University of Southampton

ABSTRACT

FACULTY OF MEDICINE

Doctor of Medicine

SIZE AT BIRTH AND NEONATAL FIBRINOGEN

by

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A raised plasma fibrinogen is strongly associated with an increased risk of cardiovascular disease. 'Programming' in early life may influence cardiovascular disease in adult life. Poor growth in infancy has been linked to high plasma fibrinogen levels in adult life. This work hypothesises that reduced fetal growth is associated with high neonatal fibrinogen concentration. A cross-sectional study of neonates over a two-year period was undertaken in the Isle of Man. The subjects were primigravida mothers and their 4 day old babies. Measurements were taken of neonatal body size and plasma fibrinogen concentrations and maternal height. Data from maternal questionnaires and hospital records were collected. The main outcome measure was plasma fibrinogen. The results showed that low birthweight was associated with low neonatal plasma fibrinogen concentrations. The relationship was the opposite of that between adult plasma fibrinogen concentrations and early growth. Neonatal fibrinogen concentrations were also associated with the acute phase reaction and the sex of the neonate. The relation of larger size at birth with higher neonatal fibrinogen concentrations may indicate improved liver function in heavier babies.

FIGURE 1

Mother and baby.

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DEFINITIONS OF ABBREVIATIONS USED

B	Regression coefficient
BMI	Body mass index
BMR	Basal Metabolic Rate
CI	Confidence Interval
CRP	C-reactive Protein
FDP	Fibrinogen Degradation Products
FpA	Fibrinopeptide A
FpB	Fibrinopeptide B
HDL	High Density Lipoprotein
HMW	High Molecular Weight
HMWK	High Molecular Weight Kallikrein
hPL	Human Placental Lactogen
IGF-1	Insulin-like Growth Factor
IUGR	Intrauterine Growth Retardation
LMW	Low Molecular Weight
LDL	Low-density Lipoprotein
mPas	Milli-Pascal seconds
p	Probability
PDGF	Platelet-derived Growth Factor
PAI	Plasminogen Activator Inhibitor
PRL	Prolactin
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
RFLP	Restriction Fragment Length Polymorphism
SMR	Standardised Mortality Ratio
SD	Standard Deviation
SE	Standard error
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
t-PA	Tissue Plasminogen Activator

NEONATAL FIBRINOGEN AND SIZE AT BIRTH

'In my beginning is my end'

T.S.Eliot

'Socially and individually the response of human beings to the conditions of the present is always conditioned by the biological remembrance of things past'

R. Dubos

STRUCTURE OF THIS THESIS

This thesis considers the relationship between size at birth and neonatal fibrinogen levels and is in six sections.

Section 1 introduces the subject by describing the two main reasons why this work is both topical and relevant.

Section 2 reviews the literature on size at birth and neonatal fibrinogen. The first part is about size at birth and the variables which influence it. The size and development of the placenta and liver are considered. Constraints on fetal growth are discussed, including undernutrition in pregnancy and intrauterine growth retardation.

The relationship between fetal growth, height and adult health are then discussed and the long term effects of poverty on health. The association of reduced early growth and height, cardiovascular disease, hypertension and impaired glucose tolerance are considered. The concept of programming is described and the contribution made by animal research is acknowledged. The debate about the fetal origins of adult disease is considered.

The next part looks at the structure, genetic determination, synthesis and functions of fibrinogen, followed by a summary of neonatal haemostasis. Sialic acid and inhibitors in blood coagulation are considered, followed by subsections on fibrinolysis and 'fetal' fibrinogen. The acute phase response, fibrinogen and C-reactive protein are then discussed with particular reference to the neonate. The rationale for measuring C-reactive protein alongside plasma fibrinogen concentrations in this study is stated. Fibrinogen in children and pregnancy is considered.

Known associations with raised and lowered plasma fibrinogen concentrations are summarised. The evidence for fibrinogen as an independent cardiovascular risk factor is presented. The association between reduced early growth and plasma fibrinogen concentrations in the adult is examined. The last part discusses methodology and a raised haematocrit as a possible causes of bias.

Section 3 states the hypothesis and study questions. The design of the study is outlined.

Section 4 is devoted to subjects and methods, with details of the criteria for inclusion and exclusion. The statistical methods are stated.

Section 5 contains the results starting with the pilot study. The mothers, fathers and neonates are characterized, and the levels of fibrinogen.
The relationships that have been found between size at birth and fibrinogen levels are stated. Other influences on these relationships are considered, starting with aspects of the mothers' life and health and continuing with an examination of the influence of perinatal variables and the acute phase reaction.

Section 6 starts with a discussion of the subjects and methods. Then the results are discussed with particular reference to possible confounders such as gender, age and neonatal infection. The results are considered with regard to the literature on fibrinogen and size at birth. Conclusions are drawn and implications for further research are suggested.

N. B. Summaries of individual sections are expressed in Italics.

1. Introduction

In recent years the epidemiology of cardiovascular disease has been enlightened by two new concepts.

The thrombotic component of vascular occlusion is now known to be of major importance in the epidemiology of vascular occlusion. Until the late 1970's lipid infiltration was deemed to be almost exclusively the cause of atherosclerosis and little attention was given to the thrombotic component of vascular occlusion. It was then proved that a high fibrinogen level in middle life is strongly associated with cardiovascular disease (Meade et al, 1986).

In recent years growth in early life and the development of cardiovascular disease in adult life have been linked by the 'Barker hypothesis'.
The concept of 'programming' in utero and infancy states that the cause of cardiovascular disease originates in the rapidly growing fetus and infant if it is constrained by its nutritional and/or hormonal environment. Reduced early growth is associated with cardiovascular disease (Barker & Osmond, 1986).

These two contributions in understanding the causes of cardiovascular disease were linked when it was shown that plasma fibrinogen levels were consistently lower in adults who were heavier at one year and that conversely underweight babies at one year grew into adults who had high fibrinogen levels in middle life (Barker et al, 1992b). In addition plasma fibrinogen levels in adults fell with increasing birthweight and abdominal circumference (Martyn et al, 1995a). However the possibility that early growth as expressed by size at birth may be a determinant of the plasma fibrinogen level in the neonate has not previously been examined. If an association can be found between early growth and a risk factor, prospective follow up studies can be planned to track changes with increasing age.

2 Review of literature

The first part of this chapter examines the literature on size at birth. It reviews the variables which influence size at birth and the associations between poverty, birthweight and health in adult life. The concept and evidence for 'programming' is discussed and the consequent importance of examining risk factors in childhood.

2.1 Fetal growth and size at birth

2.1.1 The placenta and the fetus

The placenta develops from fetal tissue and growth is most rapid at the beginning of pregnancy (Alexander, 1978). The weight of the placenta increases until near term but at a slower rate than that of the fetus; the weight ratio of placenta to fetus changes on average from 1:5 at 28 weeks to 1:7 at term. Placental function declines with advancing pregnancy and may become the limiting factor in fetal growth (Gruenwald, 1963, 1974).

The growth and function of the placenta are influenced by disruption or reduction in oxygen or nutrients (Robinson et al, 1979). Reduced oxygen supply - such as from living at a high altitude or severe maternal anaemia - produces heavier placentae and lighter babies (Kruger & Arias,1970, Beischer et al, 1970). Animal studies show that the placenta grows faster than the fetus in early gestation and is most sensitive to nutrient supply at this time. A compensatory increase in placental but not fetal size occurs when mild undernutrition or anaemia is present (Owens et al, 1989, McCrabb et al, 1992). In humans a similar compensatory mechanism has been demonstrated (Godfrey et al, 1991). In the war-induced Dutch famine of 1944-45 there was an increase in placental weight, but not birthweight, in babies whose mothers' nutrition was compromised around conception or in the first trimester of pregnancy (Lumey, 1998).

2.1.2 Fetal hormones

Many maternal protein hormones do not cross the placenta, and the fetal supply comes from placental production (Gitlin & Gitlin, 1975). Growth hormone is present in the human fetus from 12 weeks and reaches levels of 10 ng/ml by the middle of pregnancy, levels which would be associated with acromegaly in adult life (Grumbach & Kaplan, 1973). Thereafter the levels drop gradually, but are still higher in cord blood than later in childhood. Despite this abundance, growth hormone seems to have a limited effect on intrauterine growth. Insulin and growth hormone may act together to augment fetal growth indirectly. For example, it has been shown that growth hormone - from either the pituitary or the placenta - promotes beta - cell replication and insulin release in pancreatic islet cell cultures from human fetuses of 12 to 25 weeks' gestation (Sandler et al, 1987).

2.1.3 Fetal growth

Fetal age is counted from the first day of the last menstrual period and this occurs on average two weeks before fertilisation. It follows that 40.0 weeks or 280 days is the average duration of pregnancy but represents only 38 weeks of true fetal age. The human fetus grows from a fertilised egg to a full term baby as the result of an estimated 42 cell divisions (Milner & Hill, 1989).

Fetal growth differs from postnatal growth in that it involves massive cell replication and differentiation of cells into various organs, whereas postnatal growth involves mainly the multiplication of cells. Different fetal tissues have different periods of rapid growth during pregnancy and these have been termed 'critical periods' (Widdowson et al, 1972; Widdowson & McCance, 1975). Work in rats has shown that the number of functional cells in the organ may be reduced permanently with even short periods of undernutrition during these critical periods (Winick & Noble, 1966).

Male fetuses grow demonstrably faster from the two cell stage onwards, and peak velocity for weight occurs around 34 weeks with boys becoming significantly heavier from this time (Gruenwald, 1967). At term the human male is about 150 g larger than the female and this difference is measurable from 34 weeks onwards. It is independent of multiparity or race and is maintained during maternal malnutrition (Birkbeck, 1981).

2.1.4 The influence of genotype on size at birth

Size at birth is predominantly determined by the intrauterine environment of the fetus (Penrose, 1954; Morton, 1955; McCance & Widdowson, 1974). More recently, in an analysis of birthweights of offspring of primigravid grandmother/mother pairs, it was shown that there were minimal correlations between genetic factors and birthweight and it was concluded that genetic factors play only a small part in determining birthweight (Carr-Hill et al, 1987).

2.1.5 Organogenesis

Liver

In the neonate the liver is relatively larger than in the adult and clinically it is contained in the measurement of the lower chest below the nipples which indirectly includes the upper liver border at its broadest. In the midclavicular line the liver spans 4 - 5cm from the fifth intercostal space to 1cm below the costal margin in neonates (Valman, 1980). The main bulk of the neonatal liver therefore lies in the lower part of the right hypochondrium (Thibodeau & Patton, 1987). Consequently the liver's size is a large component of the lower thoracic circumference in the neonate. It has been shown on ultrasound that the longitudinal cross sectional area correlates well with anthropometry (Cortes-Gallo et al, 1993).

Unlike other organs in the body the human liver is able to regenerate throughout life. At birth the amount of DNA in the liver is approximately 600 mg whereas in the adult the range is between 2770 to 4600 mg, illustrating that

there is considerable postnatal liver growth (Widdowson et al, 1972). It has been known for many years that the fetal liver is the organ affected earliest and to the greatest extent in intrauterine growth retardation (Winick, 1971). In the last trimester of pregnancy there is a growth spurt in liver development and if nutrients are in short supply the human fetus will maintain the brain at the expense of the trunk and the liver is particularly vulnerable at this time (Dickie, 1987). This is often manifest clinically by an associated hypoglycaemia soon after birth due to depleted glycogen in the liver (Gruenwald, 1974).

Kidneys

In contrast to the liver there is no postnatal increase in the functioning units of the kidney. The number of glomeruli increases from 15 weeks to 36 weeks and then stops, when there are approximately one million glomeruli in each normal kidney. There is a profound effect upon nephron number in babies weighing under the 3rd centile (Hinchcliffe et al, 1992). Postnatal nephrogenesis does not occur. The low birthweight baby may therefore grow up with compromised renal reserves.

Lungs

Lung development is different again, for although the development of the bronchial tree is complete by 16 weeks, as many as 85% alveoli are added postnatally.

2.1.6 Maternal constraint

The size of a neonate is mainly determined by the mother rather than the father. This fact has been illustrated in both animals and humans. Over fifty years ago when Shetland ponies were crossed with Shire horses it was shown that foals were smaller when the small Shetland pony was the mother rather than the Shire horse, in spite of the genetic material being substantially the same (Walton & Hammond, 1938). More recently several studies have

indicated that the size of the neonate is determined more by maternal constraint than paternal influences (Klebanoff et al, 1984; Alberman et al, 1992; Emanuel et al, 1992).

The degree of maternal constraint on offspring size is determined when the mothers themselves were in utero (Ounsted et al, 1986). A stark example of this happened in Holland in 1944, when there was widespread famine for seven months between November 1944 and May 1945. Pregnant women were severely malnourished and their babies had reduced birthweights. The greatest decline in birthweights (about 300g) occurred in babies whose mothers had been exposed to the famine during the third trimester. The fall was mostly due to growth retardation rather than short gestation (Lumey, 1988). There was an increase in placental weight but not in birthweight, in babies whose mothers were subjected to starvation around conception or in the first trimester of pregnancy. This suggests that pregnancy undernutrition in the first trimester can stimulate compensatory placental growth with no effect on birthweight. Therefore the birthweight in such babies is not an appropriate measure of undernutrition in pregnancy (Lumey, 1998).

The offspring of mothers who were pregnant at the time of this famine were reported to have an increased perinatal mortality and a study was therefore designed to examine the late effects, if any, of such exposure. Females exposed to famine conditions when they were in utero during the first two trimesters of the gestation period, when they themselves became pregnant, had offspring of lower birthweight than females who had not been so exposed. These findings in the offspring contrast with the effects of the famine on the mothers themselves as fetuses in utero where third trimester exposure was associated with a reduction in birthweight. This study demonstrated a clear constraining effect upon birthweight of the next generation following an environmental exposure *in utero* (Lumey,1992). Further work with the Dutch families who had suffered severe malnutrition between 1944 and 1945 showed that the expected increase in birthweight with increasing birth order was not

seen after maternal intrauterine exposure in the first trimester of pregnancy (Lumey & Stein 1997). A Norwegian study has not only confirmed the maternal influence on the size of her own infant but has shown that babies who are small relative to their mother's birthweight are at increased risk of perinatal mortality (Skjaerven et al, 1997).

The importance of nutrition both before pregnancy and during pregnancy was likewise demonstrated in the Russian population in World War II. The Russians were subjected to prolonged malnutrition, had a very poor baseline nutritional status antenatally, and the birthweights of their babies were reduced by approximately 500 g (Antonov,1947). However a recent cross-sectional study of the same population showed no association of prolonged malnutrition antenatally with glucose tolerance, dyslipidaemia, hypertension or cardiovascular disease in adult life, **but** there was evidence of endothelial dysfunction (raised plasma von Willebrand factor concentration) and a stronger influence of obesity on blood pressure (Stanner et al, 1997). It is possible that endothelial dysfunction may be one biological explanation for some of these epidemiological findings.

2.1.7 Causes of intrauterine growth retardation

Clinically, fetal growth retardation is defined as a birthweight below the 10th centile of population-based standards (Neerhof,1995). The term intrauterine growth retardation (IUGR) was first defined as a birthweight below 10th centile for gestational age (Battaglia & Lubchenko,1967). Later, birthweight below the 3rd centile was used to categorise more severe growth retardation (Buck et al, 1989). When the fetus fails to meet its growth potential there is an increased risk of both short and long-term morbidity. Various epidemiological factors are associated with a baby failing to fulfil its potential. Taller heavier mothers and increasing birth order are associated with bigger babies, whereas low socio-economic group, a short inter-pregnancy interval and multiple births have a small depressing effect on birthweight (Galbraith et al, 1979).

Neonatal weight is affected by severe maternal malnutrition which may be caused not only by famine but also by bowel resection, inflammatory bowel disease, anorexia or bulimia or pancreatitis (Carlson,1988).

Smoking by pregnant women has been shown to be associated with a reduction of birthweight of babies by 150-200g. Nicotine reduces uterine blood flow which leads to placental underperfusion. Smoking tobacco also produces carbon monoxide which increases carboxyhaemoglobin in maternal and fetal blood and reduces its oxygen carrying capacity (Haddon et al, 1961). Similar results were found some years later in America where it was shown that smoking throughout pregnancy reduced birthweight by an average of 189g or 5.9% (Cliver et al, 1995). Further work has proved a dose - response relationship between the number of cigarettes smoked per day and birthweight, such that birthweight dropped by 19g for each cigarette smoked per day (Adriaanse et al, 1996).

In addition it has been reported that hypertension and pre-eclampsia in mothers leads to a reduction in birth weight (Tervila, 1973). A later study from Aberdeen, however, showed that birth weight was similar in normotensive and hypertensive women and only reduced when the mother had proteinuria in addition to hypertension (MacGillivray, 1983). Chronic maternal renal disease with associated hypertension increases the risk of reduced fetal growth and mothers with cyanotic heart disease have a ten times greater chance of producing a baby small-for-gestational age. Other causes of intrauterine growth retardation include viral infections, sickle cell disease and lupus erythematosus (Carlson,1988). Fetal infection accounts for fewer than 10% of all cases of intrauterine growth retardation. Cytomegalovirus and rubella both cause growth retardation especially when the infection occurs in the first trimester (Neerhof, 1995).

2.1.8 Forms of the nutritionally-deprived neonate

The well grown neonate has certain body proportions but if the fetus is subjected to undernutrition or hypoxia at different times in pregnancy these body proportions can be altered. The size of the impact is related to the length of time of the deprivation and the stage of pregnancy at which it occurs.

Three types of intrauterine growth retardation are described.

1. Those with Type 1 IUGR are **proportionally small neonates** who have adapted to poor maternal nutrition by establishing a low trajectory of growth from the earliest stages of pregnancy. They are small in all neonatal measurements including head circumference, length and birthweight and do not exhibit catch-up weight gain in infancy (Gruenwald, 1966). Many fetuses whose mothers have some of the above risk factors fall into this category and develop a slow growth trajectory early in pregnancy which they are able to sustain. Such neonates are proportionally small, and although underweight, are able to maintain a steady but slow growth despite chronically reduced supplies of oxygen and/or nutrients (McCance & Widdowson, 1974). Full term proportionally small babies are common in chronically undernourished communities of the third world and are often said to be genetically small, though in fact their size is more likely to reflect chronically poor maternal nutrition. For example, a big improvement in socio-economic conditions in Japan after the second world war was paralleled by a large increase in average birthweights (Gruenwald et al, 1967).

2. **The short neonate** exhibits a type of disproportionate growth characterised by a short crown-heel length in relation to the size of the head, but in which birthweight may be within the normal range (Holmes et al, 1977). A fetus of

this form has been subjected to chronic fetal distress which may have started months before delivery when there is very little muscle and fat, so no 'wasting' can occur. (Gruenwald, 1963). Birthweight may be normal and the growth of the brain, heart and lungs is protected at the expense of the trunk (Naeye,1965).

3. A third kind of impaired fetal growth leads to a **thin full term neonate** with a normal body length but a low ponderal index (Miller & Hassanein, 1971). This is known as Type II or disproportionate IUGR where the birthweight is reduced but the body length is normal. The fetus reaches its length and brain potential but then fails to gain weight and appears 'wasted' when it is born due to the lack of subcutaneous fat. Normally the ponderal index rises early in the third trimester due to an increase in subcutaneous fat, levelling out between 39 and 41 weeks and thereafter decreasing (Gruenwald, 1967). If the nutritional deprivation or subacute fetal distress in the second trimester is serious the weight loss would include muscle as well as fat and the ponderal index would be even lower. This has implications for insulin metabolism as skeletal muscle is the main peripheral site of action of insulin, which plays a vital part in stimulating cell division in the fetus (Fowden, 1989).

Growth retardation can occur at any birthweight for example a 3.5 kilogram baby might have failed to reach its full growth potential if it was meant to be 4.0 kilograms. Such babies are unlikely to be diagnosed as clinically growth retarded in a normal population sample. Does reduced early growth matter? The next part presents evidence that reduced early growth has adverse effects on adult health and then discusses the concept of programming.

2.1.9 The long-term effects of poverty in early life

Poverty is usually associated with a poor diet in pregnant women. Forsdahl (1973) in Norway was the first to show that an association existed between extremely poor social conditions during childhood and adolescence and cardiovascular disease in middle life. Present day living standards in Norway are similar throughout the country but this was not always so. There is a significant positive relationship between the cholesterol levels and cardiovascular disease of adults and the infant mortality rate previously present in the same community (Forsdahl, 1977; 1978; Arnesen & Forsdahl, 1985).

Cardiovascular disease was found to be more common in the less affluent towns of England (Gardner et al, 1969). A further study looked at area specific mortality from cardiovascular disease in relation to socio-economic conditions and infant mortality rates that prevailed fifty years earlier in the same geographical area. The relation between different causes of adult and infant death by age and sex in 212 local authorities was examined. It was found that deaths from ischaemic heart disease in 1968 -78 were strongly correlated with the neonatal and postneonatal mortality in 1921-25 (Barker & Osmond, 1986; 1987).

A paradox was also identified. Fifty years ago death from ischaemic heart disease was more common in socioeconomic groups I and II than in IV or V. By 1971 the position had reversed with SMRs for ischaemic heart disease dropping to 88 in group I and rising to III in group V. It may be that poor nutrition in early life programmes the metabolism in such a way that the individual is unable to adapt to a richer diet in later life. This would account for the high incidence of heart disease among immigrants from the Indian subcontinent who may have suffered poor nutrition in childhood (Barker & Osmond, 1986).

2.1.10 Reduced early growth, height and cardiovascular disease

Adult height is largely determined by growth in early childhood (Tanner et al, 1956) and an association has been established between height and cardiovascular disease. Male civil servants in London showed an inverse relationship between height and mortality from cardiovascular disease (Marmot et al, 1984). The same association was found in a report from Norway which showed a reduced mortality from cardiovascular disease in tall men (Waaler, 1984). A prospective study in Finland also concluded that adult height is inversely related to mortality from cardiovascular disease (Notkola, 1985). There is an inverse relationship of height with ischaemic heart disease which is consistent with risk factors for this disease being established in early life (Barker et al, 1990).

In Finland the highest incidence of coronary heart disease was found in those communities with very high infant mortality rates fifty years previously - despite the fact that prosperity today is fairly evenly spread throughout the country (Notkola,1985). Further evidence from Finland states that the sons of short heavy mothers have an excess of heart disease. Men born in the 1920s who were thin at birth and whose mothers were short and fat had a much increased risk of coronary heart disease. When conditions in the country improve following chronic malnutrition women tend to get fat because it takes more than one generation to increase height in such circumstances (Forsen et al, 1997).

2.1.11 Reduced early growth and cardiovascular disease

A large study of death rates in Hertfordshire men born between 1911-30 provided the first evidence that a slow rate of growth in infancy leading to low weight at one year is associated with an increased risk of death from ischaemic heart disease in adulthood. The average birth weight in this cohort was 7.9 pounds. Men who had weighed less than 5.5 pounds at birth had higher SMRs for ischaemic heart disease (104) compared with

those who were heavier at birth but the downward trend in SMRs with increasing birthweight was not statistically significant. Men who had low birth weights and low weights at one year had the highest SMRs of 220 and men who had weighed eighteen pounds or less at one year had SMRs of III compared with men who weighed twenty seven pounds or more who had SMRs of 42 or less. Birthweight was unrelated to social class at death (Barker et al, 1989b).

Similar findings were produced in a follow up study in Sheffield when size at birth was related to deaths from cardiovascular disease in adult life. Over fifteen hundred men who were born in the same hospital were traced and matched with their birth measurements which included crown-heel lengths and head circumferences. Cardiovascular disease death rates, especially before 65 years, fell with increasing birthweight. Those infants with small head circumferences and who were thin at birth could be separately identified as being at increased risk. These neonatal measurements suggested that growth retardation that begins early in gestation is associated with an increased mortality from cardiovascular disease (Barker et al,1993b).

The prevalence of coronary heart disease in the Hertfordshire men whose birth weight and weight at one year were known was then examined. A positive association was found between coronary heart disease and low weight at one year but not with birthweight. This effect was independent of social class and smoking (Fall et al,1995a). Women were included in another Herfordshire study to see if similar associations held. An analysis related birthweights and weights at one year in over 5000 women and 10,000 men born during 1911-30 to standardised mortality ratios for cardiovascular disease. The highest ratios for cardiovascular disease in women were in those who had been below average birthweight but above average weight at one year. The converse at one year was true in men, in whom the highest rates of early cardiovascular

death were associated with low birthweight and low weight at one year. This result accorded with the relationship found by Barker et al (1989b). Social class had no influence on these trends (Osmond et al, 1993). An American study showed similar strong evidence of an inverse association between birthweight and prevalence of cardiovascular disease and stroke in a cohort of nurses followed up from 1976 for the next 16 years (Rich-Edwards et al,1997).

It has been suggested that these associations could be explained by an interaction between influences in early life and socioeconomic factors operating in middle age. This possibility was examined in a study from Wales but no evidence for such interaction was identified. However, the risk of coronary heart disease was linked to a combination of low birthweight and a high body mass index in middle life (Frankel et al, 1996). The mechanisms underlying these associations may involve the development of vessel structure, blood pressure, glucose tolerance and/or fibrinogen levels.

2.1.12 Low birthweight and vessel structure

It is possible that the the vascular system is compromised by poor intrauterine growth as a recent study demonstrated that atherosclerosis of the carotid and lower limb arteries was more prevalent and severe in those patients who had had the lowest birthweights (Martyn et al, 1998).

