as has the elevation in fibrinogen (203,215) and plasma viscosity (216). Heilmann, Mattheck and Kurz (1977) (139) investigated the haemorheology of 27 women with pre-eclampsia and found an increase in relative blood viscosity and a reduction in erythrocyte deformability.

The cause of the observed phenomena is not clear. Certainly an increase in haematocrit may be due to the reduction in plasma volume seen in patients with pre-eclampsia (217). Increased plasma viscosity is related to raised fibrinogen levels and also to an increase in immunoglobulins which occurs in pre-eclampsia (216). Loss of water from the intravascular compartment due to abnormal capillary permeability (217) may explain the alterations in all haemorheological parameters in pre-eclampsia without implicating increased production of any factor. The protein concentration does not rise because the albumin travels with the water leaving only the higher molecular weight globulins.

The relationship between hyperviscosity and disseminated intravascular coagulation is one for speculation at present. Slowing of the microcirculation due to hyperviscosity leading to further hypoxia and hyperviscosity will predispose to the formation of thrombi in the microvasculature. This process will operate not only in the placenta but in maternal organs such as the liver, kidneys and brain which are all affected by microvascular coagulation in eclampsia (218).

Whereas the maternal blood samples were taken before therapy was started, the fetal blood was collected after the mother had been treated for a variable period of time. The fetus had also been

exposed to the hypotensive and anticonvulsant therapy given to the mother and to the rigours of labour itself. In these respects the fetal samples from pre-eclamptic mothers differed from the controls.

The only consistent effect of pre-eclampsia on fetal haemorheology was an increase in the whole blood viscosity due to elevated haematocrit. Walker and Turnbull (1953) (151) proposed that fetal hypoxia, due to placental insufficiency from whatever cause, caused a secondary polycythaemia, and this may be the main haemorheological consequence of maternal pre-eclampsia. If, however, fetal hypoxia became more pronounced, due to either a worsening maternal pre-eclampsia or the onset of labour, then a deteriorating haemorheological situation could develop, with decreased erythrocyte deformability (Chapter 5, section ii), hypercoagulability (219,220) and elevated whole blood viscosity. The situation is then ripe for acute fetal distress and for thrombosis and haemorrhage at vulnerable areas of the fetal circulation.

In conclusion, it is interesting to note that some commonly used treatment regimes used in the management of pre-eclampsia have a haemorheological basis to their actions. The restoration of normal plasma volume with salt poor human albumin (221,222) or Rheomacrodex (223) has been shown to improve fetal outcome and maternal renal function in pre-eclampsia, by increasing peripheral blood flow due to reduction in plasma viscosity, haematocrit and whole blood viscosity. In order to avoid overloading of the circulation while giving plasma volume expanders, central venous pressure monitoring is essential (222,223). Another common treatment of pre-eclampsia is to give thiazide diuretics. These will further reduce plasma volume (224,225), increase haematocrit,

plasma and whole blood viscosity, and on the basis of these changes would be expected to reduce placental blood flow. An increasing number of clinical studies (226,227) have shown that diuretic therapy is not only unhelpful in the treatment of pre-eclampsia but may, in fact, be detrimental and there is certainly a good haemorheological basis to support this.

Table 18

Haemorheological Parameters in Patients with Pre-eclampsia and Normotensive Controls - Cross-sectional Study

		Pre-eclamptic Subjects					Normal Controls				
Gestation in Weeks		32	34	36	38	40	32	34	36	38	40
Number of Subjects		20	30	30	30	10	30	30	30	30	30
Haematocrit	Mean	0.383**	0.369*	0.357	0.363	0.361	0.346	0.342	0.348	0.358	0.359
	SD	0.024	0.026	0.028	0.024	0.023	0.020	0.018	0.019	0.020	0.019
Plasma Fibrinogen (g/l)	Mean	3.73*	3.91*	3.72	3.89	3.87	3.51	3.58	3.69	3.64	3.62
	SD	0.36	0.38	0.34	0.39	0.37	0.30	0.29	0.32	0.28	0.32
Total Serum Protein (g/l)	Mean	62.4	62.9	63.8	64.2	64.7	61.3	62.8	62.4	63.0	62.8
	SD	3.4	4.1	3.9	3.8	4.2	2.7	2.8	2.8	2.9	3.1
Plasma Viscosity (centipoise)	Mean	2.19**	2.17**	2.15*	2.08	2.10	1.86	1.90	1.92	1.92	1.90
	SD	0.16	0.15	0.18	0.19	0.19	0.13	0.12	0.11	0.12	0.14
Whole Blood Viscosity (centipoise)	Mean	11.56**	10.89**	11.35**	11.79*	12.41*	8.83	7.72	8.92	9.56	9.34
	SD	2.04	2.31	2.02	2.55	2.48	1.48	1.62	1.54	1.60	1.71
Erythrocyte Deformability Index	Mean	0.98*	0.93*	0.99	0.91	0.85	1.16	1.18	1.12	1.04	0.93
	SD	0.19	0.17	0.17	0.20	0.15	0.14	0.15	0.12	0.13	0.14

Statistical Comparison Using Student's t-test * $p < 0.05$ ** $p < 0.01$

Table 19 Haemorheological Profile of Infants Born to Mothers With Pre-eclamptic Toxaemia and Normotensive Controls - Cross-sectional Study

	Normotensive Controls	Pre-eclamptic Patients	Statistical Comparison
Number of Subjects	30	30	
Gestational age (weeks) Mean ± SD	36.8 ± 0.6	36.3 ± 0.7	ns
Birth Weight (g) Mean ± SD	3175 ± 209	2885 ± 215	p 0.05
Haematocrit Mean ± SD	0.477 ± 0.024	0.537 ± 0.037	p 0.01
Plasma Viscosity (centipoise) Mean ± SD	1.42 ± 0.30	1.55 ± 0.34	ns
Whole Blood Viscosity (centipoise) Mean ± SD	15.8 ± 1.9	17.9 ± 2.1	p 0.01
Erythrocyte Deformability Index	0.72 ± 0.10	0.67 ± 0.11	ns

Statistical Comparison Using Student's t-test

CHAPTER 8

HAEMORHEOLOGICAL CHANGES IN PREGNANCY COMPLICATED BY ESSENTIAL HYPERTENSION

Sections

i) INTRODUCTION

ii) MATERNAL AND FETAL HAEMORHEOLOGY IN ESSENTIAL HYPERTENSION

iii) DISCUSSION

HAEMORHEOLOGICAL CHANGES IN PREGNANCY COMPLICATED BY ESSENTIAL HYPERTENSION

i) INTRODUCTION

Essential hypertension, unless it is severe or further complicated by superimposed pre-eclampsia, carries a good fetal prognosis (228,229).

The haemorheological consequences of hypertension have been little studied. Dintenfass and Girolami (1978) (230) noted an increase in red cell rigidity in hypertensive patients. Tibblin et al (1965 and 1966) (231,232) observed that hypertensive patients had an elevated haematocrit, plasma and whole blood viscosity and a reduced plasma volume. They suggested that there was an increased leakage of water from the intravascular compartment with hypertension resulting in reduced plasma volume and secondary hyperviscosity, similar to the mechanism postulated in the previous chapter of this thesis to explain the hyperviscosity of pre-eclampsia.

ii) MATERNAL AND FETAL HAEMORHEOLOGY IN ESSENTIAL HYPERTENSION

This study was carried out to observe the effect, if any, of essential hypertension on the haemorheology of the mother during the third trimester of pregnancy and of the fetus at birth.