2.1.13 Reduced birthweight is inversely related to blood pressure

Many studies relating blood pressure to early growth have been done. There was a positive relation between blood pressure and birthweight in 4-day old infants (Contis & Lind, 1963; Lee et al, 1976; Hulman et al, 1990). This relationship was reversed in older children and became a negative association (Cater & Gill, 1984; Barker et al, 1989a). Studies in older children showed that current systolic blood pressure fell with increasing birthweight after allowing for current size (Voors et al, 1979; de Swiet et al, 1984). In Farnborough, birthweight was positively associated with current systolic blood pressure up to

six months of age and the association became increasingly negative by 10 years (Law et al, 1993a).

In Salisbury, children showed the same negative association between systolic blood pressure at 9 years of age and birthweight that had been identified with blood pressure at 4 years, although it was weakened (Law et al, 1991; Fall et al, 1995a). An Israeli study found that there was no association between low birthweight and blood pressure in late adolescence (Laor et al, 1997).

Other work has shown that systolic blood pressure at 10 years and at 36 years was inversely related to birthweight independently of gestational age (Wadsworth et al, 1985; Barker et al, 1989a). It was also shown that although mean systolic blood pressure rose with increasing age, the inverse relation between birthweight and systolic blood pressure was amplified even further (Barker et al, 1990; Hales et al, 1991). In Sheffield these conclusions were tested again and confirmed (Martyn et al, 1995b). A systematic review of the literature showed that blood pressure in neonates was positively associated with birthweight. The relationship then changed so that blood pressure in both children and adults was inversely related to their birthweight. In adolescence the relationship was much more variable (Law & Shiell, 1996).

In Rotterdam the relationship between birthweight and blood pressure at 4 years was 'U' shaped, with the higher systolic blood pressures at low and high birthweights (Launer et al, 1993). Another Dutch study followed children from birth for 14 years and found that birthweight was inversely associated with systolic blood pressure throughout childhood, and with diastolic blood pressure in young adult life (Uiterwaal et al, 1997).

2.1.14 Reduced fetal growth and impaired glucose tolerance

Low birthweight infants have reduced numbers of beta cells and reduced insulin secretion (van Assche & Aerts, 1979; Snoeck et al, 1990). The effect of this has been explored in several studies. In Southampton men an inverse association between birthweight and plasma glucose was found (Robinson et al, 1992). The prevalence of impaired glucose tolerance or diabetes was associated with low birthweight independent of gestation or gender (Phipps et al, 1993). An association between Type 2 (non-insulin dependent) diabetes, hypertension and hyperlipidaemia is recognised (Fuller et al, 1980; Modan et al, 1985) and this triad - known as Syndrome X - has been shown to be associated with low birthweight (Barker et al, 1993a).

In the Pima Indians insulin resistance was associated with low birthweight and preceded the development of Type II diabetes, by contrast very heavy babies were more likely to develop Type 1 diabetes (McCance et al, 1994). In Sweden, poor intrauterine growth decreased and excess intrauterine growth increased the risk of childhood Type I diabetes (Dahlquist et al, 1996). A low ponderal index at birth is associated with insulin resistance as well as impaired insulin production (Phillips et al, 1994; Lithell,1996; Leger et al, 1997). 4 year old Indian children who had been growth retarded at birth had high plasma glucose levels after a glucose challenge (Yajnik et al, 1995). Similarly, 7 year old British children who had been thin at birth had high plasma glucose levels 30 minutes after a glucose challenge, independent of their current size (Law et al, 1995). Reduced cord insulin levels were associated with low placental weight, disproportionate low birthweight, and Type II diabetes in adult life.
Impaired glucose tolerance and Type II diabetes may originate through impaired fetal development in mid to late gestation and, conversely, excessive intrauterine growth increases the risk of development of childhood insulin dependent diabetes (Godfrey et al,1996a).

2.1.15 The concept of programming

The concept of programming describes the phenomenon whereby an insult or stimulus at a critical period of development has lasting or lifelong effects on the structure or function of an organism (Lucas, 1991). This idea is central to the Barker hypothesis of the fetal origins of adult disease, which proposes that cardiovascular disease, hypertension and diabetes are all 'programmed' by an inadequate supply of nutrients or oxygen in utero or during infancy. Work in animals provides supportive evidence.

Early work in this field identified 'critical periods' in both intrauterine pigs and rats in which growth could be manipulated with predictable and permanent long term effects upon the animal (Widdowson, 1974; Widdowson & McCance,1963). It was found that a reduced diet offered to pregnant and lactating rats adversely influenced the growth and metabolism of their offspring permanently (Chow & Lee, 1964). Further work in rats illustrated that dietary restriction during gestation alone was less harmful than restriction during gestation **and** lactation (Blackwell et al, 1968). Another study demonstrated that mild nutritional deficiencies in lactating mice not only reduced weight gain of the nursing young in the immediate period but jeopardized the development of the animal for the whole of its life span (Dubos et al, 1966). Other work showed that the cellular response in rats during malnutrition at various stages from birth to weaning produced rats that were proportionally reduced in size with evidence of fewer cells. These animals did not recover normal growth when refeeding started. Malnutrition introduced later did not have the same effect and normal growth resumed with refeeding. These data suggest that organs developing most rapidly at the time of malnutrition are most vulnerable to permanent stunting (Winick & Noble, 1966).

The ability of a newborn rat to produce insulin in the normal manner is permanently impaired if it is put on a low protein diet for only three weeks

(Swenne et al, 1987). It has also been shown that a low protein diet in the pregnant rat reduced the weight of the offspring. In addition the beta cells, the islet size and the vascularisation were all reduced in the fetal endocrine pancreas following a low protein diet in pregnancy (Snoeck et al, 1990). This animal work is consistent with the notion that intrauterine growth retardation has a long term effect upon insulin production.

Measured degrees of low protein diet in rats during pregnancy revealed an inverse relationship between maternal protein intake and blood pressure in offspring nine weeks after birth (Langley & Jackson, 1994). Again these results from animal research are compatible with the results in human studies (Campbell, 1924; Campbell et al, 1932).

Work done on baboons offered more evidence that nutrition in early life is critically important. Mott et al found that breast or formula feeding had permanent effects on cholesterol metabolism in young adult baboons and showed that the breast-fed had more arterial fatty streaks than formula fed animals (1986, 1991).

In summary, the concept of programming in humans is supported by a large body of evidence from animal studies which found that poor nutrition in early life can permanently stunt growth and adversely affect insulin production and the cardiovascular system.

2.1.16 The debate about the fetal origins of adult disease

The idea that coronary heart disease has its origins in fetal life has not been universally accepted. The epidemiological studies and their interpretation in support of this hypothesis have provoked considerable debate and some of the main criticisms are described below.

1. None of the studies trying to validate this hypothesis have measured the nutritional intake of the mothers, so that any observed reduced growth may be due to some other effect such as placental dysfunction (Paneth & Susser, 1995).

However, the Dutch hunger winter provides evidence that gross maternal malnutrition has profound effects on long term health of the offspring (see pages 22-23). It is also reasonable to point out that animal studies support the hypothesis that maternal nutrition is critical for fetal growth and optimal health of the offspring. Even in less extreme situations the importance of nutrition in pregnancy is demonstrable, as shown recently by a study in Southampton in which women who had a relatively high carbohydrate intake in early pregnancy and a low intake of protein in late pregnancy tended to have thin babies (Godfrey et al, 1997).

2. Inconsistent results have been cited. Although the studies quoted in support of this hypothesis show statistically significant associations between coronary heart disease (CHD) and a birth measurement, it is not always the same measurement that shows the association. For example, birth length as well as birthweight was associated with coronary heart disease in a study from India (Stein et al, 1996), whereas in Hertfordshire weight at birth and in infancy showed the most significant association. There the highest cardiovascular death rates in men were in those who had been of low birthweight and below average weight at one year, whereas in women of the same age, low birthweight and above average weight at one year had the highest rates (Osmond et al,1993). In women, low birthweight is significantly associated with some risk factors for coronary heart disease (low concentrations of high density lipoprotein cholesterol and most measures of glucose intolerance), although not with others (blood pressure and concentrations of total cholesterol, fibrinogen and factor VIII) (Fall et al, 1995b). It may be that the association between poor early nutrition and cardiovascular disease in later life is modified by other factors which influence growth, such as the ethnicity and sex of the individual.

3. Loss to follow up in the longterm studies is one of the major areas of concern to many critics as it may lead to selection bias (Kramer & Joseph, 1996).

This loss would only distort the results if the association between early growth and coronary heart disease were different in those lost to the study and those still being followed up and this seems unlikely.

4. Controlling for intervening variables may enhance an association. Body mass index and birthweight are positively associated for example, so that when the influence of body mass index is removed the perceived association between glucose tolerance and birthweight will be increased. Body mass index is a much more powerful predictor of insulin concentrations than is birthweight and is positively related to birthweight. So to control for current body mass index when assessing the effect of birthweight is to cancel out the positive effect of birthweight on body mass index and thence on risk of glucose tolerance. This then allows the effect of birthweight to be enhanced (Paneth & Susser,1995). The point was well illustrated in a follow-up study of Hertfordshire women in which plasma insulin concentration fell with increasing birthweight only when current body mass index was allowed for. (Fall et al, 1995c).

5. The persistence of poor socio-economic circumstances in some geographic areas may account for the association of previous low birthweights and associated high mortality from coronary heart disease in the U.K. In order to examine this possibility, infant mortality rates for 1895-1908 were correlated with cause-specific adult mortality for 1969 -73 in 65 -74 year old people with and without adjustment for present day social deprivation and social class. After controlling for socio-economic variables the correlation was abolished (Ben-Shlomo & Davey Smith, 1991). Further evidence for this point of view was put forward in a study of Civil Servants in London, which investigated the associations between plasma fibrinogen levels and factors operating in childhood and adulthood including psychosocial characteristics. Measures of childhood environment were inversely associated with fibrinogen levels as were measures of socioeconomic status in adult life. It was suggested that the fibrinogen level acts as an indicator which rises as the socioeconomic gradient

falls in coronary disease (Brunner et al, 1996). However this seems to be a rather simplistic interpretation, and does not reflect the plasticity of plasma fibrinogen in conditions which may have nothing to do with social class - such as in smoking or renal disease.

6. Further comment on the confounding problem has been made on the connection with early growth, clotting factors and smoking in adult life. A clear trend of increasing smoking with decreasing weight in the offspring at one year of age was found, which paralleled the known association between raised plasma fibrinogen and Factor VII concentrations in adults with low weight at one year (Barker et al,1992b). It has been suggested that men who smoke had lower weights at one year and probably had a diet containing more saturated fat than those who did not smoke. In other words the smokers had a different diet and probably a different lifestyle which could account for the relation between early growth and raised clotting factors in adult life (Davey Smith & Ben-Schlomo, 1992).

These criticisms illustrate the need for more studies in children where confounding by adult lifestyle cannot have occurred, so that data can be collected to clarify some of these issues.

2.1.17 Cardiovascular risk factors in children

Five reasons are given why cardiovascular risk factors in childhood are worthy of study.

1. Adverse influences upon growth in early critical periods of life can produce measurable effects in childhood.

2. Measurements in childhood can be related more accurately than ever before to conditions in fetal life due to the accuracy of modern obstetric monitoring - particularly ultrasound scanning.

3. Studies in children remove the possibility that these associations are due to superimposed patterns of behaviour or environmental hazards in later life. Examples of cardiovascular risk factors that have been studied successfully in childhood include blood pressure, glucose metabolism and familial hypercholesterolaemia.

4. Prospective studies of the consequences of impaired development can be completed in a reasonable time when the outcomes are measured in childhood.

5. Associations between adverse conditions in early life and the development of risk factors can be identified in childhood and compared with the enhancement or modification of such risk factors in adult life.

Numbers 4 and 5 are not addressed in this thesis.

The first part of Section 2 is summarised as follows:

2.1.1.- 2.1.8

It has been shown that the growing fetus is susceptible to modification by a variety of influences, including poor maternal nutrition, which can adversely influence placental growth and function and have profound effects. The genotype has only a small influence on birthweight whereas smoking has a greater effect in reducing birthweight. The fetal liver is particularly sensitive to nutritional supply from early in pregnancy. Maternal constraint on neonatal size due to severe malnutrition straddles generations. The physical proportions of babies at birth represent different patterns of fetal growth, which in turn, reflect nutrition and other influences during pregnancy.

2.1.9 - 2.1.16

Poverty early in life has a lifelong adverse impact on health. Historically the effects are measured by maternal and perinatal mortality rates, but poverty is also associated with reduced birthweight. Adults who have been poorly nourished in early life have an increased risk of cardiovascular disease and stroke. Other relationships have been described linking reduced fetal growth

with raised blood pressure and impaired glucose tolerance. It has been known for a long time that animals who are malnourished or injured at a critical period of development suffer long term effects. This concept is known as 'programming' and can be applied to humans. The Barker hypothesis suggests that programming of the fetus is responsible for adult cardiovascular disease. Some doubts and inconsistencies have been raised, including the effects of socio-economic factors and intervening variables. These problems may be addressed by further research into cardiovascular risk factors in children.

In recent years raised plasma fibrinogen has been identified as a risk factor for cardiovascular disease in adults. The next section describes fibrinogen and its role in health and disease.

2.2 Fibrinogen

2.2.1 Structure of fibrinogen in the adult

Fibrinogen is a large trinodular glycoprotein which includes a large number of heterogeneous but closely related dimeric molecules (Figure 2, page 42). The molecular weight of the most abundant form of human fibrinogen is 340,000 Daltons and contains 2964 amino acids. A 'dimer' (Gr) is a compound having twice the number of each atom in its molecule as another compound - especially one in which two identical molecules are joined together. Fibrinogen is such a dimeric molecule and comprises three types of nonidentical polypeptide chains: A alpha (M.W. 70,000) B beta (M.W. 60,000) and gamma (M.W. 50,000). Each of these chains occurs twice in the trinodular structure of the molecule and each pair is joined together by disulphide bonds at their amino - ends. Three main dimeric molecules can be demonstrated - HMW 340 000, LMW 300 000 & LMW 280 000. HMW has A alpha chains intact, LMW only one, and LMW none. The heterogeneity of fibrinogen is due to the following:

1. The A alpha chains may be variably degraded in three areas which are (a) the carboxyl - terminal regions (b) in the amino terminus where alanine A

alpha 1 can be absent and (c) in the degree of phosphorylation of serine residues A alpha 3 and A alpha 345.

2. The B beta chains are less heterogeneous than the A chains. Heterogeneity is only caused by variations in the sialic acid content of the carbohydrate moiety at position B beta 364.

3. The gamma chain occurs in three molecular weights: 50,000, 55,000 and 57,000. Heterogeneity is conferred in several ways, including replacing the carboxyl - terminal 4 amino acids by 20 new amino acids and differences in the sialic acid content of the carbohydrate moiety at position gamma 52 (Nieuwenhuizen, 1994).

2.2.2 Structure of neonatal fibrinogen

Differences in structure have been suggested to account for the decreased clotting capacity of neonatal fibrinogen compared to adult fibrinogen. Structurally it has been shown that both fetal and adult fibrinogen have the same number of amino-acids and hexoses and cannot be distinguished by electrophoresis (Galanakis & Mosesson, 1979).

2.2.3 Genetic determination

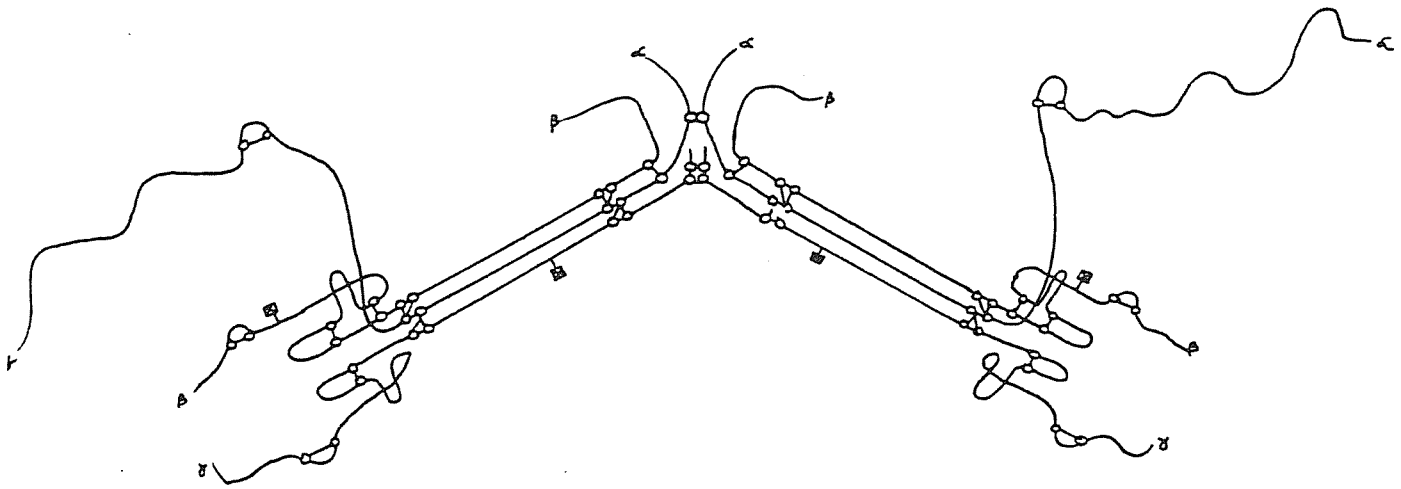
There are three genes for fibrinogen (A alpha, B beta and Gamma chains) located closely together on chromosome 4 (Henry et al, 1984) accounting for 15% of the total phenotypic variance in fibrinogen (Humphries et al,1987). However no association between genotype and fibrinogen concentration could be found in a Norwegian study (Berg & Kierulf,1989). The relationship between genetic inheritance, plasma fibrinogen levels and risk of disease has been explored and found to be variable and indirect (Fowkes et al,1992; de Maat et al,1995).

2.2.4 Synthesis

Fibrinogen is made from precursors in hepatocytes and to a smaller extent in megakaryocytes and then released into the circulation. The half-life of fibrinogen in the circulation is about four days, and during this time a significant amount is likely to be converted into fibrin by thrombin and then broken down by plasmin. The degradation products stimulate the release of interleukins by monocytes, which in turn stimulate hepatocytes to make more fibrinogen (Wang & Fuller 1991).

FIGURE 2

Artistic impression of fibrinogen molecule showing trinodular and dimeric structure



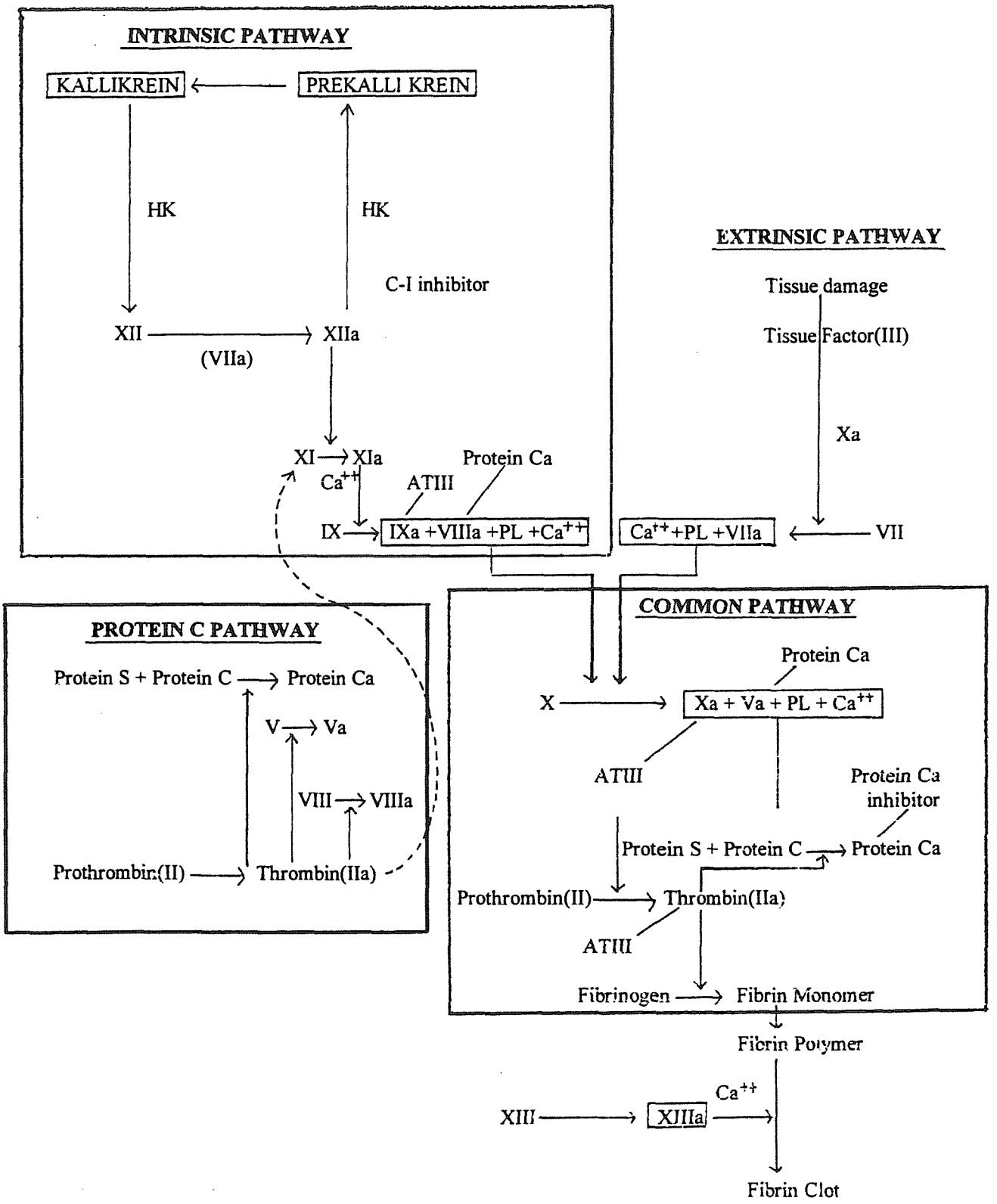
2.2.5 Function

2.2.5.1 The coagulation cascade

Fibrinogen is essential for blood coagulation and provides haemostasis for the body with plasma concentrations as low as 0.5 g/l. Fibrinogen is converted to fibrin by the protease thrombin in the last reaction of the clotting mechanism which has been called a 'cascade' due to the amplifying action of each stage (Figure 3, page 44). A damaged blood vessel activates platelets within the circulation. Primary haemostasis is achieved by platelets clumping together and sticking to the vessel wall. The next stage involves blood coagulation. It is now widely accepted that Tissue Factor (TF or Factor III) is the primary physiological initiator of coagulation. Tissue factor is produced by subendothelial cells, activated endothelial cells, monocytes in the peripheral blood and haematopoietic progenitor cells in the bone marrow. Tissue Factor serves as co-factor for Factor VII, and the TF - Factor VIIa activates Factor IX and Factor X. The coagulation cascade has been revised in the light of recent knowledge to take in the designation of Factor V and Factor VIII as co-factors and also the recognition of TF - Factor VIIa activation of Factor IX and Factor X. Factor XI, which is conventionally regarded as a component of the contact system with Factor XII, is not activated by Factor VIIa - TF complex. However a feedback mechanism has been proposed whereby Factor XI is activated by thrombin. Factor XIa then feeds back to activate Factor IX to Factor IXa. Factor IXa activates Factor X, which together with Factor V, a coagulant surface and Calcium convert Factor II to Factor IIa (Thrombin), which in turn acts on fibrinogen, converting it into fibrin.

FIGURE 3

THE COAGULATION CASCADE



2.2.5.2 Neonatal haemostasis

The development of the fetal haemostatic system starts very early in gestation. Coagulation proteins in the neonatal circulation are synthesized by the fetus and do not cross the placental barrier. Fibrinogen is synthesized by the fetal liver as early as five weeks gestation and the synthesis of all the other coagulation proteins begins later in the first trimester (Gitlin & Gitlin,1975). Plasma concentrations of Factors V and VIII reach the normal adult range from the beginning of the third trimester onwards, but all the other factors are variably reduced at birth, being lower in the preterm infants and dependent on gestational age. Infants from 24 weeks gestation onwards have mean plasma fibrinogen concentrations within the adult range, suggesting that a plasma fibrinogen concentration of 1.5 g/l at birth should be regarded as the arbitrary lower limit of normal in both premature infants and those born at term (Bonnar et al, 1970).

Plasma fibrinogen rises up to the 4th day (Buonocore et al,1991) and does not undergo a 'physiological dip' during the first four weeks of life unlike many of the haemostatic factors (Israels & Andrew, 1994). Reference values for coagulation proteins have been produced for healthy full term infants which show levels that are markedly different from those in the adult, but the differences do not show a uniform pattern. The mean values for the vitamin K - dependent factors (factors II, VII, IX and X) and the contact factors (factors XI, XII, prekallikrein - PK - and high molecular weight kininogen - HMWK) are all <70% of adult values whereas the mean values for fibrinogen, factor V, factor VIII, von Willebrand factor (vWF) and factor XIII are all >70% of adult values. Despite the lower values of these procoagulants, haemostasis is achieved in the normal full term neonate (Andrew et al, 1987).

2.2.5.3 Thrombin function in the neonate

Thrombin function in the neonate is slower than in the adult as shown by prolongation of the prothrombin time (PT) and thrombin time (TT) compared with adult values. This is due to low levels of the four contact factors and the four vitamin K dependent procoagulants (Andrew et al, 1990). Amongst term small-for-gestational-age neonates the prolongation is even greater compared with appropriately grown babies (Dube et al, 1986).

The action of thrombin allows the transformation of fibrinogen into fibrin and its subsequent polymerization. There are polymerization sites on the fibrinogen molecule that are shielded by fibrinopeptides and thrombin acts by removing them. Two fibrinopeptides A from the alpha chain and two fibrinopeptides B from the beta chain are cleaved from the central domain. The resulting fibrin dimer ($alpha_2$ $beta_2$ $gamma_2$) polymerizes spontaneously 'end to end' and then also 'side to side'. The soluble fibrin clot is stabilised by the activated Factor XIII, which crosslinks the soluble fibrin (Kudryk et al 1974; Dolittle et al,1979; Doolittle,1983).

2.2.5.4 Sialic acid and fibrinogen

Sialic acid in health

Sialic acid is a negatively charged sugar associated with the protein and lipid portions of lipoproteins (Millar et al, 1999). It is released from the terminal oligosaccharide chains of some proteins in the acute phase response, such as fibrinogen, during acute inflammation and correlates positively with C - Reactive protein (Cojocaru M, 1997). Total sialic acid also correlates significantly with the erythrocyte sedimentation rate, the neutrophil count and the platelet count and inversely with the haemoglobin concentration (Crook et al,1997). A decrease in the sialic acid content of erythrocytes may influence blood viscosity by increasing the adhesiveness of erythrocytes (Hadengue et al, 1998).

The stability and elasticity of clots is dependent upon sialic acid in the fibrinogen molecule and clots formed without sialic acid present would therefore be ineffective in repairing a vascular injury (Okude et al, 1995). In health serum sialic acid increases in pregnancy and the puerperium but not at the menopause (Crook et al, 1997; 1998). It also rises with age (Lindberg et al, 1991a).

Sialic acid in disease

In recent years sialic acid has increasingly been acknowledged as an important marker for many pathological processes. These include cardiovascular disease (Lindberg et al, 1991a, 1993; Allain et al, 1996) carotid artery disease (Rastam et al, 1996) subarachnoid haemorrhage (Kawaik et al, 1995) and silicosis (Cojocaru, 1997). Rising sialic acid levels have been associated with several aspects of non-insulin dependent diabetes (NIDDM) insulin dependent diabetes (IDDM) and kidney disease (Suzuki et al, 1995; Abshire et al, 1995; Pickup et al, 1995, 1997; Rastam et al, 1996; Chen et al, 1996; Yokoyama et al, 1996). A trend of rising titres of serum sialic acid levels has also been found in patients with colorectal malignancy as their disease became more severe (Feijoo et al,1997). Dysfibrinogenemia in patients with hepatoma was associated with prolonged coagulation times, but when sialic acid was cleaved from the fibrinogen molecule experimentally the clotting time increased (Gralnick et al, 1978).

The relationship between fibrinogen and sialic acid in adults

It has been suggested that sialated fibrinogen may be the confounder that explains the relationship between raised sialic acid concentrations and cardiovascular disease (Kario & Matsuo, 1993). This has been disputed for several reasons. Variations in serum sialic acid do not necessarily correspond to variations in plasma fibrinogen levels. Serum and plasma sialic acid levels are the same and there is no rise in serum sialic acid when fibrin is formed

(Haberli, 1984). Smokers have raised plasma fibrinogen concentrations compared with non-smokers, but not raised sialic acid levels (Lindberg et al, 1991b; Kario & Matsuo,1993). In patients with NIDDM the serum sialic acid concentrations did not differ between those who smoked and those who did not (Pickup et al, 1995).

The relationship between fibrinogen and sialic acid in infants

A study in infants establised that fibrinogen-bound sialic acid was higher in premature than in full term neonates, and enzymatic desialation corrected the prolonged thrombin times to normal or nearly normal values (Francis & Armstrong, 1982). However, when newborn samples were grouped according to high and normal-to-low sialic acid content, no significant differences were observed between the clotting rate with thrombin of the two groups. This did not support the idea that the abnormal function of newborn plasma fibrinogen was entirely due to the elevated sialic acid content (Reganon et al, 1993).

2.2.5.5 Inhibitors of blood coagulation and natural anticoagulants

The whole of the haemostatic system is held in check by a series of inhibitors. Tissue Factor pathway inhibitor (TFPI) inactivates Factor Xa and Tissue Factor complexed with VIIa in vitro, but the physiological role of TFPI remains to be elucidated. The role of three other inhibitors (Antithrombin III) and anticoagulant proteins (Protein C and Protein S) is firmly established and dependent upon gestational age, as a deficiency in any of these gives rise to venous thrombosis. These proteins are about 60% of adult values in the infant born at term and further reduced in the preterm infant (McDonald & Hathaway, 1983). Protein C and protein S are among the proteins that are dependent upon vitamin K for their synthesis. Hepatocytes synthesize proteins that are not functional and cannot be converted to their enzymatically active state. Vitamin K changes this precursor protein to a biologically competent form by directing the insertion of a second carboxyl group into the gamma-carbon of glutamic acid residues of the precursor proteins.

Protein S acts as a cofactor for protein C, which in its activated form inactivates activated factors V and VIII by limited proteolysis. Unlike protein C and protein S, factors V and VIII are not reduced at birth and consequently a potential imbalance in favour of thrombosis exists if these cofactors should be activated. This imbalance does not lead to an increase in neonatal thrombosis. Alpha-2-macroglobulin concentrations are raised above the adult range in both the term and preterm infant and this inhibitor may have a protective role against thrombosis at a time when concentrations of the other principal anti-coagulants are low (Gibson, 1989).

2.2.5.6 Fibrinolysis

A discussion of fibrinolysis follows. This is helpful in order to understand the mechanism of prolonged clotting time in the neonate. When the insoluble fibrin is formed, the components of the fibrinolytic system are incorporated into the fibrin clot. Components of the neonatal fibrinolytic system include plasminogen, alpha-2-antiplasmin, tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI). Plasminogen, alpha-2-antiplasmin and TPA all bind through lysine binding sites to fibrin. The activation of plasminogen by tissue plasminogen activator (t-PA) is the central process in fibrinolysis. Tissue plasminogen activator is a protease mainly produced by endothelial cells from which it is released in response to physiological need. It acts by cleaving the Arginine and Valine bond on plasminogen to produce the serine protease plasmin. The resultant plasmin formed can either degrade fibrin or fibrinogen or bind to alpha-2-antiplasmin (Andrew et al, 1990).

The trinodular fibrinogen molecule is then broken down by plasmin which acts by hydrolysing the lysine bonds on the alpha chains of the fibrinogen molecule. These alpha chains are in highly exposed protuberances on each side of the 'lobster shaped' dimeric molecule and are easily removed by all the proteases. At first it cleaves two thirds of the alpha chains from each end of the fibrinogen molecule thereby reducing its molecular weight to 240,000. This more compact though transient molecule is known as Fragment X. This in turn is reduced

further to an asymmetrical molecule called Fragment Y by losing Fragment D. Fragment Y consists of a remaining Fragment D and the central nodule of Fragment E (Marder & Shulman, 1969).

This description of the action of plasmin applies to the degradation of fibrinogen and non - cross linked fibrin. Fragment X is susceptible to thrombin action and is partially clottable, whereas Fragments Y, D and E are non - clottable. In vivo fibrin only exists in the cross - linked state and is also attacked by plasmin but the reaction proceeds at a slower rate. Fragment D is released and, being cross-linked to another D fragment from an adjacent molecule, the released product is D - dimer which can be used to monitor the turnover of cross-linked fibrin. The fragmentation of fibrinogen by plasmin and the thrombin - catalyzed polymerization of trinodular fibrinogen molecules is illustrated in Figure 4, page 52.

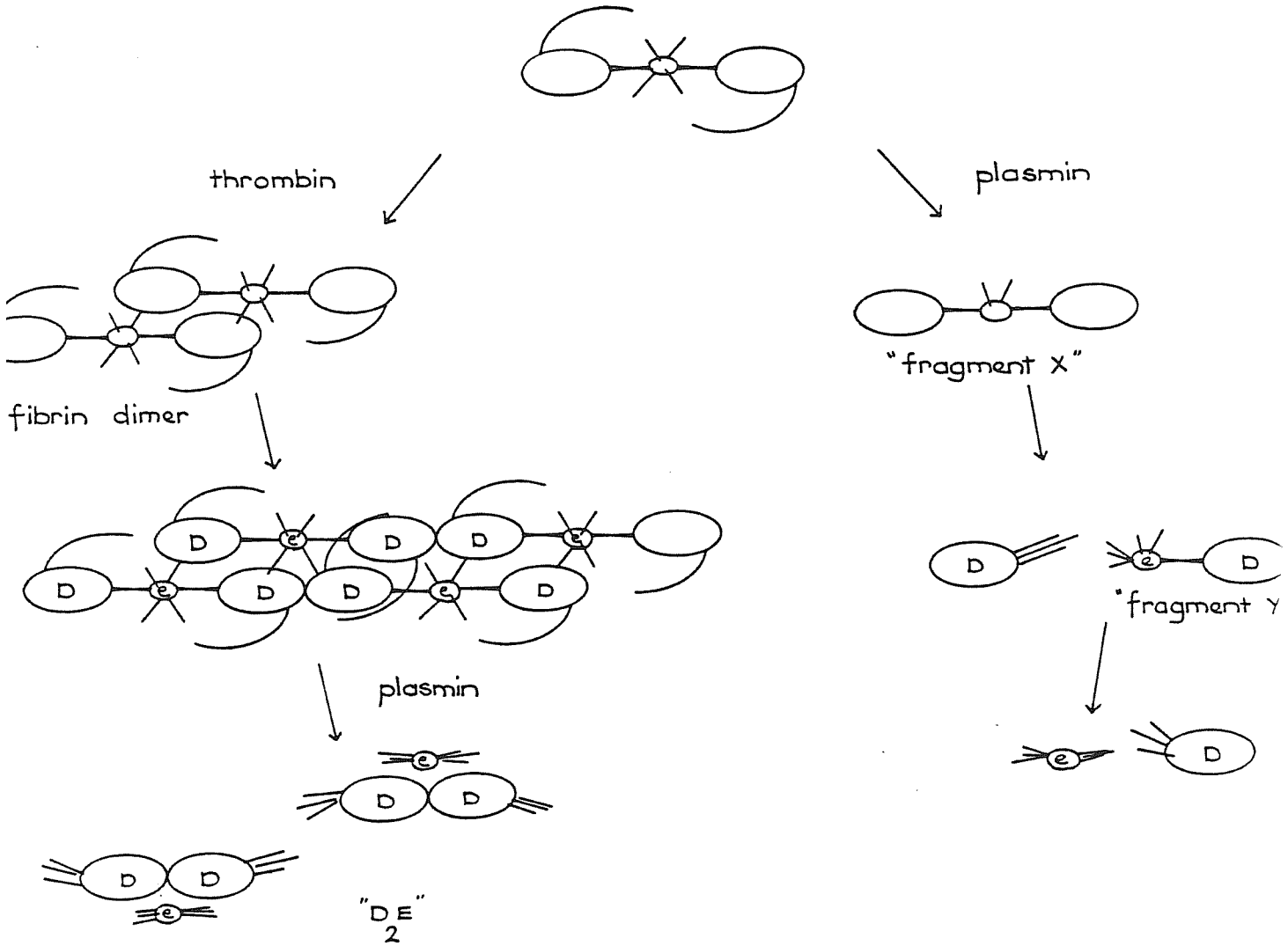
Approximately 70% of human fibrinogen has a molecular weight of 340,000 and the remaining 30% has a molecular weight of approximately 305,000 to 320,000. High molecular weight fibrinogen is decreased by about 25% in the newborn compared with the normal adult and it has also been shown that fibrinopeptide A and fibrinopeptide B are released more slowly in the plasma of the newborn compared with normal adult values, resulting in only 23% of the alpha-chain polymers taking part in the cross-linked fibrin gel and thereby forming fibrin clots at a slower rate (Reganon et al, 1993).

Although plasminogen concentrations are only about 50% of adult values at term and do not reach adult concentrations until about six months of age, fibrinolytic activity seems to be increased in the newborn. Most coagulation factors achieve adult levels by six months of age (Fisher et al, 1968). Similar results were found in Canada, when the values for the components of the

fibrinolytic system on the first day of life were observed to be similar to cord values, except for the level of t-PA and PAI which were lower than the Day 1 values (Andrew et al,1990). Further study has illustrated that not only is neonatal plasminogen relatively low compared to that in adults, but it exists in a 'fetal' form similar to 'fetal' fibrinogen, and likewise has an increased concentration of sialic acid (Ries, 1997).

FIGURE 4

Thrombin polymerization and plasmin fragmentation of trinodular fibrinogen molecule



2.2.5.7 Fibrinolysis and fetal fibrinogen

The prolonged clotting time which has consistently been observed in the neonate has, for some time, been ascribed to a separate substance named 'fetal' fibrinogen. It has been noticed that the same sample of neonatal blood would yield discrepancies in the fibrinogen levels depending upon which method of measurement was used. For example, an immunoassay method such as Laurell would give a higher value compared with an estimation using a thrombin-clottable method such as Clauss (Barr, 1978; Barr et al, 1983). Various studies have addressed this issue.

1. Evidence of enhanced fibrino(geno)lytic activity was found in cord plasma with increased levels of fibrin and fibrinogen degradation products giving 'inhibited' Thrombotest dilution curves. Addition of purified Fragment X to non - inhibited cord plasma in the laboratory reproduced the effect, reflecting the increased fibrino(geno)lytic activity in cord plasma in vivo (Hamulyak et al, 1988).

2. It was also found that newly secreted HMW fibrinogen is partly converted to LMW forms by an unidentified protease (not plasmin) and that elimination of HMW forms is greater in fetal than in adult plasma leaving more LMW forms behind (Karitsky & Kleine, 1971; Andrew et al, 1988).

3. Fibrinopeptide A is released from the carboxyl end of the A alpha chain on digestion with thrombin and one of the early cleavage sites is at the lysine - alanine bond. This removal of the carboxyl ends from the A alpha chains leads to Fragment X. It has been shown that the N - terminal alanine residues in fetal fibrinogen were only 54% that of adult values. A and B peptide release, (from the B-beta chains) was retarded when alanine was reduced to 50% in adult fibrinogen (Hasegawa & Sasake, 1989).

4. Opinion has in recent times veered away from the idea that 'fetal' fibrinogen is a separate entity. The development of new methodology using highly specific monoclonal antibodies used in the detection of intact fibrinogen alongside the Clauss test has helped in the understanding of the fibrinogen found in cord and neonatal blood. In a sub-set of samples from the cohort used in this study the associations between fibrinogen as measured by clottable, total and intact methods and fibrinogen degradation products (FDP) were examined.

Plasmin acts on fibrinogen to give fibrinogen degradation products (FDP). The first stage of this process is the formation of Fragment X which is still partially susceptible to thrombin action but polymerizes at a slower rate (see Figure 4, page 52). The FDP measured by an ELISA method increase as clottable (Clauss) fibrinogen decreases.The FDP were negatively associated with all three measurements.

The action of plasmin on cross-linked fibrin releases cross-linked Fragment D fragments, and adjacent molecules as D-dimer. The presence of this substance is therefore evidence of fibrinolysis. In this sub-set the fibrinogen degradation products and D-dimer increased together, demonstrating that both fibrinogen and fibrin were being broken down. These results are consistent with the view that 'fetal' fibrinogen is not a separate entity but is due to increased fibrino (geno) lysis giving rise to a lower level of clottable fibrinogen. These results were presented at the British Society of Haemostasis and Thrombosis meeting in Harrogate in 1992, and accord with those of Hamulyak et al (1988). A summary is presented in Table 1, p. 55. (Stirling et al, 1994).

Table 1

Clottable, total and intact fibrinogen, fibrinogen degradation products (FDP) and D-dimer (g/l) in the study by Stirling et al (1994)

Number (54)	Clottable	Total	Intact	FDP
Mean	1.75	2.09	2.00	2.22
SD	0.68	0.45	0.51	13.3
Range	0.12 - 3.17	0.31 - 2.87	0.40 - 2.90	0.21 - 55.5

The negative association between all 3 measurements and FDP

Clottable v FDP	r = -0.40	p = <0.005
Total v FDP	r = -0.19	p = 0.18
Intact v FDP	r = -0.35	p = 0.01

FDP and D - dimer in the study by Stirling et al (1994)

Number (52)	FDP	D - dimer
mean	2.22	0.47
SD	13.3	1.00
Range	0.89 - 55.5	0.08 - 3.92

The positive association between FDP and D - dimer

r = 0.89	p = <0.001

2.2.6.1 The acute phase response, fibrinogen and C-reactive protein (CRP)

One of the functions of fibrinogen is to take part of the acute phase response in which there is an increase in the concentration of liver-derived proteins in the circulation as a reaction to inflammation, insult or injury. It is accompanied by fever, leucocytosis and muscle proteolysis. C-reactive protein is also an acute phase protein but is not involved in coagulation. The measurement of CRP in the plasma is a useful biochemical marker in detecting the acute phase response.

The major acute phase proteins are C-reactive protein, complement components, Factor VIII, fibrinogen, plasminogen, Antithrombin III, Anti-trypsin, Haptoglobin, and serum Amyloid A Protein. The hepatocyte is the major site of synthesis for the acute phase proteins. Recruitment of hepatocytes to acute phase protein synthesis proceeds until between 30% and 40% of liver protein synthesis is committed to it. A simultaneous fall in synthesis of non-acute phase proteins such as albumin occurs. Synthesis of several acute phase proteins has been shown to be induced by proteins derived from macrophages and polymorphonuclear leucocytes. These mediator proteins belong to a family of closely related molecules having a molecular weight of between 12000 and 15000 called interleukins. Some acute phase proteins are stimulated in other ways, such as fibrinogen production being triggered by the products of fibrin degradation (Alving et al, 1982). Several acute phase proteins show marked increases in rates of breakdown in different types of inflammation, insult or injury. As an example, fibrinogen may have a low plasma concentration because it is being utilised in coagulation whereas C-reactive protein (CRP) is not involved in clotting.

C-reactive protein (so called because it reacts with the C-polysaccharide of the cell wall of the pneumococci) is an activator of the complement system and has a very short half-life of 4-6 hours. Its concentration is normally <10 mg/l

and it shows as much as a 4000 times increase in synthesis rate at the peak of the acute phase response to injury or infection (Whicher & Dieppe, 1985).

C-reactive protein is significantly raised in patients with unstable angina and when such patients come to autopsy they show an infiltration of inflammatory cells in the walls of the coronary arteries (Berk et al, 1990). Fibrinogen concentrations reach very abnormal levels within 3-5 days of the infarction and then return to the level usual for that patient over the next few days, whereas C-reactive protein with its very short half-life of 4-6 hours drops rapidly after the acute event.The leucocyte count is also raised in myocardial infarction and unstable angina and together with the acute rise in fibrinogen concentration above the already raised chronic level, may be considered as a marker of an inflammatory reaction in the vascular system. (Yarnell et al, 1991).

In a study of middle-aged men raised C-reactive protein levels were found to be associated with persistent Helicobacter pylori and Chlamydia pneumoniae infections as well as smoking and chronic bronchitis. These chronic insults were in turn associated with other known risk factors for cardiovascular disease such as raised plasma fibrinogen concentration, sialic acid, total cholesterol, triglyceride, glucose, and apolipoprotein B values. C-reactive protein was negatively associated with high density lipoprotein cholesterol. The inflammatory response - and C-reactive protein as a marker of it - may contribute to the progression of atherosclerosis (Mendall et al, 1996).

2.2.6.2 C - reactive protein (CRP) in the neonate

Cord blood samples from normal term neonates have serum concentrations < 0.5 mg/l. Values between 0.5 mg/l and 1mg/l are considered to be equivocal; levels > 1.0 mg/l are deemed to be abnormal.

CRP does not cross the placenta, so that a raised CRP in mother and neonate are independently produced although the same stimulus may be operating.

Cord blood CRP concentration is raised in neonates with subsequently proven bacterial or fungal infections and it has been suggested that its measurement may be helpful in the diagnosis of these conditions when the clinical signs are often vague (Sabel & Wadsworth, 1979). It is, however, clear that raised cord blood CRP levels also occur in association with fetal asphyxia, distress, shock and meconium aspiration. Thus the frequent occurrence of elevated CRP levels in the blood of uninfected neonates during the first day of life eliminates it as a useful indicator of infection at that time (Ainbender et al, 1982). For example, Caesarean Section induces a marked rise in maternal CRP, but has no effect on neonatal values. Vacuum extraction delivery, however, produces a fleeting rise of both maternal and neonatal CRP levels 24 hours after birth. Normal vaginal deliveries lead to a sharp rise and fall in CRP levels in both mother and baby within 24 hours of birth. These observations suggest that the acute phase reaction is triggered as much by varying degrees of tissue trauma as by infection in the perinatal period (Kaapa & Koistinen, 1993).

Infection is suspected in neonates when the C-reactive protein level is above 8 mg/l. Serial measurements of this protein have been shown to be more useful than changes in the neutrophil count in detecting infection whilst waiting for culture results in sick neonates (Russell et al, 1992).

Thus CRP contrasts with fibrinogen in the length of time it will persist in the blood after the acute phase response has been initiated. At four days old the neonate with a raised CRP reflects either a recent acute phase reaction such as a new infection or a serious unresolved condition persisting from birth - such as meconium aspiration. It is not likely to be caused by an acute event

surrounding birth that has been treated appropriately and resolved. A raised plasma fibrinogen concentration taken at the same time, on the other hand may well reflect an acute phase response at birth or later as fibrinogen has a half life of 4 days compared with that of C-reactive protein which is only 4-6 hours.

2.2.7 Fibrinogen in Children

Studies of fibrinogen levels in normal healthy children are uncommon. One of the earliest studies established a normal range of plasma fibrinogen levels in infants (Aballi & De Lamerens, 1962). A later study on infants and children included measurements of the fibrinogen degradation products D and E which are unclottable and yet have an antigenicity identical to fibrinogen. The test for these fragments is sufficiently sensitive to identify occult coagulopathy before it is clinically apparent. The whole group had an average value of 217 mg/dl with the only significant difference between groups being between the first week babies and the 1 week to 1 year group of infants, whose levels were 201 mg/dl and 245 mg/dl respectively. There was a correlation between serum FDP and plasma fibrinogen and normal children had FDP levels of 10.9 ug/ml, which are significantly greater than in normal healthy adults (Uttley et al, 1969). There was a graded fall in fibrinogen levels between pre-school children, school children and adults in a German study which has not been repeated (Podolsak et al, 1977).

Several studies have shown that fibrinogen levels and FDPs are elevated in illness in children. These include inflammatory conditions like asthma and bronchitis as well as leukaemia and renal disease (Famodu et al, 1994; Goldschmidt & Koos, 1984). The nephrotic syndrome is associated with grossly elevated plasma fibrinogen concentrations which revert rapidly to normal in remission (Elidrissy & Gader, 1985; Ambrus et al, 1979; Uttley et al, 1969; Ekert et al, 1972). These very high fibrinogen levels have been examined and found to contain a greater sialic acid content and a greater electronegative shift

compared to normal adult fibrinogen. In this regard the altered fibrinogen seen in these nephrotic children is similar to that of 'fetal' fibrinogen found in neonates (Abshire et al, 1995).

Fibrinogen in relation to other cardiovascular risk factors in children has been studied. As fibrinogen is a cardiovascular risk factor in middle life, attention was focussed on a possible relationship between raised plasma fibrinogen concentrations in childhood and lipid profile disturbances in a large Spanish study of over two thousand children ranging in age from two to eighteen years. It was found that fibrinogen concentrations of over 394 mg/dl - about 10% - were significantly associated with raised total cholesterol, triglycerides and very low density lipoprotein cholesterol. In addition this study illustrated that a family history of cardiovascular disease or stroke was positively associated with raised plasma fibrinogen concentrations in the children. There was a significantly higher level of fibrinogen in the groups of females aged 6 to 8 and 16 to 18 years than in males of the same ages (Sanchez-Bayle et al, 1993).

In a sub-study of The Bogalusa Heart Study plasma fibrinogen concentrations in normal children from two distinct racial groups were compared and found to be similar. There were no significant differences between the black and Caucasian groups apart from a significant rise in plasma fibrinogen concentrations in sexually mature black females of between 16 to 18 years. The main finding was a significant increase in plasma fibrinogen concentrations with rising ponderal index. In addition ponderal index, white blood count and HDL cholesterol showed independent associations with fibrinogen. An association of a raised plasma fibrinogen concentration with a family history of cardiovascular disease was not confirmed, possibly because this group of parents was younger than the age at which such disease clinically presents (Bao et al, 1993). There were significant associations between raised white count, raised fibrinogen levels and smoking amongst adolescents, as in the Oslo Youth Study (Tell et al, 1985). The mean concentrations of fibrinogen were not dissimilar to adult levels, being higher

than adult levels in the Scottish Heart Study and lower than adult levels in the Framingham Study (Lee et al, 1990, Kannel et al, 1987b). However between study comparisons are difficult due to the differing standards (calibrators) used; the amounts of the HMW, LMW and LMW components not being the same in standard and patients' samples giving rise to variability. This problem has now been overcome by the introduction of a WHO fibrinogen standard having a designated potency. Plasma fibrinogen concentrations in children with a history of familial hypercholesterolaemia were found to be similar to matched children without such a history. This implies that the rheological abnormalities seen in adults with hypercholesterolaemia are secondary to the extensive atherosclerosis and not a direct consequence of their hyperlipidaemia (Jay et al, 1991).