Patients and Methods

Firstly, 20 hypertensive patients were identified following their first visit to the antenatal clinic and were followed up with serial blood samples, as well as the routine antenatal care from 30 weeks until delivery. Essential hypertension was diagnosed on the basis of a resting blood pressure of 140/90 mm of mercury or greater in the first trimester of pregnancy, in the absence of renal or other causative disease. None of the patients were taking hypotensive therapy.

Secondly, 25 infants born to mothers with essential hypertension and 25 control infants born to normotensive mothers had blood sampled from the umbilical cord vein at birth.

Haemorheological methods were as described in Chapter 2.

Results

All the pregnant women had blood pressures varying between 140/90 and 150/105 mm of mercury and prior to the onset of labour none required regular hypotensive therapy. No patient had proteinuria.

The haemorheological parameters in the 20 hypertensive patients and 20 controls are set out in Table 20. The only difference between the two groups was a slightly elevated plasma viscosity in the hypertensive group at 40 weeks. It was common policy to induce labour in hypertensive patients between 38 and 39 weeks' gestation and only half the original reached their fortieth week.

Fetal haemorheological profiles are shown in Table 21. There was no difference in birth weight or in any of the haemorheological parameters measured between the two groups.

iii) DISCUSSION

The rise in haematocrit with hypertension found by Tibblin et al (1965) (231) is more than offset by the 'physiological anaemia' of pregnancy in the mild hypertensives who were the subject of this study. Similarly, the reduced plasma volume, increased plasma and whole blood viscosity (231,232) and the decreased erythrocyte deformability (230) previously reported in hypertension in non-pregnant subjects are swamped by the relatively large physiological change that takes place in normal pregnancy (Chapter 4, section ii).

Whether or not the haemorheological picture would be different in

severe hypertensives during pregnancy is a matter of conjecture, but as severe hypertension does cause placental insufficiency and fetal growth retardation, it is worth further investigating this problem. Fortunately, there are relatively few patients in this severely hypertensive category.

The lack of haemorheological change in pregnancy complicated by mild essential hypertension is in accord with the clinically good prognosis for the fetus.

Table 20 Haemorheological Parameters in Patients with Essential Hypertension and Normotensive Controls - Serial Study

		Hypertensive Subjects						Normal Controls					
Gestation in Weeks		30	32	34	36	38	40	30	32	34	36	38	40
Number of Subjects		20	20	20	18	14	10	20	20	20	20	20	20
Haematocrit	Mean	0.338	0.347	0.362	0.379	0.372	0.371	0.335	0.344	0.354	0.349	0.361	0.362
	SD	0.024	0.020	0.021	0.020	0.023	0.023	0.017	0.017	0.019	0.020	0.021	0.018
Plasma Fibrinogen (g/l)	Mean	3.44	3.54	3.78	3.81	3.65	3.74	3.42	3.50	3.75	3.82	3.35	3.75
	SD	0.32	0.34	0.34	0.32	0.36	0.29	0.32	0.29	0.30	0.31	0.34	0.35
Total Serum Protein (g/l)	Mean	62.5	62.8	63.0	62.9	64.1	63.7	62.5	61.3	61.0	61.8	61.9	60.6
	SD	3.8	4.3	3.9	4.2	4.3	4.1	3.1	2.9	2.8	2.9	3.4	3.2
Plasma Viscosity (centipoise)	Mean	1.98	2.02	2.03	2.10	2.09	2.13	1.95	1.94	1.95	1.85	1.98	1.87
	SD	0.21	0.23	0.19	0.18	0.19	0.21	0.13	0.11	0.12	0.12	0.14	0.15
Whole Blood Viscosity (centipoise)	Mean	8.35	8.38	8.94	9.47	11.38	11.31	7.01	7.82	7.56	8.84	9.84	9.91
	SD	1.83	1.87	1.91	1.62	1.83	1.79	1.52	1.61	1.57	1.62	1.72	1.73
Erythrocyte Deformability Index	Mean	1.15	1.14	1.16	1.12	1.09	1.02	1.15	1.18	1.12	1.09	1.04	1.04
	SD	0.18	0.18	0.16	0.19	0.17	0.17	0.14	0.13	0.14	0.15	0.14	0.11

Table 21 Haemorheological Profile of Infants Born to Mothers With Essential Hypertension and Normotensive Controls - Cross-sectional Study

	Normotensive Controls	Hypertensive Patients	Statistical Comparison
Number of Subjects	25	25	
Gestational age (weeks) Mean ± SD	37.8 ± 0.5	37.5 ± 0.6	ns
Birth Weight (g) Mean ± SD	3107 ± 201	3010 ± 285	ns
Haematocrit Mean ± SD	0.485 ± 0.032	0.493 ± 0.035	ns
Plasma Viscosity (centipoise) Mean ± SD	1.46 ± 0.33	1.48 ± 0.34	ns
Whole Blood Viscosity (centipoise) Mean ± SD	16.5 ± 2.4	16.7 ± 2.6	ns
Erythrocyte Deformability Index Mean ± SD	0.75 ± 0.11	0.73 ± 0.10	ns

Statistical Comparison Using Student's t-test

CHAPTER 9

HAEMORHEOLOGICAL CONSEQUENCES OF FETAL ASPHYXIA

Sections

i) INTRODUCTION

ii) IN VITRO EXPERIMENTS OF THE EFFECTS OF HYPOXIC ACIDOSIS

iii) ERYTHROCYTE DEFORMABILITY CHANGES WITH FETAL ASPHYXIA

iv) DISCUSSION

HAEMORHEOLOGICAL CONSEQUENCES OF FETAL ASPHYXIA

i) INTRODUCTION

In spite of the sophisticated antenatal, intrapartum and neonatal monitoring techniques designed to alert the clinician to signs of fetal or neonatal asphyxia, hypoxic brain injury remains a major cause of perinatal death or later neurological disability (233,234). Histological evidence indicates that nearly all infants dying within the first few days after birth have hypoxic brain injury (235). Computor assisted tomography has revealed that more than 40 per cent of preterm neonates, with a birth weight of less than 1500 G, have cerebral bleeds in the first three or four days of extrauterine life, but only 25 per cent of cases are fatal and many preterm infants with cerebral bleeds remain symptomless or subsequently develop communicating hydrocephalus (236, 237).

The anatomical distribution of these hypoxic vascular injuries is thought to be determined primarily by the vascular supply to these regions. They arise in the border zones between the end fields of the penetrating branches of the middle cerebral artery and of subependymal branches of the choroidal vessels (238,239). Necropsy dye injection studies of Hambleton and Wigglesworth (1976) (240) showed that the bleeding usually originated from the capillaries in these regions.

The pathogenesis of hypoxic haemorrhage remains speculative. Many workers have sought to show a relationship between hypoxic acidosis and coagulation failure (241-243), but Chessels and Wigglesworth (1972) (241) conclude that haemostatic failure is secondary and that avoidance of hypothermia and correction of acidosis are more important than treating haemostatic abnormalities.

Recent work by Lou, Lassen and Friis-Hansen (1979) (244,245) has

shown that asphyxiated neonates have both a markedly reduced cerebral blood flow, and a loss of the normal autoregulatory control. In the absence of vascular autoregulation, haemorheological factors become of prime importance (Chapter 1, section ii).

The following *in vitro* and *in vivo* studies were designed to investigate the effect of hypoxic acidosis on erythrocyte deformability, and combined with data from earlier chapters on the effect of pH change on whole blood and plasma viscosity, form the basis for a hypothesis for the pathogenesis of hypoxic brain injury.

ii) IN VITRO EXPERIMENTS ON THE EFFECT OF HYPOXIC ACIDOSIS ON ERYTHROCYTE DEFORMABILITY

Materials and Methods

Thirty 10 ml aliquots of erythrocyte suspension prepared for Erythrocyte Deformability Index measurement from fetal blood. Ten aliquots were incubated at each pH level (7.4, 7.1 and 6.8) for four hours and then the pH of all aliquots was readjusted to 7.4 for a further hour. Erythrocyte Deformability Index measurements were made on each aliquot after one, two, four and five hours' incubation. At each sampling time the pH was checked and adjustment of the carbon dioxide - oxygen mixture made as necessary. The pH adjustments and Erythrocyte Deformability Index measurements were made as described in Chapter 2.