In conclusion plasma fibrinogen concentration is a cardiovascular risk factor that is worth measuring in children.

2.2.8 Fibrinogen in Pregnancy

Normal pregnancy is accompanied by major changes in haemostasis which involve an increase in several clotting factors and a decrease in fibrinolysis. Factors VII, VIII, X and fibrinogen rise markedly during pregnancy.
In addition Factors II and V rise at the beginning of pregnancy and then fall steadily as pregnancy progresses whereas Antithrombin III C does not appear to change (Hellgren & Blomback, 1981; Stirling et al, 1984). There is a marked decline in fibrinolytic activity from II-15 weeks onwards (Bonnar et al, 1970).

Fibrinogen is converted into fibrin by the thrombin-catalyzed release of fibrinopeptide A (FpA) and fibrinopeptide B (FpB) and these substances can be measured as markers of intravascular coagulation. Levels of fibrinogen

degradation products (FDPs) rise from about 21-25 weeks (Stirling et al,1984). There is a significantly higher amount of fibrinopeptide A present in the blood samples from pregnant women and women during labour compared with levels in non -pregnant women. The rise in coagulation factors that occurs could be due to increased synthesis or increased activation by thrombin, or to both. The findings are consistent with a mild degree of local intravascular coagulation from early on in pregnancy. There was a sudden increase in fibrinolysis in maternal samples at delivery which suggested that the placenta may be a source of a placenta-mediated inhibitor and the most likely source of fibrinolysis inhibition (Stirling et al, 1984). The presence of raised FDPs and reduced fibrinolytic activity seems paradoxical, but is most likely to be due to placental digestion of fibrin. Evidence for this theory has been presented. Large deviations in clotting factors in venous blood from the placental site compared to blood from a forearm vein in the same women have shown that intravascular coagulation is likely to be confined to the placental site (Bonnar et al, 1970).

The increased plasma fibrinogen concentration and increased thrombin production as well as the inhibition of fibrinolysis may all be part of a mechanism to provide rapid and effective haemostasis in the uterus during and after placental separation. Fibrinogen rises from non-pregnant levels of about 2.5-4.0 g/l to as high as 6.0 g/l during late pregnancy and labour. This increase is estimated to be 50% higher in late gestation than in the non-pregnant state (van Buul et al,1995). Sampling from the umbilical cord showed that the plasma fibrinopeptide A (FPA) level was similar to the maternal level at parturition, suggesting that increased fetal coagulation also takes place during parturition (Yuen et al, 1989). Further research has shown that pregnancy induced hypertension in the mother is associated with a reduction in plasma fibrinogen concentration and platelet count in their offspring. These changes were accompanied by rises in fibrinogen degradation products and Prothrombin and Thrombin times. The significance of these changes has not yet been elucidated (Agarwal et al, 1995).

2.2.9 Associations with raised plasma fibrinogen concentrations

Age

Fibrinogen concentrations increase with age. In the Northwick Park Heart Study it was found that there was a marked and steady rise of fibrinogen concentration in men as they grew older (Meade & North, 1977).

Smoking

Smoking raises fibrinogen concentrations considerably. In the Framingham Study a dose-effect relation between the number of cigarettes per day and the fibrinogen concentration was demonstrated. It was also found that the fibrinogen concentration falls within two weeks when a chronic smoker stops smoking but it takes ten years or more to return to the level of a similar person who has never smoked (Kannel et al, 1987a; Yarnell et al, 1987; Ernst et al, 1987).

Female hormones

Women have slightly higher fibrinogen concentrations than men at all ages (Lee et al, 1990). The use of oral contraceptives containing oestrogen increases fibrinogen concentration, - this was particularly true of women documented in the Northwick Park Heart Study in the 1970's, when the 'pill' contained higher doses of oestrogen than those in use today (Meade et al, 1976; Mammen, 1982; Beller & Ebert, 1985; Notelovitz, 1985; Bonnar, 1987). Fibrinogen increases during pregnancy and after the menopause (Meade et al, 1983, Stirling et al, 1984).

Diabetes and Obesity

The Scottish Heart Health Study and The Atherosclerosis Risk in Communities (ARIC) Study in America found that plasma fibrinogen was higher in diabetic patients than in the normal population (Lee et al, 1993a; Folsom, 1992). Obesity is associated with raised fibrinogen concentration independently of

other characteristics (Meade & North, 1977, Meade et al, 1979; Lowe et al, 1988; 1990; Lowe, 1992; Fogari et al, 1992).

Social factors

Fibrinogen concentrations are raised in men in lower grades of employment and those without friends and hobbies (Markowe et al, 1985; Rosengren et al, 1990). In a cross-sectional study of men and women in the Civil Service in London, it was shown that adult fibrinogen concentrations were influenced by factors that operated throughout life. Adult height, father's social class and participant's education are all measures of childhood environment and were all separately and inversely related to adult fibrinogen concentrations in both sexes. The authors concluded that fibrinogen may be a marker for biological mechanisms which mediate the inverse socioeconomic gradient evident in coronary disease (Brunner et al, 1996). In Finland it was also found that fibrinogen levels were highest in those men who had been economically disadvantaged both in childhood as well as in adult life but economic disadvantage in either childhood alone or in adult life alone did not raise fibrinogen levels in this study (Wilson et al, 1993).

Seasonal variations

Sharp seasonal variations in fibrinogen levels have been demonstrated in Rotterdam where fibrinogen levels were considerably higher in winter. This difference was not dependent upon the outdoor temperature (van der Bom et al, 1997).

Infection

Evidence has been presented associating chronic chlamydial infection with acute myocardial infarction (Saikku et al, 1988). Helicobacter pylori and Chlamydia pneumoniae infection are independently associated with raised fibrinogen concentration, raised leucocyte count and coronary heart disease. The association may be due to a low grade inflammatory response which induces an acute phase reaction (Patel et al, 1995). However there is

conflicting evidence about the association between Helicobacter infection, fibrinogen levels and atheroma. In a study from Leeds no association could be found between dyspeptic patients with proven H. pylori infection and increased fibrinogen concentrations (Carter et al, 1996). Similarly patients undergoing coronary angiography for suspected ischaemic heart disease showed a positive association between Helicobacter infection and coronary atheroma but not between the infection and fibrinogen levels (Ossei-Gerning et al,1997). Furthermore even the association between Helicobacter and ischaemic heart disease was disproved in a large prospective study done in London where no association was found between H. pylori infection and death from ischaemic heart disease (Wald et al, 1997).

Atheroma

A description of plaque rupture and the formation of thrombus within and on the ruptured lesion was stated thus 'The deposit is an endogenous product derived from the blood and for the most part from the fibrin of the arterial blood'. (Rokitansky, 1852). This early observation was ignored for many years as it was thought that fibrin deposited from the blood must be on the surface of the vessel wall, whereas lesions are covered with endothelium. Many years later it was shown that thrombus organisation is a significant factor in the growth of fibrous lesions. (Duguid, 1946). Large amounts of fibrin and fibrinogen have been found in early gelatinous and fibrous plaques (Smith, 1986).

Platelet -derived Growth Factor (PDGF) stimulates growth of fibroblasts and smooth muscle cells and has been incriminated in the pathogenesis of atherosclerosis. The proliferation of smooth muscle cells is widely perceived as one of the key events in the formation of atherosclerotic plaques and it has been shown in culture that fibrin and fibrinogen degradation products - especially fragment E - can stimulate this process (Senior et al, 1986).

The cause of endothelial abnormality or permeability leading to fibrin deposition within the artery wall is not known, but it is clear that persistent or repeated endothelial abnormality is associated with excessive fibrin deposition and the development of plaque formation (Thompson et al, 1992). A further contribution to this subject was made in a study in which the plaque prevalence in a cohort of asymptomatic men with increased cardiovascular risk was measured. It was found that there was a significant and independent association between the extent of atherosclerotic plaques and the plasma fibrinogen levels (Levenson et al, 1995).

2.2.10 Associations with lower plasma fibrinogen concentrations There are a few factors which lower plasma fibrinogen levels, for example, exercise lowers plasma fibrinogen concentrations in adults (Ernst, 1987; Moller & Kristensen, 1991; Connelly et al, 1992; Elwood et al, 1993). Moderate consumption of alcohol leads to a lowering of plasma fibrinogen concentrations (Meade et al, 1979, 1987; Lee et al, 1990; Folsom, 1992) but changes in dietary fat do not influence plasma fibrinogen concentrations (Miller, 1998). Hormone replacement therapy lowers plasma fibrinogen concentration in the postmenopausal woman (Lee et al, 1993b). Fish oils have been shown to reduce the plasma fibrinogen levels in Norway (Hostmark et al, 1988).

2.2.11 Fibrinogen is an independent cardiovascular risk factor

The concept of specific risk factors for cardiovascular disease was first introduced in the Framingham Study in 1948 and measurements of plasma fibrinogen levels were added at the 10th Biennial Examination in 1968 (Kannel et al, 1987b).

The first evidence that a raised plasma fibrinogen concentration is an independent risk factor for cardiovascular disease came from the Northwick Park Heart study which started recruitment in 1972. In this study 1511 men

aged between 40 and 64 years were monitored over a period of 7.3 to 13.5 years. During this period 109 men suffered a fatal myocardial infarction. The study showed the presence of a significant relationship between the plasma fibrinogen concentration at the beginning of the period and the subsequent cardiovascular event. It was also shown that fibrinogen had a greater prognostic value than cholesterol for risk of cardiovascular disease (Meade et al, 1980; 1983; 1986).

In Goteborg, Sweden the same association between fibrinogen and the risk of myocardial infarction and cerebral infarction was demonstrated during a follow-up period of 13.5 years. Systolic blood pressure, smoking, cholesterol and fibrinogen were all significant predictors of the incidence of myocardial infarction, whereas only plasma fibrinogen concentration and blood pressure were significantly and synergistically predictive of stroke (Wilhelmsen et al, 1984). Similarly there was a strong independent correlation between fibrinogen levels and peripheral arterial disease in the lower limb (Philipp et al,1997).

In the Framingham Study it was shown that older women could tolerate higher plasma fibrinogen levels with less risk than men. Although the values for fibrinogen in women were consistently higher the associated risk seemed to be less: as women get older the magnitude of the risk relative to the plasma fibrinogen concentration decreases, such that in women over 70 years the cardiovascular risk showed no relation to fibrinogen (Kannel et al, 1987b).

The consistent finding across all the these studies - regardless of country or method of fibrinogen estimation - was that the third of the population with the highest fibrinogen concentrations has an increased relative risk for myocardial infarction or stroke of 2.3 compared to the third with the lowest fibrinogen concentrations (Ernst et al, 1993).

There are no published studies relating neonatal fibrinogen levels to the risk of cardiovascular events in adult life.

2.2.12 Raised plasma fibrinogen and other risk factors

A small study in Leigh, Lancashire examined the role of fibrinogen as a potential predictor of heart attacks in 297 men aged 40-69 years who were free from symptoms at entry into the study. They were followed prospectively for 7.3 years. There was a significant positive correlation between initial fibrinogen level and subsequent heart attacks. In addition the risk was compounded by high cholesterol and/or high blood pressure such that men who had serum cholesterol in the top third and who also had plasma fibrinogen levels in the top third had a six times greater incidence of heart attacks than those with fibrinogen levels in the bottom third. Men with systolic blood pressure in the top third who also had plasma fibrinogen levels in the top third had a twelve times increased risk of myocardial infarction (Stone & Thorp, 1985).

The Prospective Cardiovascular Munster (PROCAM) Study measured coagulation and lipid factors in 2116 healthy, employed, middle-aged men over a four year period. There was a 2.4 fold increase in coronary heart events above the upper tertile of plasma fibrinogen concentration compared below the lower tertile. Those people with values above the highest tertile of plasma fibrinogen concentration and the highest tertile of low-density lipoprotein (LDL) cholesterol had a 6.1-fold increase in coronary risk. A high LDL cholesterol was a predictor of cardiovascular disease even when plasma fibrinogen was low, but a high plasma fibrinogen was not a predictor when LDL cholesterol was low. This study confirmed that fibrinogen is an independent risk factor in the prediction of coronary heart disease (Heinrich et al, 1994).

Some studies may have overestimated the effect of raised plasma fibrinogen concentrations by not measuring the relative influence of LDL cholesterol in the same study. The Gottingen Risk, Incidence and Prevalence Study (GRIPS) clarified this potential difficulty by including LDL cholesterol in the statistical

model in a prospective study and showed that fibrinogen was still a strong independent risk factor (Cremer et al, 1992).

2.2.13 Reduced early growth and fibrinogen in the adult

Plasma fibrinogen is a cardiovascular risk factor and reduced early growth is associated with an increased risk of cardiovascular disease in adult life. It was therefore reasonable to see if an association could be shown between reduced early growth and plasma fibrinogen levels in adult life. Several studies have been done to explore this possibility.

1. A follow-up study of men born during 1920-30 in Hertfordshire was done to find out whether reduced fetal and infant growth are associated with higher plasma fibrinogen and factor VII levels in adult life. It was found that there was a significant association between the plasma fibrinogen concentration in adulthood and weight at one year, but not with birthweight. The difference in plasma fibrinogen concentrations of men who weighed 27 pounds or more at one year (2.9 g/l) and those who weighed eighteen pounds or less at one year (3.2 g/l) was equivalent to a significantly increased risk of death from cardiovascular disease of 40%. The relationship was not diminished by adjustment for cigarette smoking, alcohol consumption, body mass index and social class (Barker et al, 1992a). Studies of plasma fibrinogen concentrations in middle-aged women in both Hertfordshire and Sheffield do not show the same associations with birthweight, abdominal circumference, placental weight/birthweight ratio or weight at one year as in men (Martyn et al, 1995a; Fall et al, 1995b).

2. In Preston a similar follow up study was done by the same group comparing the plasma fibrinogen concentrations in middle-aged men with their birth measurements. As in Hertfordshire there was no association between plasma

fibrinogen concentration and birthweight but there was a significant association between fibrinogen concentration and the ratio of placental weight to birthweight. This showed that the lowest mean fibrinogen concentration (2.82 g/l) was in men who had weighed more than 7.5 pounds at birth, with a placental weight of 1.25 pounds or less. The highest level (3.11 g/l) was in men who weighed 6.5 pounds or less with a placental weight greater than 1.25 pounds. No associations could be found between birth measurements and factor VII in the Preston cohort (Barker et al, 1992b).

3. Sheffield birth records of 50-60 years ago included the abdominal circumferences of neonates. A study was designed to compare this measurement - a surrogate measurement of the liver-and birthweight with the plasma fibrinogen concentration in the middle-aged men and women. The mean plasma fibrinogen concentration fell significantly both with increasing birthweight and with increasing abdominal circumference in men but there was no association in women. There was a smaller though still significant association in men between plasma fibrinogen concentration and head circumference, length and ponderal index at birth, the shorter, smaller babies tending to have raised adult plasma fibrinogen concentrations (Martyn et al, 1995a).

It is difficult to understand why raised plasma fibrinogen concentrations and an increased cardiovascular risk in men should be associated with different measures of reduced early growth in different communities. The results in women are more consistent, in that there is no relation in women. There are distinct sex differences in the associations between plasma fibrinogen concentrations and early growth which may be hormonally controlled postnatally, as it has been shown that plasma fibrinogen concentrations are the same in cord blood from both sexes (van der Salm et al, 1994b).

Throughout life the plasma fibrinogen concentrations are higher in women than in men until the menopause when there is an even sharper rise in plasma fibrinogen concentrations in women. However, in spite of these higher levels, the risk attached to them is lower and cardiovascular disease only starts to rise to the same level as in men after the menopause (Meade et al,1983). The mechanism for this protection has not yet been identified.

Male babies start life heavier than females. Where the measurement of weight at one year is available it reinforces the message that not only fetal growth, but growth during infancy as well, is very important for the health of male adults. The significance of growth in infancy for female babies is more complex.

Cardiovascular disease is associated with low birthweight in both sexes but the association with growth in infancy is differently expressed. In a study of weight in infancy and death from ischaemic heart disease, men with the lowest weights at birth and at one year had the highest death rates from ischaemic heart disease (Barker et al, 1989b). In a study of early growth and death from cardiovascular disease in women, the highest death rates from cardiovascular disease were among those with below average birthweight but also above average weight at 1 year (Osmond et al, 1993).

In Hertfordshire women plasma fibrinogen concentration was not associated with either birthweight or weight at one year but there were significant associations between plasma glucose and insulin, higher systolic blood pressure, higher serum triglyceride concentration, lower serum high density lipoprotein cholesterol concentration and higher waist-hip ratio and low birthweight particularly if followed by a high body mass index in middle life (Fall et al, 1995b).

The second part of Section 2 is summarized as follows:

2.2.1 - 2.2.4

Fibrinogen is a large trinodular glycoprotein which has the same number of aminoacids in the neonate as in the adult and is indirectly determined by genetic inheritance. Its synthesis by the liver starts early in fetal life. and it has a vital function in blood coagulation.

2.2.5.1 - 2.2.5.7

The primary function of fibrinogen is to provide haemostasis by its conversion into fibrin at the end of the coagulation cascade. Fibrinogen levels in the neonate are within the normal adult range by the fourth day, but thrombin formation is slower which is partly due to lower levels of vitamin K dependent factors. The importance of sialic acid as a marker for various pathological processes, usually including microvascular disease, has become more generally recognised in recent years. Sialic acid is attached to the fibrinogen molecule and may have a vital role in its function, but the mechanism is not yet fully understood.

Neonatal anticoagulant factors C and S are synthesized as precursors by the liver and dependent upon vitamin K for their conversion to an active state, they are about 60% of adult values. The prolonged clotting time in the neonate is due to increased fibrino(geno)lysis giving rise to a lower level of clottable fibrinogen. 'Fetal' fibrinogen does not exist as a separate entity.

2.2.6.1 -2.2.6.2

Both fibrinogen and C-reactive protein are acute phase proteins which react to inflammation, insult or injury. C-reactive protein is a rapid marker of the acute phase response whereas fibrinogen rises more slowly in the plasma but persists for much longer. As both fibrinogen and CRP do not cross the placenta, this difference can be useful in timing the onset of the acute phase response in the neonate.

2.2.7 - 2.2.13

Fibrinogen in children is within the normal adult range and likewise rises rapidly in acute illness, particularly in the nephrotic syndrome.There is a rise in all coagulation factors in pregnancy consistent with a mild intravascular coagulation. Fibrinogen rises rapidly in late pregnancy - probably a physiological mechanism to prevent massive haemorrhage at delivery.

Raised fibrinogen levels in the adult are associated with increasing age, smoking, female hormones, diabetes, low socio-economic status, winter, infection and atheroma. Lower fibrinogen levels are associated with exercise, alcohol consumption, HRT and ingested fish oils.

Several studies have proved that a raised plasma fibrinogen level is an independent cardiovascular risk factor which acts synergistically with other risk factors in the aetiology of cardiovascular disease. Reduced early growth in male babies - as measured by weight at one year, the ratio of placental weight to birthweight, and abdominal circumference at birth - is associated with raised plasma fibrinogen levels in adult life. The same is not true of female babies.

2.3 Measurement and interpretation of neonatal fibrinogen levels

2.3.1 Methodology

There are many different methods of measuring plasma fibrinogen concentrations. They can be categorised into three groups (a) fibrin formation by the action of thrombin or snake venoms (b) physicochemical methods (c) immunological techniques.

Fibrin formation

This method involves either the collection of fibrin (which can be measured by weight, colorimetry or nitrogen content), or measuring the clotting time (Clauss method), the plasma-fibrinogen titre or the clot turbidity. Methods based on fibrin formation using thrombin time measure functional fibrinogen. Some provide a rapid result - e.g. the Clauss technique - whereas the colorimetric

and nitrogen determination are time consuming. All the methods based on fibrin formation underestimate fibrinogen by about 10% due to loss of fibrinopeptides.

When thrombin is added to fibrinogen a clot is formed.The method of Clauss utilises this reaction and measures the clotting time which is inversely proportional to fibrinogen content of the plasma being tested. It is widely used and measures functional fibrinogen. The clotting time of a test sample is compared to that of a standard. Partially clottable X and Y breakdown products are also measured but this is not relevant in normal, healthy subjects (Clauss, 1957).

Physicochemical methods

Fibrinogen may be measured by salting out the protein (using either ammonium sulphate or sodium sulphite) or by using quantitative electrophoretic techniques. 'Salting out' methods are no longer in regular use and are not suitable for a large number of samples. In general, physicochemical methods measure total fibrinogen and may not reflect functional activity.

Immunological methods

Fibrinogen may also be measured by passive agglutination (using latex particles or haemagglutination inhibition) radial immunodiffusion (when the diameter of the antigen/antibody complex is measured) or quantitative immunoelectrophoresis (such as the Laurell 'rocket' technique) (Laurell, 1966).

The original immunological methods measure not only total fibrinogen but molecules with immunological identity, i.e. degradation products. More recently specific antibodies have become available which will not react with the degradation products. Passive agglutination techniques can be adapted to provide quantitation and are rapid, but do not measure functional activity. Immunological measurements can be useful in identifying dysfibrinogens by the discrepancy between functional and total fibrinogen measurements.

In 1995 an automated method for the determination of plasma fibrinogen was introduced which is based on the Clauss principle combined with photometric detection. After the addition of thrombin, the coagulation time is determined by measuring the change in light absorption at 405 nm by spectrophotometry. A laboratory in the Netherlands evaluated and compared this method with two other techniques, the original Clauss assay and with the prothrombin time (PT)-derived automated method. The inter-assay coefficient of variation of the Clauss-derived assay was lower than the PT-derived assay. The effects of fibrinogen degradation products on the Clauss-derived assay were comparable with the effects on the Clauss assay, in contrast to the effects on the PT-assay which were much greater. The Clauss-derived assay proved to be a specific and precise automated method to determine fibrinogen concentrations in plasma, which is not liable to interference from different pathophysiological substances (Oosting & Hoffman, 1997).

The WHO international standard prepared by assessment of absolute fibrinogen in an isolated and washed plasma clot is now available against which all commercial and 'in house' standards can be calibrated. This has greatly improved comparison of 'within study' and particularly 'between study' results. This WHO method is time consuming and is not suitable for routine use in a clinical laboratory (Gaffney & Wong, 1992).

In Russia the original Clauss method of measuring fibrinogen has been compared with three other techniques (using an Echis snake venom, a gravimetric and a radial immunodiffusion method) in normal subjects and patients with a variety of haemstatic disorders. It was found that the data of all methods coincided if the initial levels of fibrinogen were normal, whereas in manifest hypo- or hyperfibrinogenaemia, Clauss' method was the most accurate and informative (Tseimakh et al, 1997).

Another aspect of the subject was examined by the MRC laboratories in Penarth, where the relative power of two fibrinogen assays in predicting

ischaemic heart disease was compared. It was found that the heat-precipitation nephelometric assay was more significantly associated with the risk of developing heart disease than the functional Clauss assay. The authors reflected that clottable fibrinogen as measured by Clauss may not measure all the mechanisms which mediate between fibrinogen and cardiovascular disease (Sweetnam et al, 1998).

The Clauss technique (or the automated Clauss-derived version) is one of the most commonly used methods for estimating plasma fibrinogen levels in healthy subjects. The mean of fibrinogen in the adult using the Clauss method is 2.7(1.5 to 4.0 g/l) but during acute inflammation values may rise up to 10 g/l (Ernst,1992). Plasma concentrations of fibrinogen in neonates are similar to adults.The mean (range) values of plasma fibrinogen concentrations in 118 healthy full term neonates were established by a study in Canada (Andrew et al, 1987) and were as follows:

Day 1: 2.83 g/l (1.76-3.99)
Day 5: 3.12 g/l (1.67-4.62)
Day 30: 2.70 g/l (1.62-3.78)

The reason for undertaking the present study was to determine whether there is an association between size at birth and fibrinogen in the neonate as has been proved between size at birth and fibrinogen in the adult male. Virtually all - quite possibly all - of the prospective studies showing the association between fibrinogen and coronary heart disease, and the studies relating fibrinogen in adults to measurements in early life, have used methods based on thrombin time. It was therefore logical to use a method based on thrombin time to measure plasma fibrinogen in this study. The Clauss method is commonly used and provides a rapid measure of functional fibrinogen in healthy subjects, and was therefore an appropriate choice for this study of a large cohort of neonates.

2.3.2 Raised haematocrit as a possible cause of bias

The range of haematocrit values for cord blood is usually between 0.45 and 0.51 (Foley et al,1978). An haematocrit reading of >0.65 from a central vein is diagnostic of polycythaemia and it is possible that such high readings may bias the measurement of plasma fibrinogen concentrations as there will be an increasing proportion of citrate diluting a smaller volume of plasma in samples with higher haematocrits.

The haematocrit is traditionally measured on an EDTA sample with dry anticoagulant, and in this case there is no dilution factor. Fibrinogen may be measured on a citrate sample using a liquid anticoagulant which gives a dilution factor of 10/9, however reference values used volumes of citrate adjusted for haematocrit (Hathaway & Corrigan,1991). The effect of different haematocrit (PCV) readings on the dilution of clotting factors in the plasma can be illustrated as follows:

First consider a 5ml blood sample with a haematocrit of 50%. This should yield 2.5ml cells and 2.5ml plasma. When collected into 0.5ml citrate, a smaller volume of whole blood (4.5ml) would be drawn into the sampling tube. This sample with its 50% PCV would then only have 2.25ml cells in the tube (2.25ml plasma + 2.25ml cells + 0.5ml citrate) and the total liquid volume = 2.75ml. Assume that the apparent plasma fibrinogen as measured on this diluted sample was 240mg/ml. Therefore the concentration in undiluted plasma would be 2.75/2.25 x 240 = 293.3mg/dl.

Now say that the PCV increases to 60% in the sample of 4.5ml blood. In citrate this would be 2.7ml cells + a total liquid volume of 2.3ml (1.8ml plasma + 0.5ml citrate). The total liquid volume has therefore decreased.
The dilution factor is 2.3/1.8 and the apparent fibrinogen is 1.8/2.3 x 293.3 = 229.5mg/dl. The dilution factor is therefore greater when the haematocrit is 60% than when 50%.

If the haematocrit falls to 40%, then the plasma volume recovered from 4.5ml blood is 2.7ml. This is diluted in 0.5ml citrate to give a total volume of liquid of 3.2ml. The dilution factor is 3.2/2.7 and the apparent fibrinogen concentration is 2.7/3.2 x 293.3mg/dl = 247.5mg/dl. Therefore variation in haematocrit from 40% to 60% is associated with a change in apparent fibrinogen concentration of 247.5 - 229.5 = 18mg/dl.

Normally the haematocrit levels rise in the first 2 hours after birth and return to cord blood levels by 18 hours in appropriate for gestational age (AGA) neonates. In a study from Texas the prevalence of polycythaemia at 6 hours in IUGR neonates, was 12-15%, in AGA neonates it was between 2-4% and in macrosomic neonates it was up to 8% (Ramamurthy & Brans, 1981; Ramamurthy & Berlanga, 1987). The author described an occasional transient polycythaemia in well term neonates which falls by four/five days and may cause mild hyperbilirubinaemia by that time. Another researcher described a fall in haematocrit and a small rise in plasma fibrinogen concentration by 4 days (Buonocore et al, 1991). However reference values for clotting factors in fetuses, premature and full term neonates have all been performed in citrated plasma with a fixed ratio of citrate: blood of 1:9 (Andrew et al, 1990; Reverdiau-Moalic et al, 1996). Secondly although it is known that plasma clotting factors become diluted by the fixed volume of anticoagulant at very high levels of haematocrit in adults (Komp & Sparrow, 1970), there are no published studies on the effect of haematocrit on coagulation factors in normal appropriate for gestational age (AGA) neonates (Ries, 1998).

The third part of Section 2 is summarised as follows:

2.3.1 - 2.3.2

There are three categories of methods for measuring fibrinogen. Two of these do not measure the functional capacity of the molecule, whereas those which are based on the formation of thrombin measure the function rather than structure. The Clauss method is one of these. Some breakdown products of fibrinogen are partially clottable using this test but are not normally important in healthy subjects. An automated version of the Clauss method is now in general use which involves photometric detection of the forming clot and is both reliable and precise.

A high haematocrit value can artificially dilute clotting factors. Ill and/or growth retarded neonates have a higher prevalence of polycythaemia than appropriately grown neonates at birth. Neonatal polycythaemia usually leads to jaundice by the fourth postnatal day.

In conclusion

The review has discussed the influences that affect fetal growth and the evidence that reduced early growth has profound implications for the health of the adult. It has illustrated the physiology of fibrinogen in health and disease and has shown that reduced early growth is associated with raised plasma fibrinogen concentrations in middle life. It was therefore reasonable to examine the possibility that there may be a relationship between size at birth and neonatal fibrinogen concentrations.

The Clauss method of fibrinogen estimation was shown to be an appropriate choice for this study.

3 Hypothesis

Reduced size at birth is associated with high neonatal fibrinogen levels.

3.1 Study Questions

The following study questions were investigated:

1. Is there an association between size at birth and plasma fibrinogen levels in the four day old neonate?

2. Is there an association between familial, educational, social or other perinatal variables and plasma fibrinogen levels in the neonate?

3. Do these associations mediate the relationships between size at birth and plasma fibrinogen levels?

4. Does the method of measurement of fibrinogen affect these relationships and what implications does this have for interpretation of the associations?

5. To what extent are plasma fibrinogen levels in neonates a function of the acute phase reactant properties of fibrinogen?

6. Are the relationships between size at birth and significant perinatal factors and plasma fibrinogen levels a consequence of the acute phase reactant properties of fibrinogen?

3.2 Design of study

A cross-sectional study of neonates was designed to test the above hypothesis and answer the study questions. The main data were collected over a two-year period and consisted of

1. Measurements of mother and neonate
2. Blood sampling from neonate
3. Questionnaire completed by mother
4. Data extraction from mother's and neonate's notes.

The data collection was done in the Isle of Man which is a Crown Dependency situated in the Irish Sea midway between the Northwest coast of England and Ireland. It has complete autonomy over domestic matters but the United Kingdom has responsibility for its international affairs and the overall good government of the Island. The population is approximately 70,000 people of whom about 50% are Manx born and almost all are Caucasian. The community is served by a small busy general hospital, Noble's Isle of Man Hospital, Douglas, which provides services disproportionate to the size of the population due to the relative wealth of the island and its isolation. There are between 850 and 880 births per year and home deliveries are a rarity.

3.3 Ethical Permission and Consent

Details of the proposed study were submitted to the Local Ethical Research Committee for approval which was given. Permission was also sought from and given by the Consultant Obstetrician and the Consultant Paediatrician before any patient was approached. Each mother was asked if she would like to be included in the study. The nature of the measurements and the questionnaire were explained to her.

4 Subjects and Methods

4.1 Pilot study

In 1988/89 a cross-sectional pilot study consisting of 94 blood samples was done in order to establish the most appropriate day for sampling and the feasibility of transporting specimens to the MRC Unit in Northwick Park Hospital, Harrow, United Kingdom, from the Isle of Man. Primigravida mothers were asked if they would allow their infants to have a single blood test before discharge from hospital and sampling was randomly allocated to one of the first six postnatal days. A free-flowing sample of 0.9 mls blood was taken by venepuncture from the back of the infant's hand and put into a bottle containing 0.1ml 3.8% trisodium citrate. The sample was then spun down and deep frozen at -70^0 C in the laboratory in Noble's Isle of Man Hospital. 94 samples thus collected were sent by air and courier to the MRC Unit for the plasma fibrinogen levels to be estimated. Plasma fibrinogen concentrations were estimated for each of the six postnatal days (Figure 6, page 96).

4.2 Main Study

Subjects

The source of mothers and babies was the Jane Crookall Maternity Wing of Noble's Isle of Man Hospital. Data were collected every day for the two year period from January 1991 to December 1992 including Bank Holidays and weekends but excluding leave periods when the researcher was away from the Island. In 1991 there were 860 births of whom 385 were primigravidas. In 1992 there were 848 births of whom 365 were primigravidas. The total population of first-borns in the study period was 750 babies and 542 (72%) of their mothers were interviewed.

4.2.1 Criteria for inclusion

The criteria for inclusion in the study are summarised in Table 2.

Table 2

Criteria for inclusion

1. Consent
2. Primigravida mother
3. Single pregnancy
4. Gestational age 37-42 weeks
5. Available 84-108 hours postnatally

Consent

Each primigravida mother was interviewed soon after she had recovered from the delivery and the nature and purpose of the study were explained. It was emphasised that only one attempt for a suitable sample of her baby's blood would be made and that babies were rarely distressed by the technique used in this procedure. It was also stressed that the study was optional and would not affect the treatment of mother or baby in any way.

Gestation

Gestation is the period between conception and birth and the included neonates were required to have a gestational age between 37 and 42 completed weeks (259-294 days). Gestational age was estimated by the number of completed weeks of gestation from the first day of the last menstrual period (LMP) and this was usually confirmed by ultrasound scan at 7-8 weeks. If this was not available - for instance if the mother had no antenatal care - a full Dubowitz assessment was done within 24 hours of birth to establish the baby's gestational age (Dubowitz & Dubowitz, 1977). This was needed in fourteen cases.

4.2.2 Measurements

Height of mother

The equipment used to measure the height of each mother was the fixed Harpenden Stadiometer (Holtain, U.K). This consists of a rigid vertical backboard with a rigid horizontal wooden headboard which moves up and down the backboard using small ball bearing rollers. The horizontal cursor is weighted precisely enough to allow it to move downward slowly with gravity. The headboard is fixed at 600 mm from the ground at its lowest level and 2100 mm at its highest level and the accuracy of the installation checked against a standard metre rod. The counter displays the measurement in millimetres and is read to the last completed unit.

The mother was asked to remove her footwear and any topknots, hair slides or 'ponytails' so that she could be measured with her feet flat on the floor and the headboard could slide down to touch the head with minimal effect from hair style. She stood in front of the stadiometer so that the buttocks and shoulders were in contact with the vertical backboard and the medial malleoli were touching. The shoulders were held in a relaxed natural position and the arms held loosely with the palms facing medially. The mother was asked to inhale and exhale deeply and then relax and stand as tall as possible, considering that she had delivered a baby only four days earlier.

The spine was stretched slightly by pressing upwards behind the mastoid processes before positioning the head in the Frankfurt plane, so that the lower margin of the orbit of the eye is in the same horizontal plane as the upper margin of the external auditory meatus. The measurement was read from the counter to the last completed unit, the lower figure being recorded if the counter was between two values. This procedure was repeated three times for each mother and she stood at ease between each measurement, and the mean value was used. The quality control of the instrument was ensured by a six-monthly check to see if there had been any change in its accuracy.

Birthweight of neonate

Birthweight was done as soon as the baby's condition allowed, usually within minutes of birth. The baby was dried, the cord clamped and cut, and then the baby was weighed to the nearest 5 g with digital scales (Seca 727). These scales were serviced every six months.

Occipito-frontal circumference of neonate

A disposable paper tape measure was used for each baby. It was firmly fixed with the left hand over the most anterior protuberance of the forehead whilst the right hand adjusted the tape over the most posterior protuberance of the occiput so that the measurement was the maximum head circumference. The tape was read to the nearest completed millimetre. The measurement was taken three times for each baby and the mean was used for analysis.

Lower chest circumference of neonate

This measurement was taken using a disposable paper tape measure at right angles to the axis of the body below the nipple line, such that the lower edge of the tape was at the lower edge of the xiphisternum. This was done three times, on each occasion when the baby was in expiration. The results were recorded to the nearest completed millimeter and the mean used in analysis.

Length of neonate

The equipment used to measure the length of babies was a neonatometer (Holtain, U.K). This instrument consists of a light alloy frame within which are a curved fixed headboard and a ball-bearing-mounted free moving foot board operated by a constant pressure lever. The lever is kept in the locked position when not in use and may be unlocked ready for use by rotating the lever to its full extent towards the rear of the instrument.

The naked baby was placed within the neonatometer frame on a warm surface and an assistant held the head gently but firmly against the centre of the

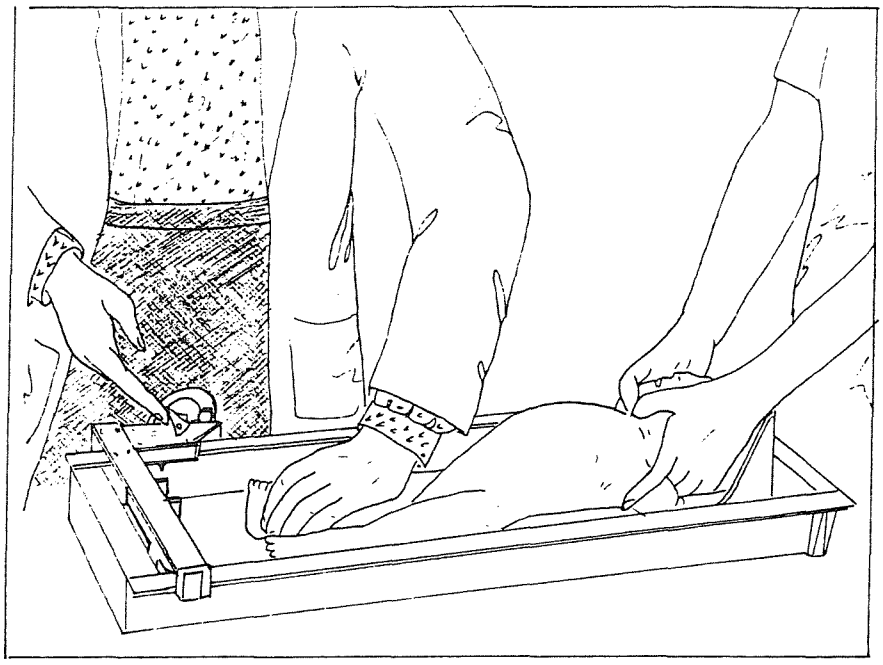
curved head piece so that the lower margins of the orbits lie in a vertical plane with the external auditory meati. At the same time the assistant's index fingers held the baby's shoulders down to touch the table and prevent arching of the back. The baby's body was maintained parallel to the long axis of the instrument and the legs were straightened with the ankles held at right angles and the toes pointing directly upwards. The carriage was released and one heel was placed against the footboard with the knee held firmly in extension (Figure 5, page 87).

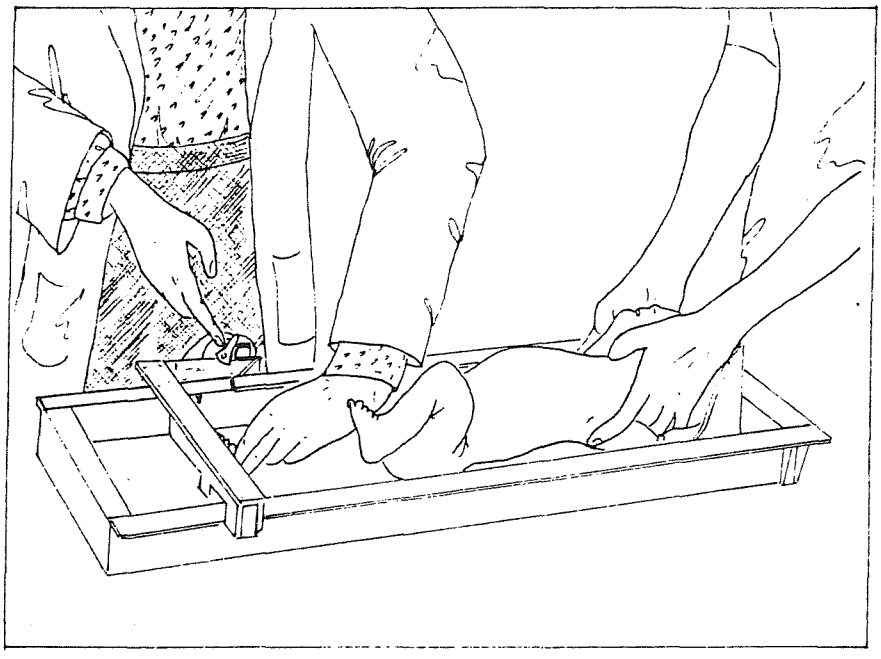
The position of the head was checked again at this stage, as it commonly slips slightly from its position against the headboard. The reading was then taken to the nearest millimetre. Each baby was measured in this way three times and the mean used in the analysis. The accuracy of the instrument was checked every six months by measuring a piece of wood of known length - 4.3 cm wide and 40.4 cm long (Appendix 2).

Maternal height and all the neonatal measurements apart from birthweight were done by a single observer (the author).

FIGURE 5

Measuring the length of a neonate.





Blood for fibrinogen estimation from neonate

The Medical Research Council Epidemiology and Medical Care Unit at Northwick Park Hospital, Harrow, agreed to measure the plasma fibrinogen concentrations and C-reactive protein levels were measured on a subgroup of samples.

Methodology

The Clauss test is the method most generally used to measure fibrinogen concentration in cord or neonatal blood and this was the chosen method for the main thesis. It measures the amount of clottable - that is haemostatically active - fibrinogen at the time of blood collection and as such is clinically relevant. For example fibrinogen as assessed by a functional assay (Clauss) alone is strongly associated with risk of ischaemic heart disease in middle life. Plasma fibrinogen measured in this way determines the polymerizing time to clot formation on addition of thrombin. This polymerizing time is subject to interference from other sources such as sialic acid and, in particular, the partially clottable X and Y Fragments. It does not give any information about the degree to which fibrinogen is altered and gives no information as to the cause of the prolonged thrombin time which is characteristic of so-called 'fetal' fibrinogen. After due consideration of these factors and consultation with the experts in the field (Patrick Gaffney, John Bonnar, Willem Nieuwenhuizen, David Lane, Peter Esnouf) it was decided to do the following methods to estimate fibrinogen concentrations (Stirling, 1989):

(a) Functional assay Clauss method (Clauss, 1957)
(b) Immunoassay Laurell 'rocket' method (Laurell, 1972)
(c) Monoclonal antibody 'Intact' assay (Hoegee-de-Nobel et al, 1988).

and to estimate C-reactive protein by an immunoassay.

Functional assay: Clauss method

All the blood samples in the study had plasma fibrinogen concentrations done by the Clauss method (Clauss, 1957). The Clauss method for thrombin - clottable fibrinogen measures the thrombin time which is inversely proportional to the fibrinogen level. The larger fibrinogen degradation products X and Y may prolong the clotting time, being only partially clottable by thrombin. The test measures haemostatically active fibrinogen.

Method for collection of blood

The equipment used for the estimation of plasma fibrinogen concentrations by this method was as follows:

No 25 gauge needle
Bottle containing 0.1 ml of 3.8% trisodium citrate
Owren's veronal buffer, pH 7.35
Thrombin with a dilution of 50 iu/ml
Fibrinogen of known standard value (Freeze-dried Immuno)
Fibrinogen from internal reference plasma

The venepuncture was usually done without disturbing the neonate and the failure rate was 3%, necessarily higher than in usual clinical practice when a further attempt would be made. The samples were taken from babies when they were between 84 and 108 hours old. A No 25 gauge needle was used to take 0.9 ml of free-flowing blood from a vein on the back of the baby's hand and put into a bottle containing 0.1 ml of 3.8% trisodium citrate.

Each sample was centrifuged and deep frozen at -70^0 C in the Pathology Laboratory within one hour of collection. When approximately fifty samples had been accumulated they were sent in dry ice by air and courier to the Medical Research Council laboratory at first situated in Northwick Park

Hospital and from 4th March 1992 transferred to St Bartholomew's Hospital, Charterhouse Square, London. The specimens were frozen on departure and still frozen on arrival.

Fibrinogen assay method (Clauss)

Doubling dilutions (1/5, 1/10, 1/20) of Standard were prepared in Owrens buffer. Each dilution was assayed in duplicate by adding reagents to KC10 cuvettes as follows:

0.2ml Standard dilution
0.1ml Bovine Thrombin (50NI Hu/ml)
Record clotting time

Prepare 1/10 dilutions of Reference and Test plasmas in Owrens buffer and assay in duplicate as above. If the clotting time of the test plasma was shorter than the 1/5 of standard it was repeated at a dilution of 1/20 and the final fibrinogen result multiplied by 2.

If the clotting time of the test plasma was longer than the 1/20 of standard, it was repeated at a dilution of 1/5 and the final fibrinogen result was divided by 2. In long runs, the Reference plasma would be included after every 20 test plasmas and the Standard re-assayed at the end of the run to check for activation or deterioration of Standard or reagents. (The 'Standard' is based on the mean value of the results from 12 laboratories using the same blood sample).

Calculation

The Standard curve was plotted on log-log paper so that the 1/10 dilution corresponded to 100%. The Test plasmas were read off as '% Standard Fibrinogen', then multiplied by assigned value of the Standard.

i.e. Test Fibrinogen = 110% Standard

Assigned value of Standard = 243mg/dl

Therefore Test Fibrinogen = 110/100 x 243 = 267mg/dl.

Immunoassay: Laurell 'rocket' method

The total fibrinogen (as opposed to the functional) was measured on all the samples in the whole cohort using a modification of the Laurell 'rocket' electroimmunoassay test. This assay provided the best measure of total fibrinogen available at the time, although the antibody used was not totally specific. Aliquots of standard and neonate plasmas were incubated with an equal volume of 3M potassium cyanate for 30 minutes at 37 degrees C. The plasmas were then diluted 1 in 10 in electrophoresis buffer. Duplicate 5 micro litres (ul) aliquots were applied to wells cut in a 1% agarose gel containing 0.5% Dako rabbit antihuman fibrinogen serum. The gels were run overnight at 2 volts/cm then dried, stained and the 'rockets' measured. Results were calculated against the standard plasma and expressed as g/l.

Monoclonal antibody assay: Intact method for non - plasmin degraded fibrinogen

This assay was developed by Niewenhuizen and his colleagues at the Gaubius Institute in Leiden,The Netherlands (Hoegee-de-Nobel et al, 1988). 110 samples were sent to Leiden for this analysis. The capture antibody, used to coat the wells of microtitre plates, is directed against the carboxyl-terminal of the fibrinogen A-chain. The second antibody, conjugated with horse-radish peroxidase, is specific for covalent-bound fibrinopeptide A in the amino-terminal of the A-chain. Because of the specificities of the two antibodies the assay is specific for fibrinogen molecules having totally intact A- chains.

C-Reactive Protein (CRP)

This protein is not associated with the clotting system and was measured as a biochemical marker for the acute phase response, in order to distinguish how much of the fibrinogen estimation was associated with an acute phase response in a group of 157 randomly chosen from the whole cohort. The CRP was measured by particle-enhanced turbidometric immunoassay in which the specific antibody is bound to latex particles.

4.2.3 Questionnaire

A questionnaire was completed and designed to collect information about both parents, their marital and educational status, weights, smoking habits, places of birth, occupations and any family history of hypertension, coronary artery disease (CAD) or stroke (Appendix I).

The occupation of the baby's father was used to categorize social class according to the Office of Population Censuses and Surveys, Classification of Occupations (1980). In a few cases the occupation of the father was unknown or the father was not living with the family. In these cases the occupation of the mother was used to categorize social class.

4.2.4 Data extraction

Data extraction sheets were completed from hospital notes (Appendix I).

Mother

The maternal notes summarised the mother's health during her pregnancy and included blood pressure readings, weight gain, delivery details, haemoglobin status and placental weight. The first blood pressure recorded in the second trimester often coincided with the first blood pressure recorded by the hospital. When recording maximum levels of maternal blood pressure the highest diastolic pressure was taken as the maximum and not the highest systolic pressure. Pre-eclampsia is diagnosed as a condition in which the first diastolic blood pressure recorded on booking is below 90 mm Hg and is followed by a

subsequent rise of at least 25 mm Hg in patients whose systolic blood pressure was less than 140 mm Hg on booking (Redman & Jefferies, 1988). This definition helps to distinguish pre-eclampsia from chronic hypertension. The first haemoglobin was often the same as the highest haemoglobin, as it was taken before the physiological haemodilution of pregnancy.

Placentae were weighed with all the attached membranes untrimmed and with the cord severed at approximately 50% of its length (which is usually about 20 cm). The weight of the placenta was then recorded to the nearest 10 g.

Neonate

Data extraction from the neonate's notes included records of sex, birthweight and Apgar scores, details of feeding practices, oxygen therapy, jaundice, hypoglycaemia or suspected infection.

The Apgar Score is a clinical method of assessing the state of a baby at one minute and again at five minutes after birth (Apgar, 1953). The score at one minute reflects any distress that the baby may have endured and the score at five minutes reflects the baby's response to extrauterine life. There are difficulties with this scoring system if the baby needs resuscitation and these are considered in the discussion.

The oxygen therapy refers to oxygen needed after any resuscitation that may have been necessary and does not include any oxygen used by face mask or for ventilation purposes in the first five minutes after birth. For the purposes of this study, jaundice was defined as clinical jaundice confirmed by a laboratory estimation of the total bilirubin level which was >169 umol/l up to the time of blood sampling for the fibrinogen level.

Hypoglycaemia was looked for in symptomatic babies or in babies who were small for dates or had had a particularly difficult birth and/or low Apgar scores.

It is defined as a blood glucose of <1.5 mmol/l in the first 48 hours and <2mmol/l thereafter (Roberton, 1993).

At the time of this study mothers were screened antenatally for beta-haemolytic streptococcal infection and if found to be positive the babies were given penicillin prophylactically at birth. In addition babies with clinically suspected infection were given antibiotics after a septic screen was done.

4.2.5 Data Entry

Analysis was done under the guidance of the Medical Research Council Environmental Epidemiology Unit in Southampton. The samples for fibrinogen assay in which macroscopic clots could be identified were removed from the file, leaving 390 sets of data which were entered twice on to computer by two individuals. This double entry ensured that errors would be more easily recognised and then corrected for non-matching cases.

4.2.6 Data protection

In order to comply with current regulations with regard to collection and storing of information about individuals, these data are registered under the Isle of Man Data Protection Act,1986, with the Registration Number 960012.09 for the purposes of epidemiological research and statistical analysis.

4.2.7 Statistical methods

The study was analysed using SPSS/PC version 6 (SPSS Inc; Chicago, Illinois, USA). After completion of the entry procedures the 390 sets of data were then cleaned. Any outliers or inconsistencies were checked against the original data and a decision made as to whether to leave them unaltered, change them or recode them as missing. Continuous variables were described by summarising them using the mean and standard deviation. A frequency distribution was plotted for each variable with mean and maximum values together with standard deviation. Multiple regression and analysis of variance

were used to decide how much of the observed overall variation in fibrinogen concentration could be accounted for by possible predictor variables.

The frequency distributions of the plasma concentration of C-reactive protein (CRP) were skewed and logarithmic transformation was used before analysis. Groups were compared using the two-tailed t-test and analysis of variance. All the results are expressed to two decimal points. Percentages are presented to the nearest whole number.