Results

The Erythrocyte Deformability Index measurements in the samples are shown in Table 22. Decrease in pH was associated at each measurement time with a significant decrease in the Erythrocyte Deformability Index ($p < 0.001$). The time of incubation also

affected the deformability at each pH level, with a significantly lower Erythrocyte Deformability Index after four hours' incubation at pH 7.4 ($p<0.05$), pH 7.1 ($p<0.001$) and pH 6.8 ($p<0.001$). Reincubation of anoxic erythrocytes at a pH of 7.4 for one further hour resulted in a return of the Erythrocyte Deformability Index to the same level as erythrocytes that had been at pH 7.4 for the full five hours.

Figure 27 is a repeat of Figure 26 (Chapter 5, section ii) and shows the effect of pH change on adult and fetal erythrocyte deformability.

Figure 28 is a repeat of Figure 18 (Chapter 2, section iii) and shows the effect of pH change on plasma viscosity, Erythrocyte Filtration Rate and Erythrocyte Deformability Index.

iii) ERYTHROCYTE DEFORMABILITY CHANGES IN CLINICAL FETAL ASPHYXIA

Patients and Methods

Blood was collected from 30 neonates who were delivered following cardio-tocographic evidence of fetal distress. The haematocrit, pH and Erythrocyte Deformability Index were measured in cord blood.

Results

The cord blood pH and Erythrocyte Deformability Index are shown in Figure 29. Comparing the reduction of Erythrocyte Deformability Index *in vivo* (Figure 29) with that *in vitro* (Figure 27) with decreasing pH levels gives a good comparison, with similar degrees of reduction in both situations.

The mean haematocrit of the 30 samples was 0.597, SD 0.048. Comparison with the haematocrits in infants of similar gestational ages, as shown in Table 14 (Chapter 5, section iii), shows that these infants

who showed evidence of fetal distress in labour had a significantly higher haematocrit ($p < 0.001$).

iv) DISCUSSION

The in vitro section of this study further illustrates the sensitivity of fetal erythrocytes to hypoxic acidosis shown in Chapter 5, section ii. This reduction in deformability is, however, a reversible phenomenon, as shown in Table 22. This reversibility of decreased erythrocyte deformability has been shown in several studies on stored blood bank specimens and is due to the highly sensitive metabolic dependency of the erythrocyte membrane enzyme systems (246,247).

The clinical study confirms the in vitro effects of acidosis on erythrocyte deformability and confirms previous observations that infants who exhibit fetal distress in labour have higher cord haematocrit levels (151). This indicated that many of these infants have been under chronic hypoxic stress for some time before the onset of labour, and the stress of labour has exacerbated an already compromised hypoxic state.

The clinical implications of this study are interesting and a hypothesis for a possible mechanism whereby hypoxic acidosis may cause intracerebral and intraventricular haemorrhage can be constructed.

Hypoxic acidosis by causing a pronounced decrease in erythrocyte deformability (Figures 27, 28 and 29) and an increase in whole blood viscosity, through this action on erythrocyte deformability, in association with an already elevated haematocrit in the case of growth retarded fetuses, will create a situation of hyperviscosity which, when combined with the already lowered cerebral blood flow,

lack of autoregulation and hypotension of fetal asphyxia (245) is ideal for thrombosis in the vulnerable microcirculation of the periventricular region. Capillary thrombosis may rapidly lead to haemorrhage in a situation now complicated by secondary coagulation failure (242,243).

This vicious circle of hypoxia and hyperviscosity may operate during antenatal development as a chronic situation, during labour in a more acute way and also in the neonatal period. Hypoxia from whatever cause is associated, in the neonate, with hyperbilirubinaemia (248), impaired peripheral circulation (249), oliguria (250) and, if severe enough, with disseminated intravascular coagulation (251).

Table 22

Reversibility of Erythrocyte Deformability Change Caused by Hypoxic Acidosis in vitro

Incubation Time	Erythrocyte Deformability Index (Mean ± SD)			
	1 Hour	2 Hours	4 Hours	Back to pH 7.4 for 1 Hour
pH 7.4	0.71 ± 0.17	0.69 ± 0.17	0.60 ± 0.11	0.58 ± 0.10
pH 7.1	0.58 ± 0.15	0.47 ± 0.14	0.33 ± 0.12	0.55 ± 0.11
pH 6.8	0.38 ± 0.13	0.29 ± 0.11	0.23 ± 0.09	0.53 ± 0.13

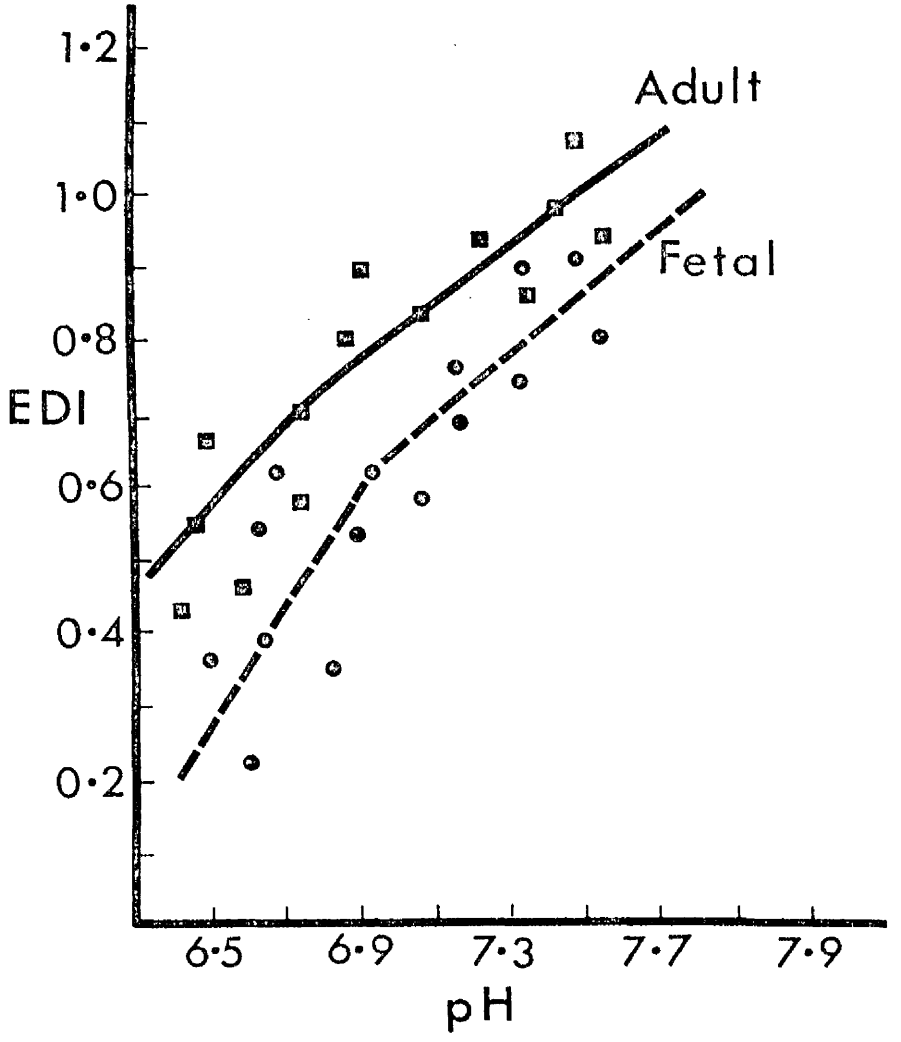

Figure 27 The Relationship Between pH Level and Erythrocyte Deformability Index (EDI) in Fetal and Adult Blood

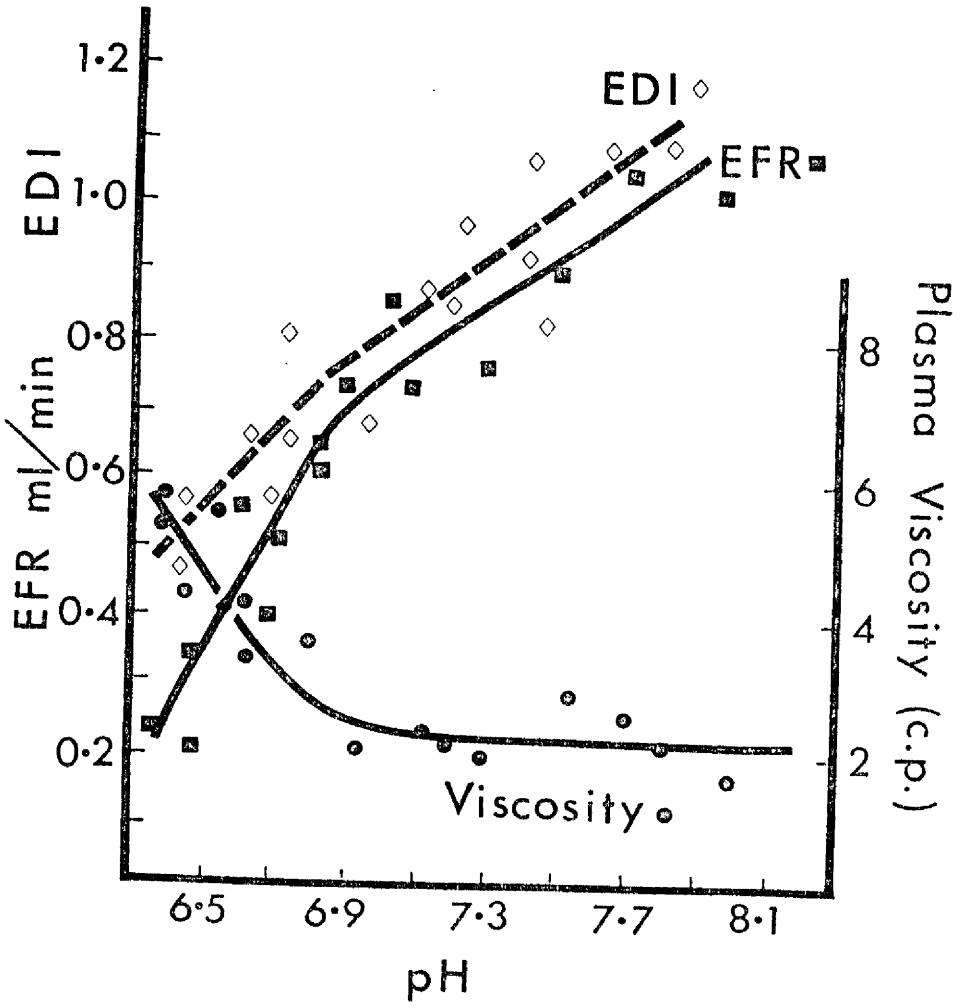

Figure 28 The Effect of Variation in Sample pH Level on Plasma Viscosity, Erythrocyte Filtration Rate (EFR)

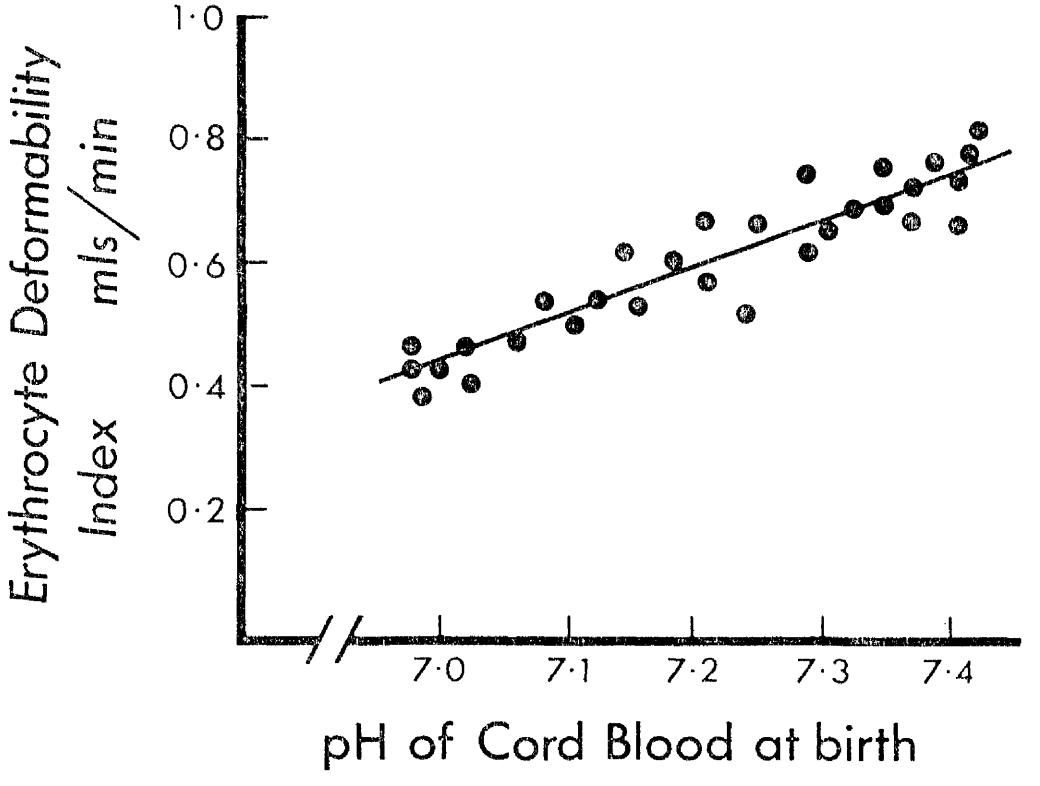

Figure 29 The Relationship Between Cord Blood pH and Erythrocyte Deformability Index

CHAPTER 10

THE PATHOGENESIS OF NEONATAL HYPERBILIRUBINAEMIA FOLLOWING INDUCTION OF LABOUR

Sections

i) INTRODUCTION

ii) HAEMORHEOLOGICAL HAEMATOLOGICAL AND BIOCHEMICAL MEASUREMENTS IN CORD BLOOD FOLLOWING ELECTIVE CAESARIAN SECTION, SPONTANEOUS AND OXYTOCIN INDUCED LABOUR

iii) THE EFFECT OF OXYTOCIN, BUPIVICAINE AND PROSTAGLANDIN E2 ON FETAL ERYTHROCYTE DEFORMABILITY IN VITRO

iv) DISCUSSION

THE PATHOGENESIS OF NEONATAL HYPERBILIRUBINAEMIA FOLLOWING INDUCTION OF LABOUR

i) INTRODUCTION

Several iatrogenic factors have been implicated in the aetiology of neonatal hyperbilirubinaemia.