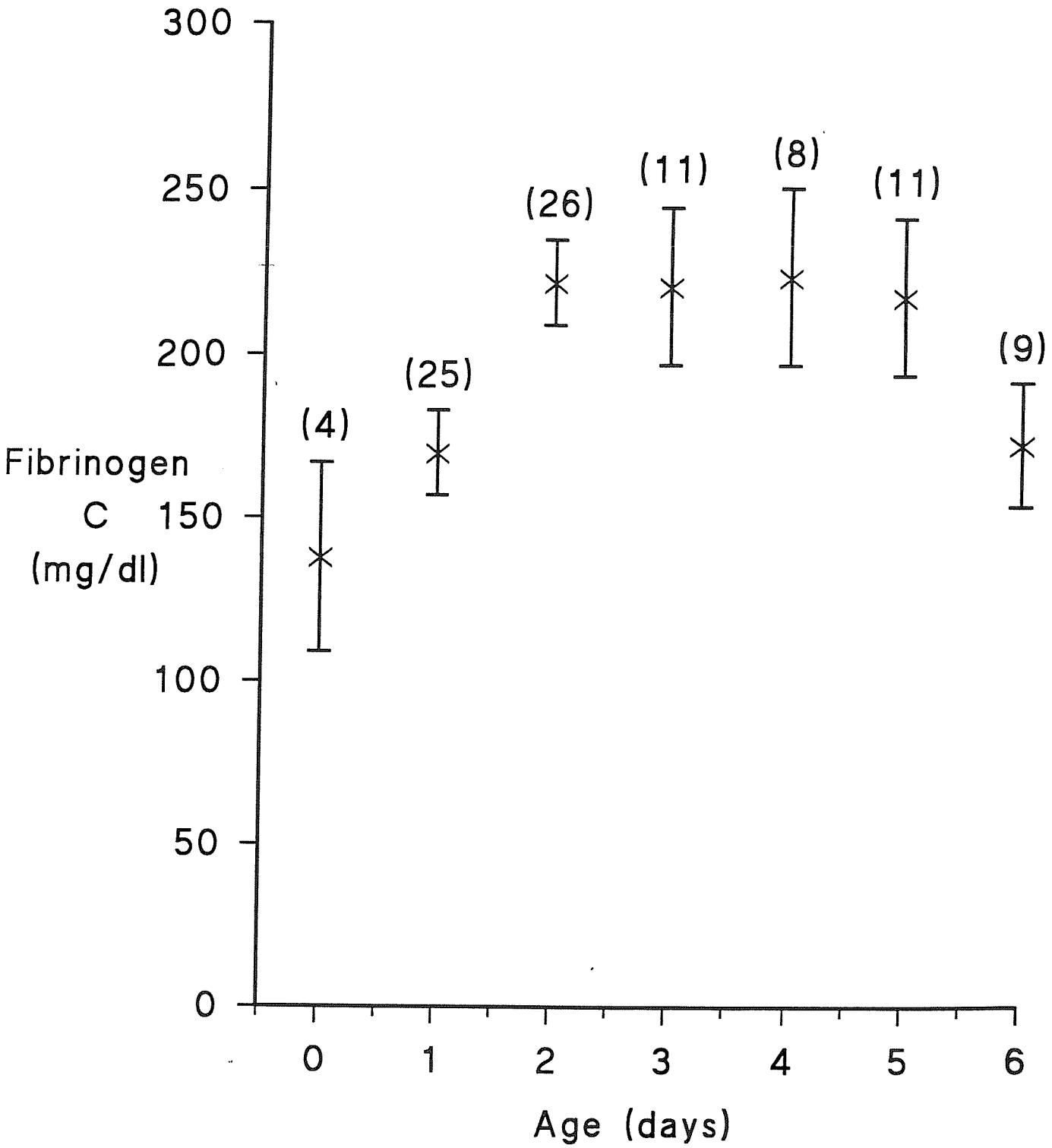
5. Results

5.1 Pilot study

As fibrinogen is an acute phase reactant it is possible that stressful birth events may induce a rise in this protein in the neonatal period and neonatal fibrinogen is known to rise in the first 4 days of life (see page 45). However the results of the pilot study show that the levels on days 2, 3, 4 and 5 were very similar. Figure 6 (page 96) illustrates the mean plasma fibrinogen concentrations according to Clauss (C) and age in days. All the fibrinogen levels are measured by the Clauss method unless stated otherwise. In order to minimise any residual rise in fibrinogen that may be due to an acute phase reaction it was decided to use the samples for the main study as late as possible before discharge - that is on the fourth day. Delay beyond four days was not practicable as healthy primigravida mothers were usually discharged in the Isle of Man on the fourth or fifth postnatal day at this time. Measurements of head circumference are also more accurate by the fourth day as the effect of moulding and caput succadaneum will have disappeared (Forfar, 1984).

Figure 6

Pilot Study Mean Plasma Fibrinogen Concentration According to Age in Days



Bar length is one s.e. No of infants in brackets

5.2 Non-participation

542 mothers were interviewed and of these 474 mothers were eligible to take part in the study. Of these 84 mothers did not participate. Data sets and blood samples were collected from 390 mother and baby pairs, but this number was reduced to 322 when the blood specimens were examined in the laboratory. 64 had plasma fibrinogen results which included the comments 'microscopic clots' or 'lysis'. These 64 sets were withdrawn. In addition 4 blood samples were reported missing and these data sets were also withdrawn.The final number of data sets and blood samples for analysis was 322. Details are given in Table 3, page 98. The characteristics of the whole cohort of 390 mothers, fathers and neonates are tabulated in Appendix 3 by way of comparison. The differences between the final sample of 322 and the remaining 68 were not great and were not statistically significant. The rest of the analysis in this study is based on this sample of 322.

Table 3

Non-participation

Total number eligible at interview	474
Refusals	8
Agreed but went home early	20
Unable to get blood sample	12
Completely/partially clotted specimens	44
Total number at time of blood sampling	390

Further reduction in numbers due to laboratory findings

Microscopic clots or lysis	64
Missing specimens	4

Final number of complete data sets analyzed	322

5.3 Description of the sample

5.3.1 Characteristics of the mothers

Maternal ages ranged from 16.4 to 41.4 years.

The booking weights were expressed in pounds in the mothers' notes and were not changed into metric values in order to avoid any spurious effect by 'rounding up or down' of the measurements.

30% of the group of mothers were smoking at the beginning of pregnancy, but this had dropped to 21% by the end of pregnancy. Smoking was more common in the younger mothers. The mean age of mothers still smoking more than 10 cigarettes per day in the third trimester was 23 years, compared with the mean age of the whole sample of 26.4 years.

98% of the cohort were Caucasian mothers and of these 43% were born in the Isle of Man, 47% were born in the U.K and 10% were born elsewhere. 43% of the mothers were in employment at the time of the birth of their child. Only 3% of the mothers had a history of chronic illness: none had a history of heart disease or hypertension.

Social class was based on the occupation of the head of household, which was the father's occupation where the parents are living together and on the mother's occupation if she was living alone. 117 (55%) mothers had no occupation outside the home and 18 (5.6%) of the fathers were unemployed. 25% of the fathers had no qualifications compared with 12% of the mothers.

These characteristics are summarized in Table 4, page 100.

Table 4

Characteristics of mothers

	Mean (SD)
Age (yrs)	26.4 (5.1)
Height (cm)	163 (6.2)
Booking weight (pounds)	141 (22.9)
Own birthweight (ounces) (known in 247 mothers)	115 (19.3)
	Number (%)
Living with baby's father	265 (82)
	Number (%)
Social class I, II, IIIN	113 (35)
Social class IIIM, IV, V	32 (10)
Social class unclassified/unrecorded	177 (55)
Qualifications: None	40 (12)
CSE, O-level, Secretarial/Clerical	190 (59)
A-level, RGN, SRN, Technical	66 (21)
Teaching, Degree	26 (8)

5.3.2 Characteristics of the fathers

The place of birth was recorded and showed that 46% of the fathers were born in the Isle of Man, 43% were born in the United Kingdom and 11% were born elsewhere. 94.4% of the fathers were employed. Social class was numerically weighted to the lower end with 52% of men in the lower half of the scale.

There were 25% of fathers with no qualifications compared with only 12% of the mothers. A similar shift was observed with CSE, O-levels and secretarial or clerical qualifications when only 45% of men were educated up to this standard, whereas 59% of the women in the 322 sample had at least this level of achievement. Men with A-level or equivalent qualification numbered 18% compared with 20% of women. When the range of education from A-levels to degree level is considered, the percentage was the same at 29% in both mothers and fathers.

Only 2 fathers in this group had a history of heart disease (1%) and 6 fathers had a history of high blood pressure (2%).

These findings are summarized in Table 5, page 102.

Table 5

Characteristics of fathers

Fathers	Mean (SD)
*Height (in)	69.4 (2.7)
+Age (yrs)	28.9 (6.4)
	Number (%)
Social class I, II, IIIN	136 (42)
Social class IIIM, IV, V	168 (52)
Social class unclassified/unrecorded	18 (6)
	Number (%)
Qualifications: None	81 (25)
CSE, O-level, Secretarial/Clerical	145 (45)
A-level, RGN, SRN, Technical	58 (18)
Teaching, Degree	34 (11)
Qualifications not known	4 (1)

* Fathers' heights were based on recall by the mother and were always quoted in feet and inches, whereas the mothers' heights were measured by the author in centimetres. Three mothers did not know fathers' height.

+ One father's age was not known.

5.3.3 Characteristics of the neonates

These are summarized in Table 6, page 108.

Birthweight

The mean (SD) birthweight (g) was 3396 (443).The mean difference between the sexes was 35g, the male neonates being the larger

The frequency distribution of birthweights is illustrated in Figure 7.

Outliers

The mean birthweight for each sex was on the 50th centile line on the Gairdner-Pearson centile chart but there were twenty six babies whose birthweights were outside the 3rd-97th centile range using these charts. These were accounted for by twenty one 'small-for-dates' and five very large infants.

Length

The mean (SD) length (cm) of the sample was 50.2 (1.9).

The mean difference between the sexes was 0.4 cm, the male neonates being longer.

Outliers

The mean length for each sex was on the 50th centile line on the Gairdner-Pearson centile chart but there were six babies whose lengths were outside the 3rd-97th centile range using this chart. These were accounted for by four 'small-for-dates' (SFD) and two very large infants.

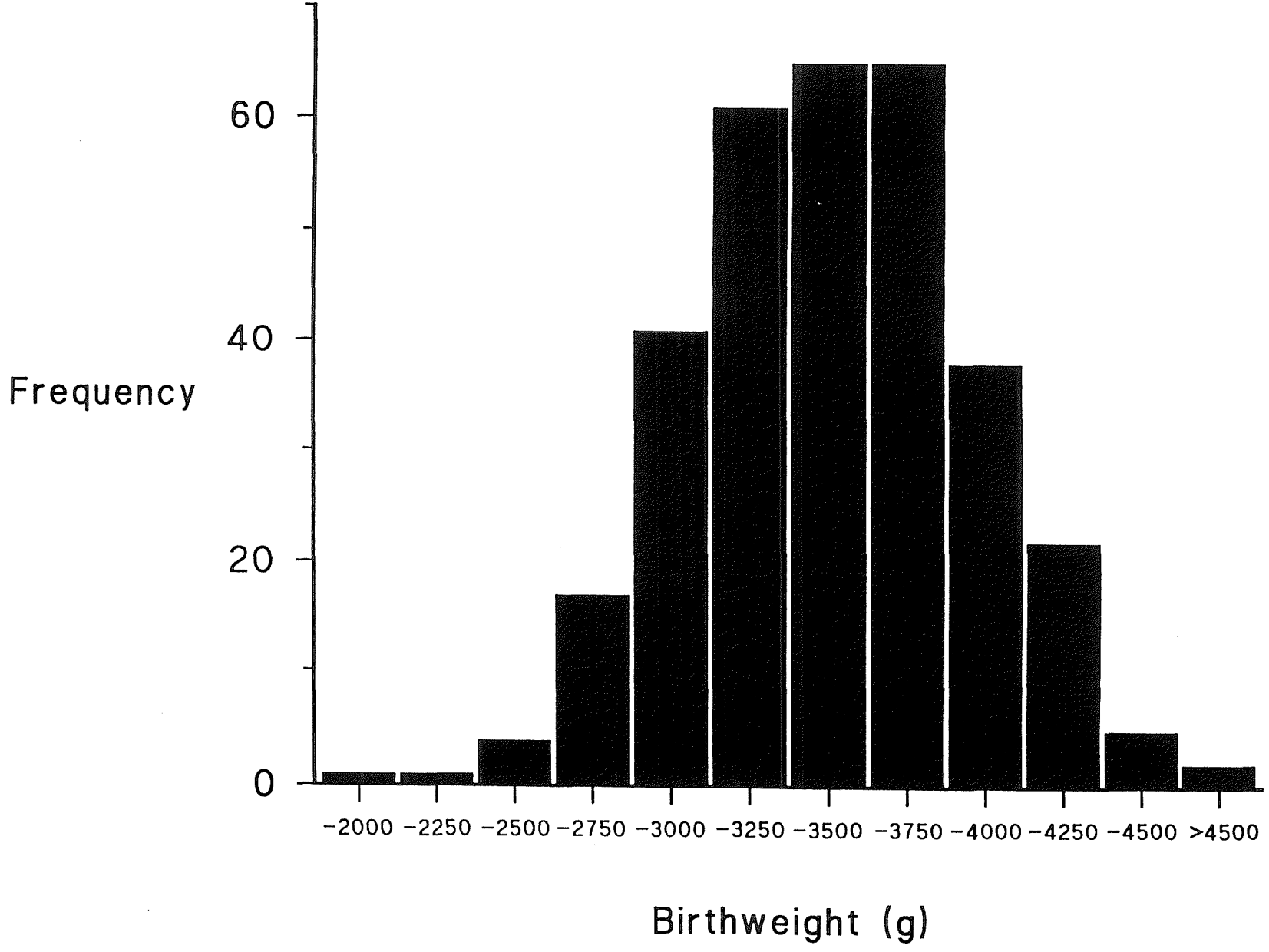


Figure 7 Frequency distribution of birthweights.

Head circumference

The mean (SD) head circumference (cm) of the sample was 34.7 (1.1).
The mean difference between the sexes was 0.4 cm, the male neonates being larger.

Outliers

The mean head circumference for each sex was on the 50th centile line on the Gairdner-Pearson centile chart but there were six babies whose heads were outside the 3rd-97th centile range using these charts. These were accounted for by four 'small-for-dates' and two very large infants.

Lower chest circumference

The mean (SD) lower chest circumference (cm) of the sample was 32.6 (1.6).
The mean difference between the sexes was 0.2 cm, the male neonates being larger. The frequency distribution of chest circumferences is illustrated in Figure 8.

Outliers

There were seventeen infants with chest measurements that were outside the 3rd -97th percentage of the whole group. These were accounted for by eight infants with chests measuring 29.2 cm or less including an isolated one with a chest measurement of 26.8 cm. Nine infants had chests measuring 35.8cm or more.

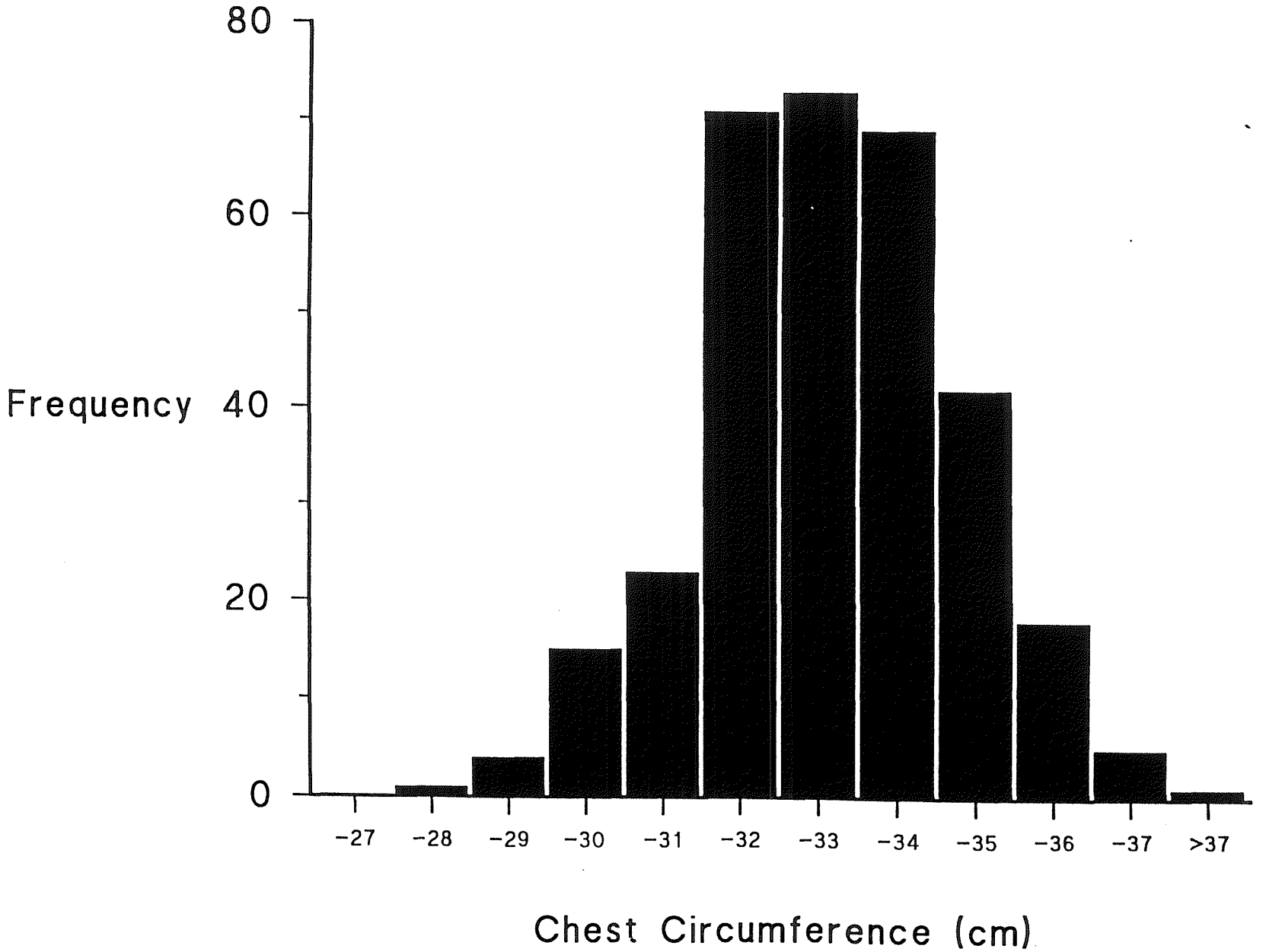


Figure 8 Frequency distribution of chest circumferences

Weight of Placentae

The mean (SD) placental weight (g) for the sample was 607.3 (123.8) . The mean (SD) placental weight (g) of the males was 607.5 (124.3) and for the females it was 607.0 (123.6).

Outliers

There were sixteen placentae that were outside the 3rd - 97th percentage of the whole sample. These were accounted for by seven placentae weighing less than 370g including an isolated one weighing 140g. Nine placentae weighed 850g or more.

Neonatal measurements were highly correlated, with the strongest positive correlation occurring between birthweight and chest circumference.

The mean (SD) gestational age at birth was 280.0 (7.8) days.
The Caesarean Section rate was 10% and other instrumental deliveries were needed in 27% births. The social class of the baby was taken as the social class of the head of the household. Breast feeding had been started in 53% and there was recorded jaundice in 35% and hypoglycaemia occurred in 8%.

Table 6

Characteristics of neonates

	Males	**Females**
	Mean (SD)	Mean (SD)
Birthweight (g)	3414 (446)	3379 (441)
Length (cm)	50.3 (1.8)	49.9 (1.7)
Head circumference (cm)	34.9 (1.2)	34.5 (1.1)
Chest (cm)	32.7 (1.6)	32.5 (1.6)
Gestational age (days)	280.0 (8.0)	279.6 (7.5)
Number	155 (%)	167 (%)
Breast fed	72 (27)	101 (31)
Infection suspected	11 (3)	10 (3)
Jaundice	54 (16)	58 (18)
Hypoglycaemia	12 (3)	14 (4)

Both sexes combined

Social class I, II, IIIN	136 (42)
Social class IIIM, IV, V	168 (52)
Social class unclassified/unrecorded	18 (6)

5.3.4 Plasma fibrinogen concentrations

There was no association between hours from birth and the plasma fibrinogen concentrations. The mean (SD) plasma fibrinogen concentration (mg/dl) of the sample was 243 (72) and ranged between 57 mg/dl and 506 mg/dl. The mean (SD) plasma fibrinogen concentration (mg/dl) in the males was 232 (72) and in the females it was 254 (71). The difference in plasma fibrinogen concentrations between the sexes was 21.9 mg/dl (95% CI 6.3 to 37.6, p = 0.006). The mean range for the first week of life in full term healthy infants is between 283 - 314 mg/dl (see page 76).

The frequency distribution of plasma fibrinogen concentrations is illustrated in Figure 9.

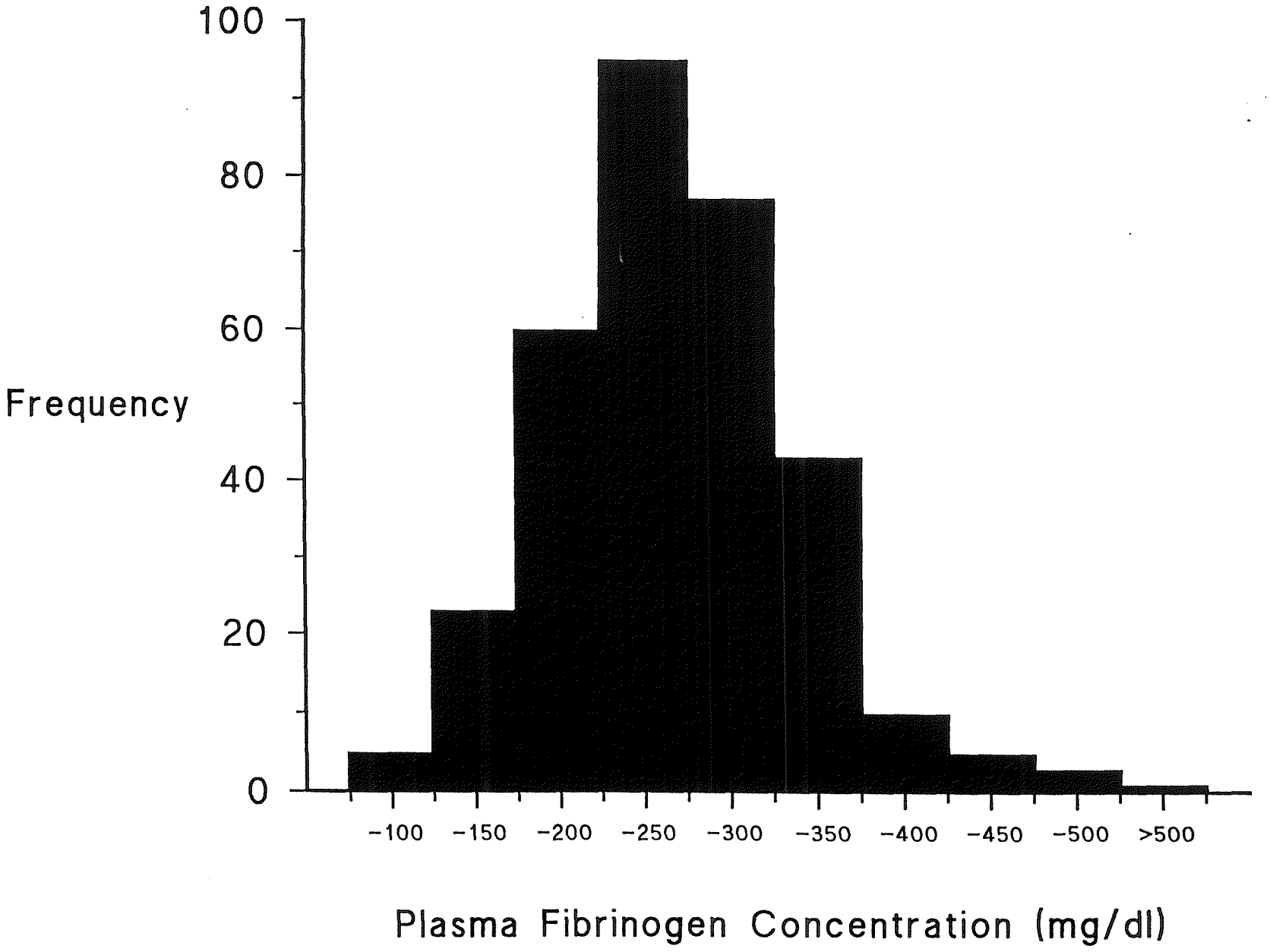


Figure 9 Frequency distribution of plasma fibrinogen concentrations

5.3.5 Relationship between size at birth and neonatal fibrinogen

5.3.5.1 Birthweight

Plasma fibrinogen concentrations were significantly associated with the birthweight of the neonate and the regression is illustrated in Fig. 10, page 112. For every kilogram rise in birthweight the fibrinogen level rose by 33.3 mg/dl (95% CI 15.9 to 50.9) p = 0.0002.

5.3.5.2 Placental weight

Each gram increase in placental weight was associated with a rise in plasma fibrinogen concentration of 0.2 mg/dl (95% CI 0.01 to 0.41, p = 0.02). The possibility that the effect of placental weight was mediated through birthweight was analysed by multiple linear regression and proved to be the case. Placental weight was no longer significantly associated with plasma fibrinogen concentration (p = 0.6) whilst birthweight still had a strong association (p = 0.009).