The association between oxytocin-induced labour and neonatal hyperbilirubinaemia is well documented (252-259). Several reports (254-256) have shown that the degree of hyperbilirubinaemia is related to the total dose of oxytocin given to the mother during labour. The actual mechanism whereby oxytocin caused hyperbilirubinaemia has been a matter of considerable debate. Ghosh and Hudson (1972) (257) suggested that induced labour was associated with anoxic damage to the fetal hepatic glucuronyl transferase enzyme system which is necessary for bilirubin excretion. Davies et al (1973) (256) proposed that the hormonal factors which initiate spontaneous labour are necessary for the induction of fetal hepatic enzymes which remain inactivated following induced labour. Oski (1975) (258) put forward the theory that the strong uterine contractions induced by oxytocin caused an increased placento-fetal transfusion with a resultant polycythaemia in the neonate. Singhi and Singh (1977) (259) reported that fetal erythrocytes were more osmotically fragile following oxytocin-induction and D'Souza et al (1979) (255) found evidence of active haemolysis in cord blood and suggested that the uterine contractions were in some way damaging the erythrocytes and so accelerating their destruction.

Prostaglandin E2 given intravenously for the induction of labour was shown in a study by Calder et al (1974) (260) to be associated with neonatal hyperbilirubinaemia, but this was not confirmed by Beazley and Weeks (1976) (261).

Epidural analgesia with bupivicaine was implicated as a cause of neonatal hyperbilirubinaemia by Friedman et al (1978) (252) but Calder et al (1974) (260) had found no such association.

Instrumental and breech delivery are well known causes of neonatal hyperbilirubinaemia (262,263) because of the tissue bruising caused by forceful handling.

This study was stimulated by the observation that fetal erythrocytes were less deformable following oxytocin-induced labour than after spontaneous labour. Erythrocyte deformability is an important determinant of erythrocyte lifespan in vivo (65-67) and decreased erythrocyte deformability has been shown to be the precursor of haemolytic anaemia in acute copper intoxication (264), cyanate poisoning (265) and uraemia (266).

ii) HAEMORHEOLOGICAL, HAEMATOLOGICAL AND BIOCHEMICAL MEASUREMENTS IN CORD BLOOD FOLLOWING ELECTIVE CAESARIAN SECTION, SPONTANEOUS AND OXYTOCIN-INDUCED LABOUR

Patients and Methods

Ninety-five healthy newborn infants, who weighed more than 3 Kg at birth and who had an apgar score of greater than seven at one minute, had 20 ml of blood collected from the umbilical cord vein as soon as the cord had been clamped and before the placenta had separated, using a wide bore needle and gentle suction to avoid haemolysis during sampling. The blood was anticoagulated with lithium heparin.

Forty of these infants had been delivered following spontaneous labour and 40 following induction of labour by amniotomy and intravenous oxytocin (Syntocinon) administered by Cardiff infusion pump. The average dose of oxytocin given was 4,500 mU (range 3,000 to 7,000 mU). In the cases selected for this study the duration of

labour was between six and 14 hours and analgesia was achieved using intramuscular pethidine, no patient had epidural analgesia. In none of the cases was there clinical or cardiotocographic evidence of fetal distress and all had spontaneous vertex deliveries. The remaining fifteen infants were delivered by elective caesarian section. Within two hours of delivery haematocrit, plasma osmolality, Erythrocyte Deformability Index, plasma bilirubin, plasma haptoglobin and plasma lactate dehydrogenase were measured on all samples by the methods described in Chapter 2.

Results

The 95 infants, 44 male and 51 female, had a mean birth weight of 3,420 g (range 3,005 to 4,108 g). Haematological, haemorheological and biochemical values in cord blood from these infants are shown in Table 23.

There was no significant difference in any of the measured parameters between the elective caesarian section and spontaneous labour groups. The oxytocin-induced group, however, showed statistically significant evidence of active haemolysis during labour with a decreased haematocrit ($p < 0.001$), increased plasma bilirubin concentration ($p < 0.001$), decreased plasma haptoglobin concentration ($p < 0.01$), and increased plasma lactate dehydrogenase activity ($p < 0.001$). These infants also showed a significant reduction in mean Erythrocyte Deformability Index ($p < 0.001$) and plasma osmolality ($p < 0.001$).

iii) THE EFFECT OF OXYTOCIN, BUPIVICAINE AND PROSTAGLANDIN E2 ON ERYTHROCYTE DEFORMABILITY IN VITRO

Materials and Methods

In vitro studies were made on fetal blood obtained at birth from

the umbilical vein of a number of normal infants.

Firstly, 3,000 μU of oxytocin (Syntocinon) were added to 20 ten ml aliquots of erythrocyte suspension and 500 μl of normal saline were added to a duplicate 20 ten ml aliquots prepared from the same cord bloods. The samples were incubated at 37°C and the Erythrocyte Deformability Index measured at intervals on both sets of samples.

Secondly, 50 five ml aliquots of erythrocyte suspension were prepared and 500 μl of normal saline, 500 μU, 1,500 μU, 2,500 μU or 4,000 μU of oxytocin were added to groups of ten aliquots which were then incubated for four hours and the Erythrocyte Deformability Index of each sample measured in duplicate.

Thirdly, 2,000 ng of bupivicaine were added to 10 ten ml aliquots of erythrocyte suspension and 500 μl of normal saline to a duplicate set of aliquots. The samples were incubated at 37°C and the Erythrocyte Deformability Index measured at intervals on both sets of samples.

Fourthly, 25 five ml aliquots of erythrocyte suspension were prepared and 250 μl of normal saline, 250 ng, 500 ng, 750 ng or 1,000 ng of bupivicaine were added to groups of five aliquots which were then incubated for four hours and the Erythrocyte Deformability Index of each sample measured in duplicate.

Fifthly, 3,000 ng of prostaglandin E2 were added to 10 ten ml aliquots of erythrocyte suspension and 500 μl of normal saline were added to a duplicate set of samples. The samples were incubated at 37°C and the Erythrocyte Deformability Index measured at intervals on both sets of samples.

Sixthly, 25 five ml aliquots of erythrocyte suspension were prepared

from the same cord bloods and 500 µl of normal saline, 500 ng, 1,500 ng, 2,500 ng or 4,000 ng of postaglandin E2 were added to groups of five aliquots which were then incubated for four hours and the Erythrocyte Deformability Index of each sample measured in duplicate.

Results

Incubation of erythrocytes with oxytocin showed both a time related (Figure 30) and a dose related (Figure 31) reduction in Erythrocyte Deformability Index. The incubation duration affected the degree of deformability reduction. There was no difference between the oxytocin and control samples at one and two hours, but there was a significantly greater reduction in deformability in the oxytocin treated samples at four hours ($p < 0.05$) and at six and eight hours ($p < 0.001$). Varying the dose of oxytocin in the erythrocyte suspensions showed that a dose of 100 µU/ml caused no significant reduction in deformability, but 300 µU/ml caused a significant fall in deformability ($p < 0.001$) and a further decrease at doses of 500 and 800 µU/ml ($p < 0.001$).

Incubation of erythrocytes with bupivicaine showed both a time related (Figure 32) and dose related (Figure 33) reduction in Erythrocyte Deformability Index. The incubation duration affected the degree of deformability reduction. There was no difference between the bupivicaine treated and control samples at one, two and four hours, but at six hours the bupivicaine treated samples showed a significantly greater reduction in deformability ($p < 0.05$) and at eight hours the difference was even greater ($p < 0.001$). Varying the dose of bupivicaine showed that it was only at the maximum dose tested, 200 ng/ml, that the Erythrocyte Deformability Index was just significantly reduced ($p < 0.05$).

Incubation of erythrocytes with prostaglandin E2 showed an initial

reduction in Erythrocyte Deformability Index ($p < 0.001$) after 30 minutes (Figure 34), after one hour the deformability in prostaglandin E2 treated samples was still slightly less than in the controls ($p < 0.05$) but at two and three hours there was no differences between treated samples and controls. Variation of the dose of prostaglandin E2 gave a biphasic response (Figure 35) with no effect of 100 ng/ml, a significant depression of deformability at 300 ng/ml ($p < 0.001$) an increase from that level back to the control level at 500 ng/ml and an elevation in deformability above the control level at a dose of 800 ng/ml of prostaglandin E2 ($p < 0.05$).

iv) DISCUSSION

The clinical section of this study gives clear evidence of active haemolysis during oxytocin-induced labour. The reduced haematocrit and elevated plasma bilirubin concentration after oxytocin-induced labour confirm the findings of D'Souza *et al* (1979) (255), and the low plasma haptoglobin concentration and increased plasma lactate dehydrogenase activity confirm that accelerated erythrocyte destruction was taking place during induced labour. The plasma haptoglobin level in neonates is normally low (267), but the very low values observed in the infants after induction suggests an active process of haemolysis with release of haemoglobin into the circulation. The finding that fetal erythrocyte deformability was reduced after oxytocin-induction and the knowledge that decreased deformability leads to accelerated haemolysis indicate the pathway of haemolysis in the induced group, and only the cause of the reduced deformability remained to be proved.

The levels of oxytocin found in maternal blood during oxytocin-induced labour are between 300 and 800 μU/ml (268) and although

absolute evidence of materno-fetal transfer of oxytocin is lacking in the human, in the sheep (269) and guinea pig (270) there is clear evidence of placental transfer of oxytocin from mother to fetus. The in vitro experiments demonstrate both a time and dose related effect of oxytocin in a dosage range of 300 to 800 μU/ml on fetal erythrocytes. The mechanism whereby oxytocin reduces erythrocyte deformability cannot be absolutely defined, but the finding of a lowered plasma osmolality in cord blood following oxytocin-induced labour confirms the observations of Singhi and Singh (1977) (259) and suggests that the vasopressin-like action of oxytocin (271) causes activation of electrolyte and water transport across the renal tubules but also across the erythrocyte membrane with consequent osmotic swelling. Osmotic swelling is a well recognised cause of decreased erythrocyte deformability and leads to accelerated erythrocyte destruction (272,273). In the neonate whose hepatic enzymes are not able to cope with the increased bilirubin production, clinical hyperbilirubinaemia ensues.

Placental transfer of bupivicaine from mother to fetus is well documented (273-275) with fetal levels following caesarian section under epidural anaesthesia with bupivicaine of up to 210 ng/ml (274). Following epidural analgesia in labour, the bupivicaine concentration in fetal blood was up to 110 ng/ml (275) and similar levels have been detected following paracervical block with bupivicaine (276). The half life of bupivicaine in the neonate is in the order of two hours (274), so after four hours after caesarian section, under bupivicaine epidural anaesthesia, the fetal blood level will be around 50 ng/ml. In the in vitro studies, it was shown that bupivicaine in a dose of 200 ng/ml incubated with fetal erythrocytes for four hours caused

only a small reduction in deformability which just reached statistical significance. It is questionable whether such small changes in deformability would have a significant effect on haemolysis *in vivo*, but as epidural analgesia is commonly used during induced labour, so the effects of oxytocin and bupivicaine may combine.

Placental transfer of prostaglandin E2 from mother to fetus has been demonstrated (277) with widely varying levels of prostaglandin E2 in cord blood being reported. Seigler *et al* (1977) (278) reported levels up to 1,480 pg/ml, and Pokoly and Jordan (1975) (279) found levels between 400 and 1,000 pg/ml. Using these levels in preliminary *in vitro* experiments gave absolutely no effect on erythrocyte deformability and it was at levels over 200 ng/ml that the first alterations in erythrocyte deformability were seen. Allen and Rasmussen (1971) (280) and Gruber and Gilbertson (1978) (281) used similar ng levels in their experiments on the influence of prostaglandin E2 on deformability and sickling of erythrocytes. The effect of prostaglandin E2 in reducing deformability is short lived, being maximal at 30 minutes and absent after two hours. This agrees with earlier work (281) as does the finding of a biphasic dosage response (280). It seems highly unlikely that prostaglandin E2 significantly effects erythrocyte deformability in induced labour and if it is responsible for neonatal hyperbilirubinaemia (260), then some other mechanism of action must be sought.

In conclusion, oxytocin is an important therapeutic agent in obstetrics and probably its effect on erythrocytes cannot be prevented other than by keeping the total dose used to a minimum. The use of prenatal drug treatment with either phenobarbitone (282) or antipyrene (283) to activate fetal hepatic glucuronyl transferase and so increase the neonate's ability to eliminate bilirubin has

been suggested, but it would be more logical to prevent the hyperbilirubinaemia by reducing the dose of oxytocin rather than treat it with potentially toxic drugs. This may be safely done with no added risk of hyperbilirubinaemia by the pre-induction use of prostaglandin E2 given vaginally (284), or by the combined use of prostaglandin and oxytocin in the induction of labour (285). The combined use of epidural analgesia and oxytocin-induction of labour will continue to present a higher risk of neonatal hyperbilirubinaemia but awareness of the problem should enable the clinician to minimise the risks.

Table 23 Haemorheological, Haematological and Biochemical Measurements in Cord Blood

Cord Blood	Elective Caesarian Section Mean ± SE (n)	Spontaneous Labour Mean ± SE (n)	Oxytocin-Induced Labour Mean ± SE (n)	'p'
Haematocrit	0.52 ± 0.012 (15)	0.53 ± 0.013 (40)	0.47 ± 0.014 (40)	0.001
Erythrocyte Deformability Index	0.83 ± 0.03 (15)	0.81 ± 0.04 (40)	0.61 ± 0.06 (40)	0.001
Plasma Bilirubin u mol/l	31 ± 1.2 (15)	33 ± 1.3 (40)	38 ± 1.5 (40)	0.001
Plasma Haptoglobin g/l	---	0.091 ± 0.024 (25)	0.023 ± 0.006 (25)	0.01
Plasma Lactate Dehydrogenase I U/l	---	223 ± 8 (25)	346 ± 25 (25)	0.001
Plasma Osmolality mosmol/l	286 ± 4 (15)	290 ± 6 (40)	277 ± 6 (40)	0.001

'P' was derived using Student's t-test comparing oxytocin induced with spontaneous labour