5.3.5.3 Length

Plasma fibrinogen concentrations were regressed on neonatal length. For every 1 cm rise in neonatal length the plasma fibrinogen rose 4.4 mg/dl (95% CI 0.2 to 8.6, p = 0.04).

5.3.5.4 Head circumference

There was no significant association between head size and plasma fibrinogen concentration. For every centimetre decrease in head size the fibrinogen level dropped by 1.0 mg/dl (95% CI - 7.7 to 5.5, p = 0.8).

5.3.5.5 Lower chest circumference

Amongst the measurements of neonatal size the lower chest circumference was most strongly associated with neonatal fibrinogen concentration and the regression is illustrated in Figure 11, page 114. One cm increase in chest circumference was associated with a 10.3 mg/dl increase in plasma fibrinogen concentration (95% CI 5.5 to 15.0, p = <0.0001). Table 7, page 115, shows that the babies with the smaller chests tended to have the lowest fibrinogen concentrations and that those with the bigger chests tended to have higher fibrinogen concentrations.

Figure 11

Plasma Fibrinogen Concentration According to Chest Circumference

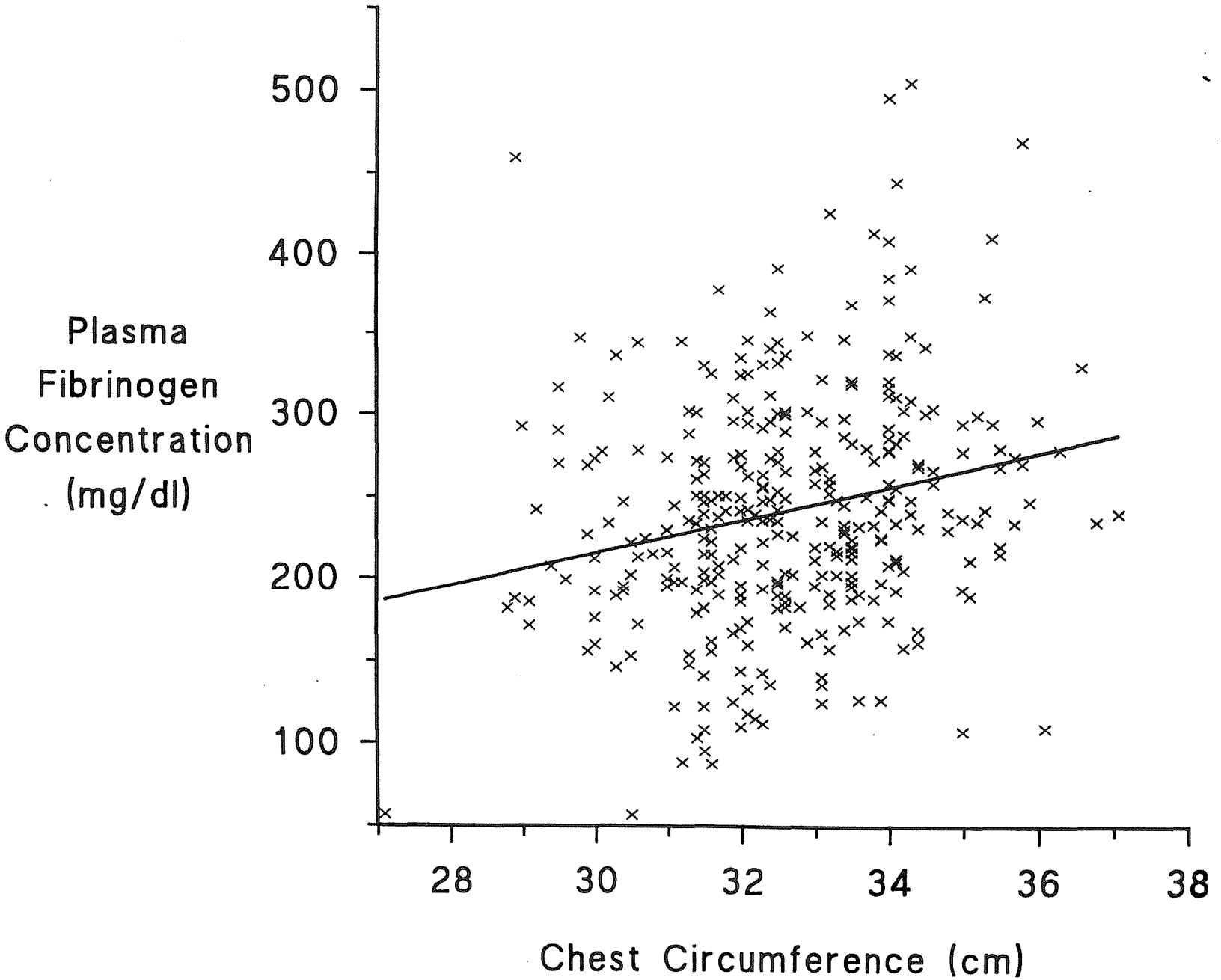


Table 7

Plasma fibrinogen concentrations according to chest circumference in 7 groups

Chest circumference (cm)	Mean (mg/dl)	SD	Number of cases
For whole sample	243	72.2	322
Group 1 = <30 cm	234	86.0	20
Group 2 = -31 cm	219	63.3	23
Group 3 = -32 cm	218	64.8	71
Group 4 = -33 cm	249	64.0	73
Group 5 = -34 cm	244	70.7	69
Group 6 = -35 cm	277	79.2	42
Group 7 = >35 cm	273	73.1	24

5.3.5.6 Fibrinogen and sex

This study has shown that the highest plasma fibrinogen concentrations were in the heaviest babies (see 5.3.5.1, page 111) and that female babies had significantly higher fibrinogen concentrations than the males (see 5.3.4, page 109). As male babies were heavier than the females the association with sex could not be explained by weight (seeTable 6, page 108).

To explore the relationship between sex and fibrinogen while taking account of the differences in birthweight between the sexes a multiple regression analysis was done combining sex, birthweight and plasma fibrinogen concentration. This showed that the significance of sex increased from p = 0.006 to 0.003, and the significance for birthweight increased from p = 0.0002 to 0.0001. A female baby would have a mean fibrinogen level of 23 mg/dl more than the mean for the whole population, after taking birthweight into account (95% CI 7.8 to 38.5, p = 0.0001). Also for every kilogram increase in birthweight the plasma fibrinogen concentration would rise by a mean of 34 mg/dl after adjusting for sex.

The relationship between fibrinogen and birthweight is independent of the effect of sex. Further regression analyses were done to see if the relationship between these was different in male and female infants. Plasma fibrinogen concentration was regressed on birthweight firstly in males and then in females. The regression coefficients were 0.035 and 0.033 respectively, that is in the same direction and of the same magnitude in both males and females. There was reduced statistical power with p values of 0.006 and 0.007 as expected due to the smaller numbers.

Therefore the relationship between fibrinogen and birthweight in both males and females is very similar.

A significant association was observed between neonatal length and plasma fibrinogen concentrations, so that the longer the baby the higher the plasma fibrinogen level (5.3.5.3, page 113). This association was attenuated when birthweight was included in the model. When the three variables of sex, birthweight and length were entered simultaneously into a multiple regression, increasing birthweight was the predominant predictor of plasma fibrinogen level ($p = <0.0001$).

Length was dropped from the calculation and plasma fibrinogen concentration was regressed with sex, birthweight and lower chest circumference. Birthweight then ceased to be significant ($p = 0.6$). The influence of sex increased so that a female neonate had 23.9 mg/dl more fibrinogen than a male in this sample (95% CI 8.7 to 39.2, $p = 0.002$) and the lower chest circumference maintained a strongly independent and significant association with plasma fibrinogen concentration so that for 1 cm increase in chest size the fibrinogen level rose by 9.1 mg/dl (95% CI 1.0 to 17.3, $p = 0.03$).

The lower chest circumference and sex are the only factors which survived in this multiple model, and once these were known, birthweight and length did not add any further significance.

5.4 Mechanisms which may influence the relationship between size at birth and neonatal fibrinogen

Groups of variables were analyzed by univariate regression to demonstrate any separate association with birthweight and/or neonatal plasma fibrinogen. Analysis was then done using the variables with statistically significant associations with size at birth and/or plasma fibrinogen levels in order to ascertain if the relationships between size at birth and neonatal fibrinogen levels were altered by these variables.

5.4.1 Rationale for using birthweight rather than chest circumference

Birthweight was used as a variable rather than chest circumference for two reasons:

1. Birthweight is the summary of intrauterine growth most commonly used in the literature.

2. Using birthweight as a variable, it is possible to construct an hypothesis to determine which variable might confound birthweight.

3. Raised plasma fibrinogen concentrations in male adults are associated with low birthweight. It is therefore reasonable to see if the relationship is the same in the neonatal period.

5.4.2 Maternal sociodemographic variables

5.4.2.1 Ethnic origin and birthplace

317 (99%) mothers were Caucasian and the remaining five were of different racial origins, consequently there were too few non-Caucasians for any valid analysis. Birthplace of the mother had no association with birthweight ($p = 0.5$) or any significant effect on the

plasma fibrinogen concentration. The mean value for mothers born in the Isle of Man was 238 mg/dl, for mothers born in the U.K. the mean was 245 mg/dl and for those born elsewhere it was 254 mg/dl. Analysis of variance showed that the variance ratio (F) was 0.8, showing that the association between the groups was not significant.

5.4.2.2 Education and social class

28% of mothers were educated to A-level standard or above and there was a positive relationship between their education and birthweight. 72% mothers did not reach 'A' level standard or equivalent, and the mean birthweight of their babies was 73 g less than those whose mothers were educated up to that standard (95% CI 10 to 135, p = 0.02). Neonatal fibrinogen concentration was not significantly dependent on the education of the mother, rising only 0.7 mg/dl for each category of rising qualifications on regression analysis (95% CI -9.6 to 11, p = 0.9).

The social class of the neonates was based on the occupation of the head of the household. Birthweight was not significantly dependent on the baby's social class (regression coefficient = -18, 95% CI = -57 to 20, p = 0.3) and did not have a significant influence on plasma fibrinogen concentration (regression coefficient = -2.8, 95% CI = -9.2 to 3.5, p = 0.4).

5.4.2.3 Living alone or with the father

82% of mothers were living with the fathers of their babies. Using the two-sample t-test there was no significant association between birthweight and living alone or with the father of the baby. Babies of single mothers living alone had mean fibrinogen levels of 251 mg/dl and those with two parents at home had levels of 241 mg/dl, giving an increase of 10 mg/dl (95% CI - 31.2 to 10.3, p = 0.3) in the fibrinogen levels in babies of single mothers living alone. Education, social class and living alone may only be markers for other determinants of size at

birth and fibrinogen levels. For example 18% mothers were living without the support of the baby's father and of these 95% were below 27 years (p = < 0.0001), were likely to have a lower social class (p = 0.005) and have fewer, if any, qualifications (p = 0.0002). They were also more likely to smoke (p = 0.0001) (see 5.4.4.3).

5.4.2.4 Working outside the home

45% mothers worked outside the home in this pregnancy and this was associated with a higher birthweight by 81g (95% CI -16 to 179, p = 0.1). Neonatal fibrinogen was lower in babies of working mothers by 4.7 mg/dl but this was not significant (95% CI -21 to +11, p = 0.6).

5.4.3 Health history of mother

5.4.3.1 Maternal birthweight

It is possible that maternal birthweight - either directly or indirectly - may have an effect on a mother's ability to nourish her own fetus and therefore the birthweight of her baby (Lumey, 1992); consequently plasma fibrinogen concentrations may be indirectly affected. This possibility was explored. 247 mothers knew their own birthweight and these were examined to see if there was any association between their own birthweight and that of their baby (see Table 8, page 121).

Neonatal plasma fibrinogen concentration rose by 0.4 mg/dl (95% CI -0.07 to 0.9, p = 0.09) for every ounce (28.3g) increase in mother's own birthweight, which was not significant.

Table 8

A group frequency comparison between maternal birthweight and birthweight of neonates

	Mothers	Neonates
Mean birthweight	3262 g (recalled)	3396 g (recorded)
Number	322 (%)	322 (%)
<2500	21 (7)	6 (2)
2500-2950	54 (17)	45 (14)
2950-3400	84 (26)	108 (33)
3400-3800	61 (19)	109 (34)
<3800	27 (8)	54 (17)
Unrecorded	75 (23)	None

The mean (recalled) birthweight of the mothers was 115 oz (3.3 kg), which was 134 g less than the mean (recorded) birthweight of neonates of both sexes in this sample. A mother's birthweight was significantly associated with the birthweight of her own baby and to a lesser extent with its length, chest and head circumference. For every ounce (28.3g) increase in maternal birthweight the neonatal birthweight rose an extra 6.6 g (95% CI 3.8 to 9.4, p = <0.0001).

Birthweight was strongly associated with mother's height, and both maternal grandparents' height (Table 9, page 122). The cardiovascular health of the maternal grandparents did not have a significant effect upon the birthweight of their grandchildren in this sample. The numbers of strokes were small but have been included for completeness. Maternal grandmothers had the following prevalence of cardiovascular disease:

high blood pressure (20%), stroke (2%) heart disease (7%). Maternal grandfathers had prevalences as follows: high blood pressure (18%), heart disease (18%) and stroke (3%). These findings are summarized below.

Table 9

Univariate analysis of the effect of maternal and grandparental variables on birthweight

x-variable	B	SE B	95% CI B	p value
Mother's height (cm)	19.5	3.9	11.8 to 27.1	<0.0001
Maternal grandm.ht (in)	24.5	10.2	4.3 to 44.7	0.01
Maternal grandf.ht (in)	29.9	7.9	14.5 to 45.5	0.0002
Mat. grandm. high BP	-129.1	62.3	-251.8 to -6.4	0.04
Mat. grandm. stroke	-11.4	201.0	-406.9 to 384.1	0.95
Mat. grandm. heart d.	-19.1	98.6	-213.0 to 174.8	0.85
Mat. grandf. high BP	77.3	65.2	-51.1 to 205.6	0.24
Mat. grandf. stroke	202.8	142.9	-78.3 to 484.1	0.16
Mat. grandf. heart d.	-51.3	65.7	-180.6 to 78.1	0.44

5.4.3.2 Menarche

The age at which periods started in the mother had no statistical association with either the birthweight of her baby (regression coefficient = 2.9, 95% CI -30 to 36, p = 0.8) or the plasma fibrinogen concentrations (regression coefficient = - 2.8, 95% CI -8.2 to 2.6, p = 0.3).

5.4.3.3 Diabetes, hypertension and chronic ill health

There were four well controlled diabetic mothers (1%) in the sample, insufficient for analysis. None of the mothers had a history of hypertension before starting this pregnancy. Thirteen mothers (3%) had a history of chronic illnesses, these included rheumatoid arthritis, renal problems, epilepsy and depression. These diverse chronic illnesses did not statistically affect birthweight which was lower by 68 g (95% CI -348 to 213, p = 0.6) or the fibrinogen levels of the neonates which was lower by 20 mg/dl in the cases (95% CI -65 to 25, p = 0.4).

5.4.4 Antenatal health

5.4.4.1 Age

Birthweight was not significantly related to maternal age. It decreased by 2.8 g for each year of increase in maternal age (95% CI -12.2 to 6.7, p = 0.6). There was a weak relationship with neonatal fibrinogen levels which decreased by 1.5 mg/dl for each year of increase in maternal age (95% CI - 3 to 0.08, p = 0.06). After adjusting for maternal age in a multiple regression analysis, the significance of the association between birthweight and plasma fibrinogen levels dropped from p = 0.0002 to p = 0.0003 and for every kilogram increase in birthweight the plasma fibrinogen concentration rose by 32.9 mg/dl.

5.4.4.2 Maternal size

Height

The mean (SD) height of the mothers was 163 cm (6.2). The association between maternal height and the baby's birthweight was significant with an increase in birthweight of 19.5 g for each cm increase in maternal height (95% CI 12 to 27, p = <0.0001). Mothers' height alone was not statistically associated with neonatal fibrinogen levels and when regressed with plasma fibrinogen in the neonate there was a rise of 0.5 mg/dl for each cm rise in maternal height (95% CI -0.8 to 1.8, p = 0.5).

Weight and body mass during pregnancy

The variables which measured weight in this study were

(a) weight of the mother at booking (lb)

(b) mother's normal weight (lb) (reported not measured)

(c) the maximum weight (lb) during pregnancy

from these were calculated the derived variables

(d) weight gain (maximum weight - normal weight)

(e) normal BMI (normal reported weight (kg) / height (m^2)

Birthweight rose by 3.6g for each pound (lb) increase in the normal (reported) weight of the mother (95% CI 1.4 to 5.8, p = 0.001) and 3.2g for each pound (lb) increase in the weight at booking in the antenatal clinic (95% CI 1.2 to 5.3, p = 0.003). This rose to 3.7g for each pound (lb) of the maximum weight before delivery (95% CI 1.8 to 5.6, p = 0.0002).

The association of birthweight with derived maternal variables was less significant. Birthweight rose by 3.6g for each lb weight gain (95% CI 0.01 to 7.2, p = 0.05). The association was less in the calculation with the (non-pregnant) body mass index. For each unit rise in the BMI the birthweight rose by 7.5g (95% CI -6.7 to 21.6, p = 0.3) (Table 10, page 125).

Measured and derived variables of maternal size were analyzed against neonatal fibrinogen levels. There was no statistically significant association between the usual weight, booking weight or maximum weight of mothers and neonatal fibrinogen levels.

Table 10

Univariate analysis of differences in birthweight (g) of neonates according to maternal variables

x variable	B	SE B	95% CI B	p value
Normal weight (lb)	3.6	1.1	1.4 to 5.9	0.001
Wt at booking (lb)	3.2	1.1	1.2 to 5.3	0.003
Max wt (lb)	3.7	0.9	1.8 to 5.6	0.0002
Mother's height (cm)	20	3.9	11.8 to 27	<0.0001
Weight gain (lb)	3.6	1.8	0.01 to 7.2	0.05
Normal BMI (kg/m^2)	7.5	7.2	-6.7 to 21.6	0.3

Table 11

Univariate analysis of differences in neonatal fibrinogen levels (mg/dl) according to variables of maternal size

x variable	B	SE B	95% CI B	p value
Normal weight (lb)	-0.28	0.18	-0.64 to 0.08	0.1
Wt at booking (lb)	-0.25	0.18	-0.60 to 0.09	0.2
Max wt (lb)	-0.14	0.16	-0.47 to 0.18	0.4
Mother's height (cm)	-0.5	0.65	-0.8 to 1.8	0.5
Weight gain (lb)	0.28	0.16	-0.31 to 0.88	0.3
Normal BMI(kg/m^2)	-2.34	1.17	-4.64 to 0.05	0.5

5.4.4.3 Smoking

30% of the mothers were smoking at the beginning of pregnancy and 21% were still smoking in the third trimester. The mean birthweight of babies whose mothers smoked at any time in pregnancy was 192g lower (95% CI -296 to -88, p = 0.0003) than the mean birthweight of the babies of non-smoking mothers. The mean birthweight of babies whose mothers smoked more than 10 cigarettes per day in the last trimester was 280g (95% CI 239 to 321, p = 0.0001) lower than the mean birthweight of the babies of non-smoking mothers. When age, height, normal weight and weight gain as well as smoking in the third trimester were regressed simultaneously, only smoking and height were associated with significant changes in birthweight. Smoking in the third trimester had the

greatest effect on birthweight and maternal height was the next most significant.

The effect of smoking on plasma fibrinogen concentrations was analyzed.The mean fibrinogen concentrations from neonates whose mothers were non-smokers was 246 mg/dl and from neonates of mothers who smoked at the beginning of pregnancy was 237 mg/dl. Statistically this difference was not significant - 9 mg/dl (95% CI -26 to 8, p = 0.30).

When the fibrinogen levels of babies whose mothers persisted in smoking more than 10 cigarettes per day in the third trimester were analyzed with those of fibrinogen levels from babies of non-smoking mothers, the association was still insignificant (regression coefficient 8 mg/dl, 95% CI -20 to 3.6, p = 0.2). The babies of these mothers had a mean fibrinogen level of 219 mg/dl.

So far we have shown that neonatal fibrinogen concentrations rise with increasing size at birth. Those variables which are statistically associated with increased birthweight may - by this association - indirectly have a positive effect upon neonatal fibrinogen concentration. We have also shown that smoking affects birthweight adversely and that this may be the reason why babies of smoking mothers have lower plasma fibrinogen concentrations - the reverse of the effect of smoking on fibrinogen concentrations in adult life.

In order to explore this further, a multiple regression analysis was done using birthweight as the dependent variable and including mothers' age, height, weight gain, smoking in the third trimester and sex in the regression analysis, the significant associations of birthweight were with smoking (p = 0.0003) and height (p = <0.0001). There was a drop of 159g

in birthweight in neonates of smoking mothers, and an associated rise of 17g for each 1 cm rise in maternal height.

Multiple regression analysis was done using plasma fibrinogen concentration as the dependent variable and including mothers' height and age, smoking in the third trimester as well as birthweight and sex. The significant positive associations which have already been shown were maintained with birthweight ($p = 0.007$) and sex ($p = 0.002$) as well as an associated small drop in fibrinogen with each year's rise in maternal age ($p = 0.02$). Mothers' height and smoking in the third trimester were not significant.

5.4.4.4 Blood pressure

The variables which measure blood pressure in this study were

(a) Systolic blood pressure on booking at hospital
(b) Diastolic blood pressure on booking at hospital
(c) First systolic blood pressure in 2nd trimester
(d) First diastolic blood pressure in 2nd trimester
(e) Maximum systolic blood pressure before labour
(f) Maximum diastolic blood pressure before labour
(g) Pre-eclampsia

Pre-eclampsia was defined as a condition in which the first diastolic blood pressure was below 90 mm Hg followed by a subsequent increase of at least 25 mm Hg and a maximum reading of at least 90 mm Hg (Redman & Jefferies, 1988).

Each of these variables was analysed in univariate regression with both birthweight and then with plasma fibrinogen concentration. No statistically significant associations were identified. Pre-eclampsia was diagnosed in 20 (6%) mothers. The mean reduction in birthweight of 145g

in their babies' birthweight was not significant (95% CI -346 to 56, p = 0.2). Using the last menstrual period (LMP) the following derived variables were calculated

(h) Gestational age in days at 1st BP in second trimester

(i) Gestational age in days at first hospital BP

(j) Gestational age in days at maximum BP

These derived variables were used to calculate mothers' blood pressure at different stages of pregnancy and the results were analyzed to see if there was an association between raised blood pressure at different stages of pregnancy (I) with birthweight and (2) with plasma fibrinogen concentrations. No significant associations were found.

5.4.4.5 Haematology

The WHO definition of anaemia in pregnancy is a haemoglobin level below 11 g/dl (World Health Organisation,1968) and only one mother had a level below this on booking at the hospital and which persisted throughout pregnancy.

It is known that maternal anaemia in pregnancy may lead to an enlarged placenta and a high ratio of placental weight to birthweight (Godfrey et al, 1991). The placenta/birthweight ratio was analyzed with the haematology variables in this sample and the finding was consistent showing that the higher the ratio the lower the haemoglobin (regression coefficient = -3.6, 95% CI -6.2 to -1.0, p = 0.04).

Anaemia and reduction of haemoglobin in pregnancy conceivably could affect birthweight and consequently could confound the relation between size at birth and fibrinogen. This possibility was examined with all the haematology variables separately in relation to birthweight and fibrinogen concentrations and no significant relationships could be identified.

5.4.5 Health of the father

The birthweight of the neonate was analyzed with paternal information to see if there was any association between the two. Social information such as social class ($p = 0.36$) employment ($p = 0.28$) and highest qualification ($p = 0.66$) and whether the father of the child lived with the mother ($p = 0.97$) were examined. It was found that none of these had any significant effect on the birthweight of the neonate. A small effect of paternal height on birthweight was recorded ($p = 0.06$), but there was no association with paternal age ($p = 0.7$). Paternal high blood pressure (6 cases) and heart disease (2 cases) did not provide sufficient data to analyze. The same calculations were performed between the paternal variables and neonatal fibrinogen levels and no significant associations were found.

5.5 Perinatal variables and neonatal fibrinogen

There was no statistically significant association between the gestational age of the neonate and plasma fibrinogen concentrations (regression coefficient = 0.5, 95% CI -0.6 to 1.5, $p = 0.4$).

5.5.1 Labour

For every hour in labour up to 18 hours the neonatal plasma fibrinogen rose by 8.3 mg/dl with a mean value of 243 mg/dl for the group (95% CI 0.15 to 16.4, $p = 0.05$). When prolonged labour was analyzed with plasma fibrinogen concentrations there was a sharp rise, such that for those women who were in labour 18 hours or more, their babies' plasma fibrinogen concentrations were higher by a mean of 58 mg/dl (95% CI 23.5 to 93.2, $p = 0.001$). In 13 women who were in labour between 19 and 24 hours, the mean was 296 mg/dl, and in four women who were in labour in excess of 24 hours the fibrinogen level was 305 mg/dl.

The length of labour was classified in 6 groups of 6 hours. Increasing birthweight was associated with longer labour, so that for each 6 hour increase there was an associated increase in birthweight of 67.7g (95% CI 17.7 to 117.7, p = 0.008). However when labour longer than 18 hours was separately regressed on birthweight it ceased to be significantly associated (regression coefficient 38.3, 95% CI -176 to 256, p = 0.7).