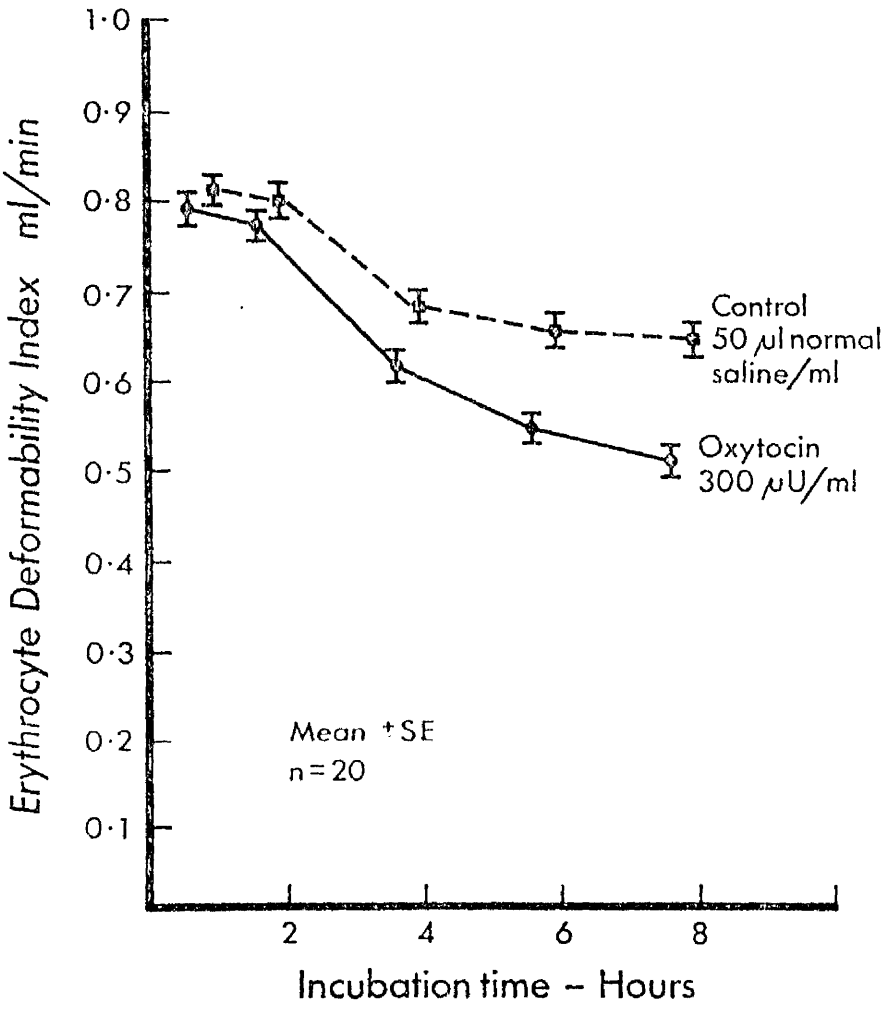

Figure 30 Effect of Increasing Incubation Time With Oxytocin on Erythrocyte Deformability

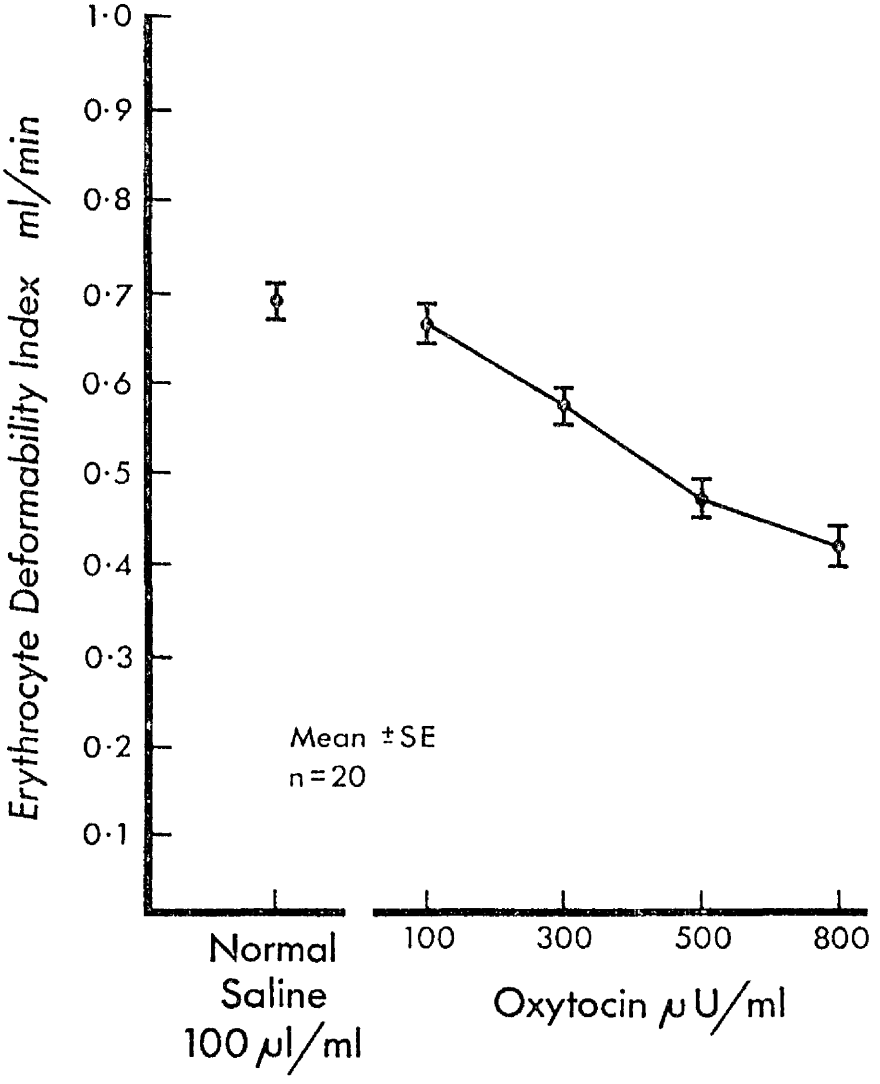

Figure 31 Effect of Increasing Oxytocin Dosage on Erythrocyte Deformability

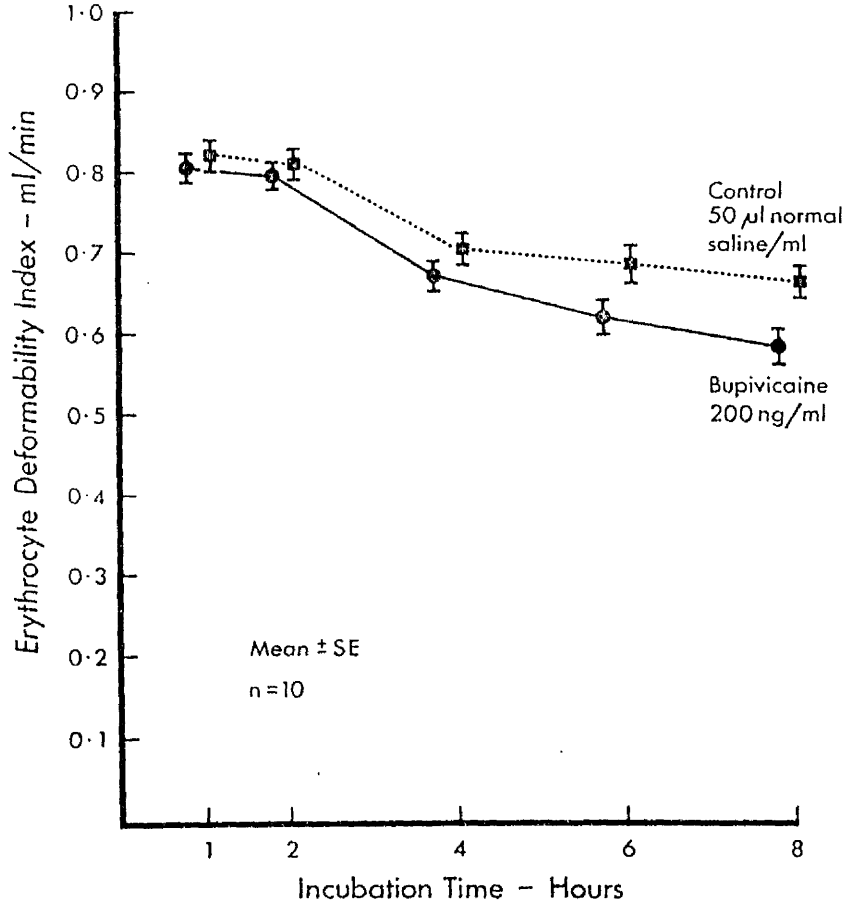

Figure 32 Effect of Increasing Incubation Time With Bupivicaine on Erythrocyte Deformability

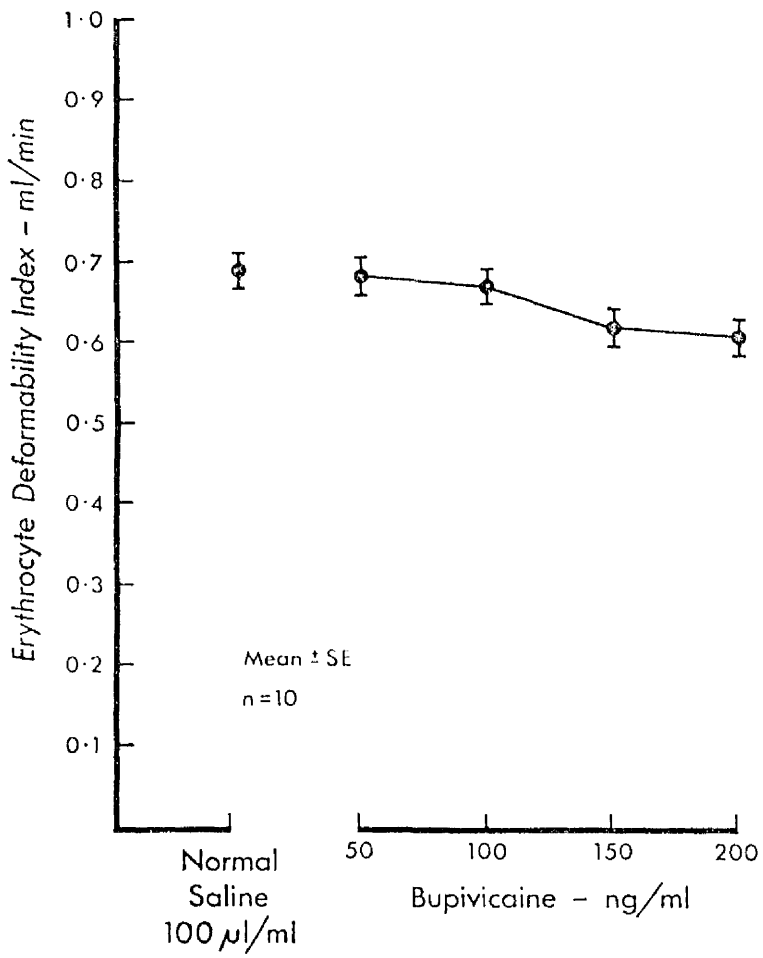

Figure 33 Effect of Increasing Bupivicaine Dosage on Erythrocyte Deformability

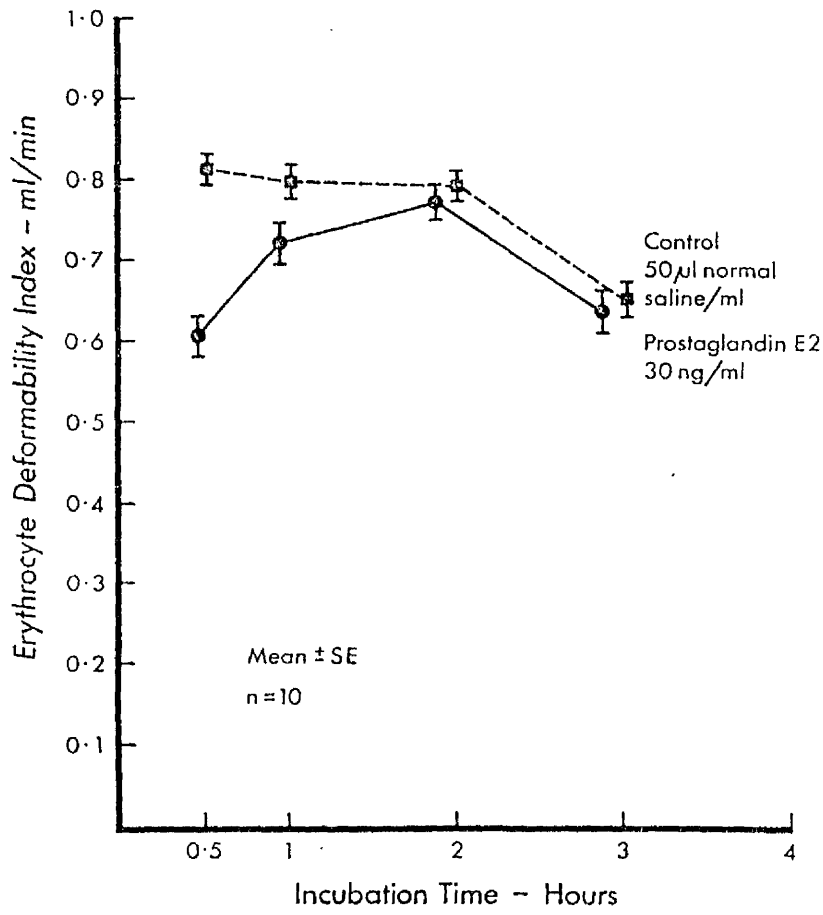

Figure 34 Effect of Increasing Incubation Time With Prostaglandin E2 on Erythrocyte Deformability

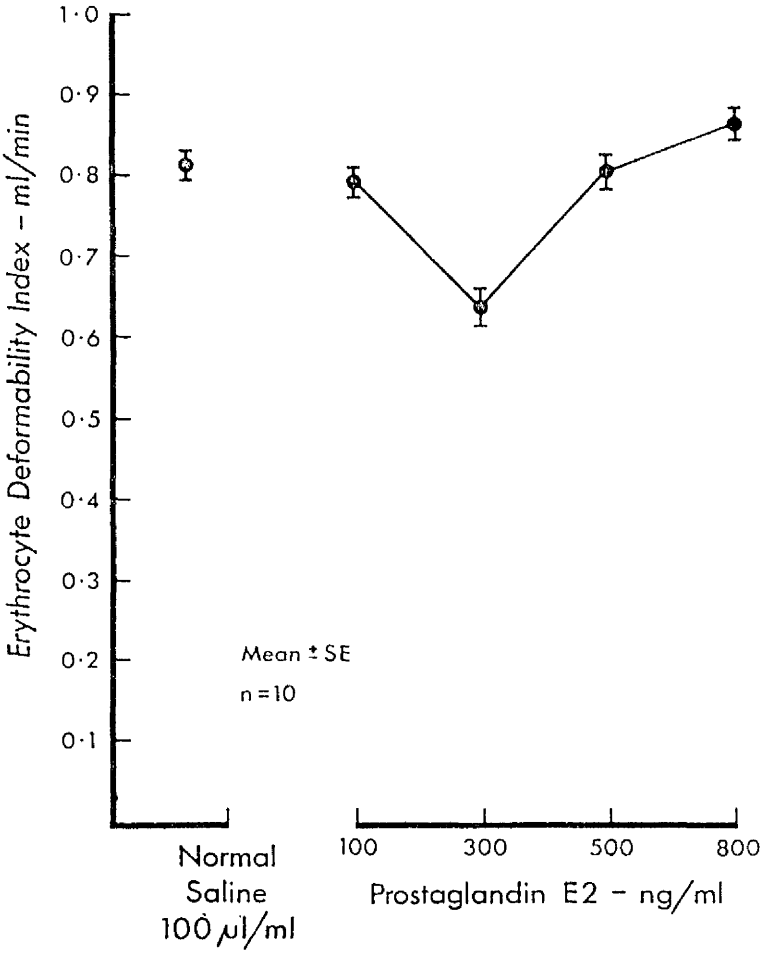

Figure 35 Effect of Increasing Prostaglandin E2 Dosage on Erythrocyte Deformability

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