When birthweight and hours in labour were in multiple regression with plasma fibrinogen, birthweight maintained its association (p = 0.005) but the length of labour was not significant (p = 0.13). If the length of labour was classified according to whether or not it was longer than 18 hours however, the influence of birthweight on plasma fibrinogen rose (p = 0.0002) and that of the long labour became significant in its influence on plasma fibrinogen (p = 0.001).

5.5.2 Delivery and condition at birth

The mode of delivery had no effect on plasma fibrinogen concentrations in the neonate. The mean fibrinogen concentration for the whole sample was 243 mg/dl (95% CI 235 to 250, p = 0.5), for the 203 normal deliveries it was 243 mg/dl, for the 80 delivered by forceps or Ventouse extraction it was 249 mg/dl, for the 5 breech deliveries it was 228 mg/dl, for the 2 elective LSCS deliveries it was 231 mg/dl and for the 32 emergency LSCS deliveries the mean was 233 mg/dl. Plasma fibrinogen concentrations were not significantly associated with mode of delivery (p = 0.5).

A gross estimate of blood loss from the mother was recorded by the midwives as less than 500 ml or more than 500 ml (24 cases). The babies of the former group of mothers had mean fibrinogen levels of 243 mg/dl and the smaller group whose mothers lost much more blood at delivery had mean fibrinogen values of 240 mg/dl. Maternal blood loss

was associated therefore with a statistically insignificant drop of 3 mg/dl in the neonatal plasma fibrinogen level (95% CI -33 to 27, p = 0.8).

The Apgar scores at 1 minute in this sample were put into three groups: 1-5 = group 1; 6 and 7 = group 2; and 8, 9 and 10 = group 3. These were then analyzed in regression with plasma fibrinogen concentrations. There was an associated rise of 14.6 mg/dl in plasma fibrinogen concentration with each group rise in Apgar score (95% CI 3.3 to 25.9, p = 0.01).

When birthweight was separately regressed with the Apgar score coded into three groups in the same way, the birthweight rose by 52.6 g for each group rise which was not significant (95% CI -17 to 122, p = 0.14).

Birthweight, Apgar Score and fibrinogen concentrations were analyzed in multiple regression which showed that the influence of the Apgar score on the plasma fibrinogen dropped from 14.6 mg/dl to 12.9mg/dl (95% CI 1.8 to 24.0, p = 0.02) and birthweight maintained its strong association with plasma fibrinogen concentration (regression coefficient = 0.03, 95% CI 0.01 to 0.05, p = 0.0003).

Only six babies needed oxygen beyond 5 minutes after birth so further analysis was not possible.

5.5.3 Feeding

53% of mothers in this sample breast-fed their babies. There was no significant influence on fibrinogen which could be related to method of feeding or the time since the last feed (p = 0.6). Likewise amongst the 'bottle feeders' there was no change in the fibrinogen levels which could be related to the type of milk used.

5.5.4 Jaundice

35% of the neonates became clinically jaundiced. 99% were physiologically jaundiced and had total bilirubin levels between 169 and 240 umol/l. There was no significant association between jaundice and fibrinogen levels (regression coefficient = 8.6, 95% CI = -8.0 to 25.2, p = 0.3).

5.5.5 Hypoglycaemia

8% of the sample developed hypoglycaemia and when regressed against fibrinogen levels there was no statistically significant association (regression coefficient = -6.7, 95% CI -35.5 to 22.2, p = 0.7).

5.5.6 Blood pressure

On regression of plasma fibrinogen levels on neonatal systolic blood pressure no association was found (regression coefficient = 0.2, 95% CI -0.9 to 1.4, p = 0.7).

5.5.7 Infection

7% of the neonates had suspected infection and these showed a positive association with plasma fibrinogen levels with a mean fibrinogen of 285 mg/dl, so that there was a rise of 45 mg/dl in babies with suspected infection (95% CI 13.3 to 76.7, p = 0.006). Only half of these later proved to have had a positive septic screen. It is possible that either the true rise of fibrinogen in proven infection was higher or that the septic screen did not pick up all the important infections. All neonates with suspected systemic infection were treated with antibiotics whilst awaiting the results of the septic screen.

5.6 The acute phase reaction in the neonate

In order to examine the hypothesis that the main cause of variable fibrinogen levels in the neonate is a function of the acute phase response, another acute phase protein - C-reactive protein - was estimated in a subset. This is a particularly useful biochemical marker in this instance as not only is it **not** associated with blood coagulation (see 2.2.6.1, page 56) but it has a half life of 4-6 hours only, compared with the half life of fibrinogen which is about four days. It was therefore possible to separate out the acute phase response induced by a perinatal event and the acute phase response caused in the later postnatal period by comparing the levels of these two substances. If they were both significantly raised it is likely that an acute phase response had been triggered during the previous few hours, whereas if only plasma fibrinogen was raised the acute phase response may have been any time after birth up to the time of sampling at four days.

5.6.1 C-reactive protein

A random selection of 157 neonates had extra blood for CRP levels taken. The frequency distribution of the plasma concentration of C-reactive protein (CRP) in the sub-set was very skewed, ranging from 20 neonates with <2 mg/l to four neonates with levels >10 mg/l. The raw data were transformed for analysis using logarithms, so that the mean log value became 1.27, with a minimum of zero and a maximum of 2.7.

First it was shown that there was a significant association between the two acute phase proteins CRP and fibrinogen, which was illustrated by regression analysis. This showed that for each unit rise in the log of C-reactive protein there was an increase of 31 mg in plasma fibrinogen (95% CI 7.61 to 53.70, p = 0.01), see Figure 12, page 136.

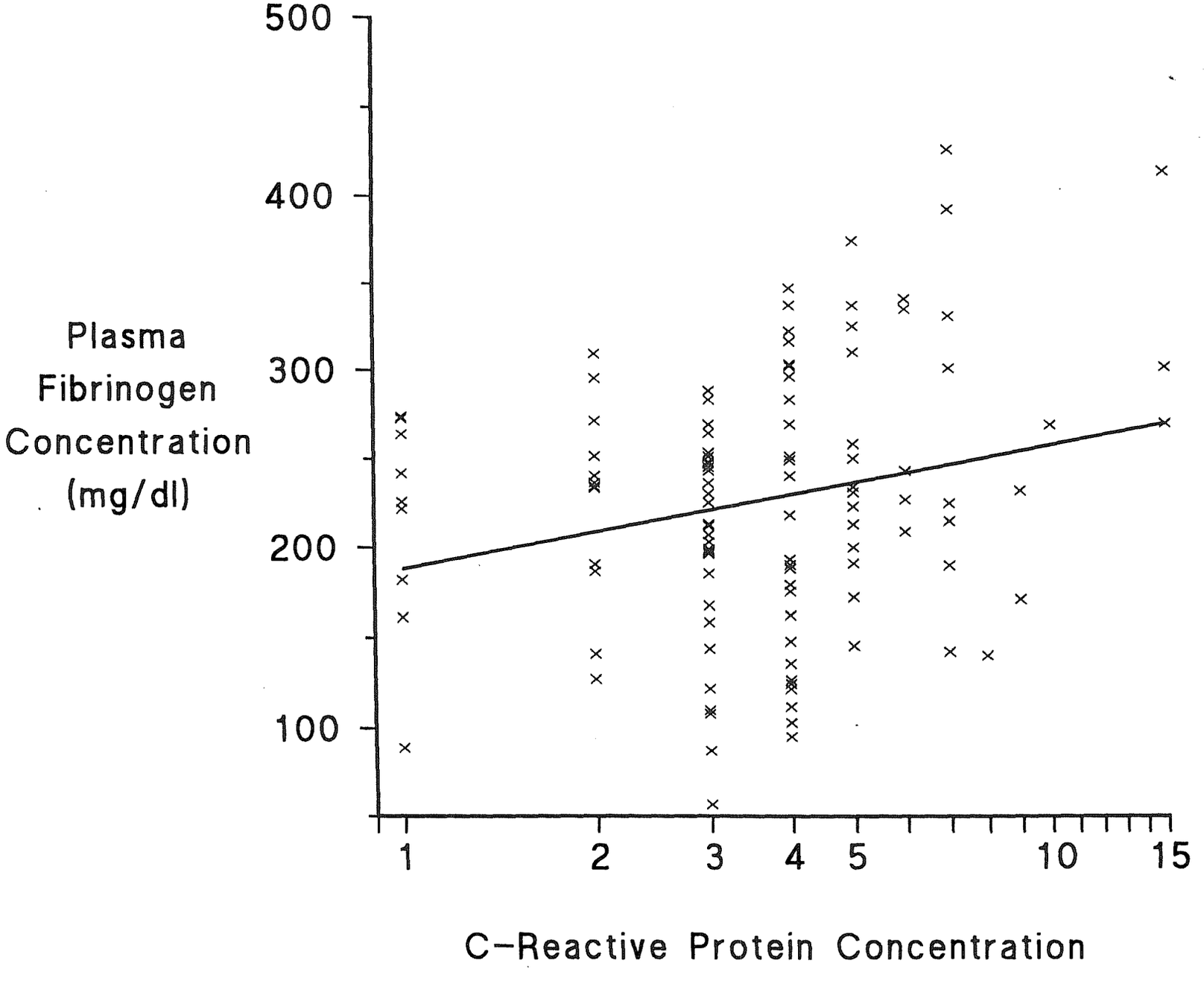
Next the birth measurements in the subset were analyzed to see if there was an association between them and the corresponding CRP estimations. It was found that the greater the birthweight the higher the log of the C-reactive protein (regression coefficient = 154, 95% CI 11 to 297, p = 0.04) but there was no significant association between chest size and CRP (regression coefficient = 0.02, 95% CI - 0.05 to 0.09, p = 0.6) or between length and CRP (regression coefficient = 0.03, 95% CI -0.03 to 0.08, p = 0.4).

5.6.2.1 The effect of a long labour

The group mean fibrinogen levels for the 17 babies whose mothers had been in labour longer than 18 hours was 300.5 mg/dl compared with the mean for the whole sample of 243.1 mg/dl. On analysis this was positively significant (regression coefficient = 58.3, 95% CI 23.5 to 93.2, p = 0.001). The 8 neonates whose mothers had long labours **and** C-reactive protein measured had a mean log C-reactive protein level of 1.43 compared with 1.25 for the remaining 111 neonates who had C-reactive protein measured but whose mothers had shorter labours. For each case of a mother labouring for longer than 18 hours the log C-reactive protein rose by 0.18 (95% CI -0.23 to 0.58, p = 0.4). The association was not significant, which is not surprising as the half-life of CRP is only 4-6 hours and the blood was taken four days after the birth following the long labour. The group mean of the birthweights of the babies whose mothers had been in labour longer than 18 hours was 3416g compared with the mean of the whole sample of 3396g.

Figure 12

Plasma Fibrinogen Concentration According to C-Reactive Protein



5.6.2.2 The effect of infection

Infection in the neonate is likely to induce a rise in both acute phase proteins, fibrinogen and C-reactive protein. Eight neonates who had C-reactive protein measured also had suspected infection. These babies had a mean value of log C-reactive protein of 1.73 compared with the remainder of the group who had this measurement done and whose mean log C-reactive protein was 1.23. There was therefore an associated rise in log C-reactive protein of 0.49 in the babies with suspected infection compared with those who were not infected (95% CI 0.10 to 0.88, p = 0.01).

Some of the variability of plasma fibrinogen in the four-day old neonate is therefore a function of the acute phase response.

5.6.3 Sex, size and the acute phase reaction

These three variables each have an effect upon neonatal fibrinogen levels. In order to ascertain whether or not they were independent and significant in their influence, multiple regression was done and included sex, lower chest circumference, birthweight, duration of labour and infection. The sex of the baby and its chest circumference maintained a significant relationship with plasma fibrinogen such that for female babies the plasma fibrinogen was 24 mg/dl higher than in male babies (p = 0.002) and for each 1cm increase in chest measurement the plasma fibrinogen rose by 10 mg/dl (p = 0.02) whereas both birthweight and duration of labour lost significance (p = 0.9 and p = 0.07 respectively). Birthweight and duration of labour were dropped from the regression analysis and lower chest circumference, infection and sex were kept in the model with plasma fibrinogen as the dependent variable. Each variable remained significant. Plasma fibrinogen concentrations rose by 10.3 mg/dl for each unit increase in chest circumference (95% CI 5.6 to 14.9, p = <0.0001), rose by 24.4 mg/dl in female neonates (95% CI 9.3 to 39.5, p = 0.02)

and rose by 41.2 mg/dl in those neonates with suspected infection (95% CI 10.6 to 71.8, p = 0.008).

5.7 Associations using different methodology

5.7.1 Associations between fibrinogen measured by 3 methods

Samples from the whole cohort were measured by the immunoassay method of Laurell as well as Clauss, in order to ascertain whether or not the associations of size at birth and 'total' as opposed to 'functional' fibrinogen as measured by Clauss were different. In 110 of the cohort some of the blood was sent to Leiden where a new assay was used to measure the Intact fibrinogen molecule (see page 91). When these results were compared they showed that the mean values of Laurell and Intact fibrinogen did not differ significantly; Clauss values were on average lower, but correlated with the other two measurements. These results are expressed quantitatively as follows:

The mean (SD) plasma fibrinogen concentration was 2.24 (0.63) g/l using the method developed for Intact fibrinogen. The mean (SD) plasma fibrinogen concentration of the same subset using the Clauss method was 2.08 (0.79) g/l. When the regression of Intact fibrinogen on Clauss fibrinogen was examined the association was found to be highly significant (correlation coefficient = 0.88, B = 1.11, 95% CI 1.00 to 1.22, p = < 0.0001), see Figure 13, page 140.

The results from the Laurell method were regressed on those using the Intact method and also proved to be highly correlated (correlation coefficient = 0.73, B = 0.57, 95% CI 0.56 to 0.58, p = < 0.0001) (see Table 12, page 139).

The mean (SD) plasma fibrinogen concentration on the 322 samples using the Laurell method was 2.28 (0.49) g/l. When the Clauss results were regressed

on those done by the Laurell method the association was again highly significant (correlation coefficient = 0.72, B = 0.96, 95% CI 0.86 to 1.06, p = < 0.0001).

The significant associations between (a) chest size and plasma fibrinogen and (b) birthweight and plasma fibrinogen as measured by Clauss were then compared to the associations between the same measurements and Intact plasma fibrinogen. It was shown that these associations were similar in magnitude and direction and remained highly significant (Table 13, p.141 and Figure 14, p.142). Therefore the conclusion in the main study that reduced fetal growth is associated with low plasma fibrinogen concentrations is upheld when fibrinogen concentration as measured using the Intact method is analyzed with chest circumference and birthweight, although the significance was reduced as there were fewer subjects included.

Table 12

Associations between plasma fibrinogen concentrations in a subset of 110 neonates using 3 different assay methods on the same sample

Method	Correlation coefficient	p value
Clauss/Intact	0.88	<0.0001
Intact/Laurell	0.73	<0.0001
Clauss/Laurell	0.72	<0.0001

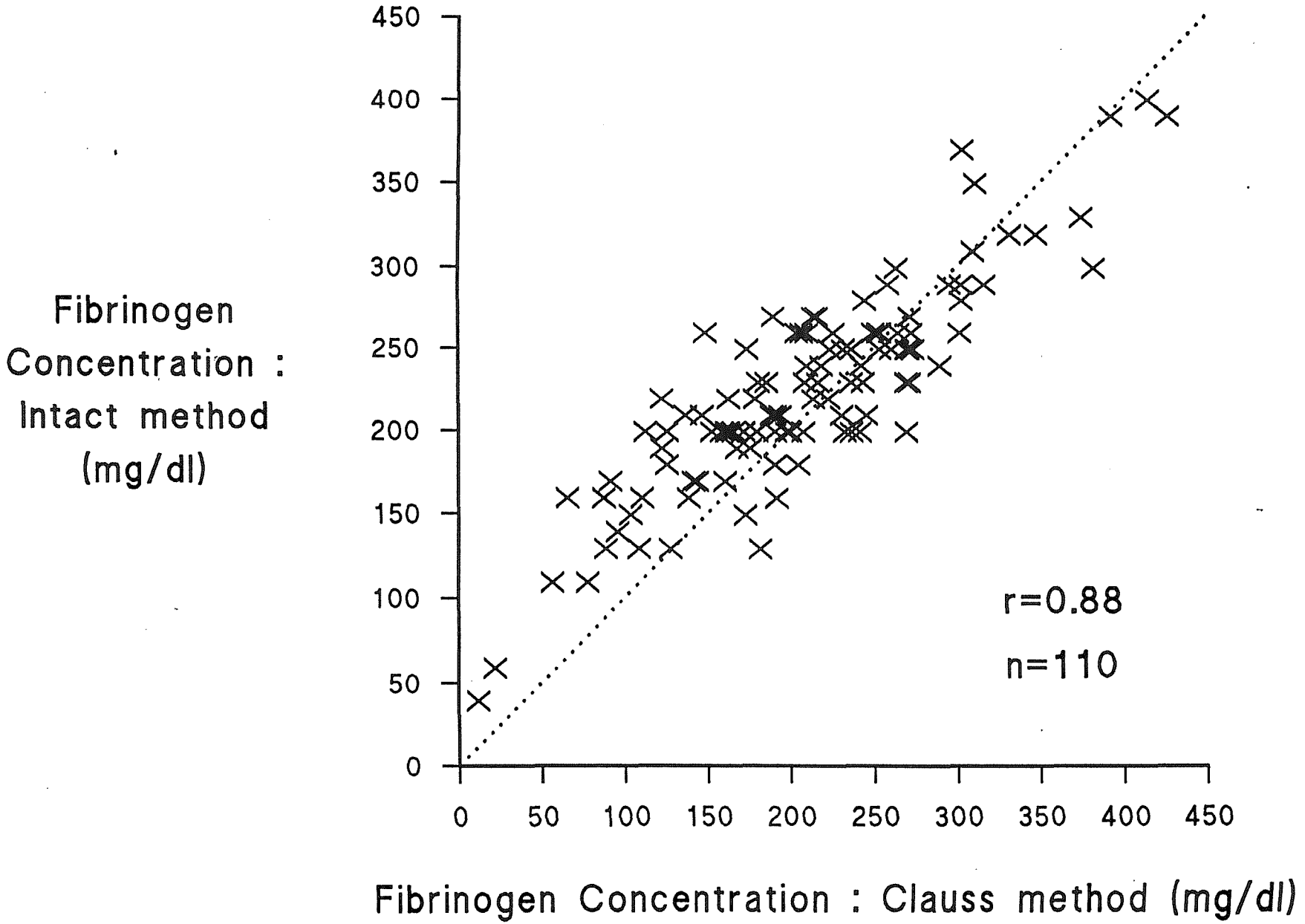


Figure 13 The relationship between fibrinogen measured by the Intact and Clauss methods

Table 13

The univariate associations of chest and birthweight with fibrinogen in the main cohort of 322 neonates using the Clauss method to measure plasma fibrinogen concentrations

x variable	B	SE B	95% CI	p value
Chest (cm)	10.30	2.41	5.55 to 15.05	< 0.0001
Birthweight (g)	0.03	0.01	0.02 to 0.05	< 0.0002

compared with

The univariate associations of chest and birthweight with fibrinogen in a subset of 84 neonates using the Intact method to measure plasma fibrinogen concentrations

x variable	B	SE B	95% CI	p value
Chest (cm)	9.91	4.43	1.23 to 18.59	0.03
Birthweight (g)	0.03	0.02	-0.01 to 0.07	0.10

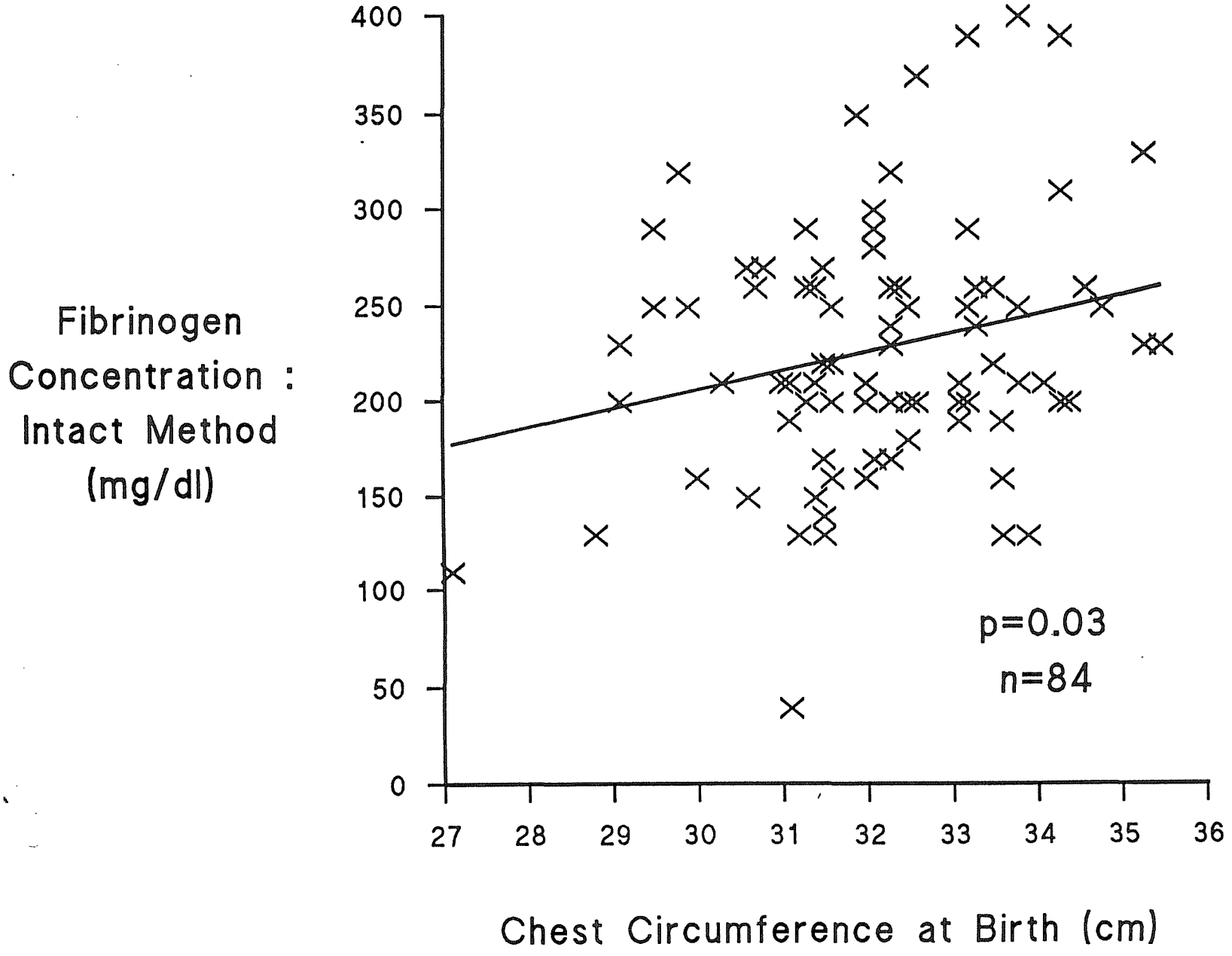


Figure 14 The relationship between fibrinogen concentration and chest circumference at birth

5.7.2 Possible bias of plasma fibrinogen with growth retardation

It has been shown that plasma fibrinogen in the neonate as measured by the Clauss method is lower than when measured by other comparative methods. It is possible that whatever causes the prolongation in thrombin time as measured by the Clauss method may be more expressed in growth retarded babies than in appropriately grown babies. In order to examine this possibility, the difference between the Intact and Clauss measurements of fibrinogen concentration by regression analysis was explored. The difference was regressed as the y variable, on the mean value of the two concentrations, birthweight and gestational age. The resulting p values were: mean value $p = 0.82$; birthweight $p = 0.87$; gestational age $p = 0.78$. This suggests that the difference between Intact and Clauss measurements of fibrinogen concentration depends neither upon the total amount of fibrinogen present nor upon the weight of the baby at birth, even adjusting for gestational age.

When the same approach to investigate the difference between the results using Laurell and Intact methods was done, the p values for birthweight and gestational age were 0.46 and 0.61 respectively, again suggesting that the difference between the two methods was not dependent upon birthweight even adjusting for gestational age.

5.7.3 C-reactive protein and plasma fibrinogen using 3 assays

In a sub-set of 157 samples the associations between the plasma fibrinogen as measured by three different assay methods and C-Reactive protein were explored. The non - haemostatic acute phase reactant C-Reactive protein (CRP) was found to be positively associated with all three plasma fibrinogen measurements (see Table 14, page 144). An acute phase reaction is evident by the correlation between CRP and Clauss fibrinogen but there was no evidence of an association between CRP and plasma fibrinogen as measured by the Laurell or Intact methods.

Table 14

Associations between fibrinogen as measured by Clauss, Laureil and Intact methods and CRP estimations in a subset of 157 neonates

Number (157)	Clauss	Laurell	Intact	CRP
Mean	2.19	2.29	2.24	3.75
SD	0.74	0.49	0.63	2.28
Range	0.12 - 4.27	0.31 - 3.52	0.40 - 4.	<2.0 - 16.0

Clauss v CRP	r = 0.21	p = <0.005
Laurell v CRP	r = 0.14	p = <0.04
Intact v CRP	r = 0.21	p = <0.01

Section 5 is summarized as follows:

5.1 - 5.3.4

The sample was described.

5.3.5.1 - 5.3.5.6

There was a strong and graded relationship between birthweight, lower chest circumference and plasma fibrinogen concentration. The lower chest circumference, which contains the bulk of the neonatal liver, was the strongest predictor of plasma fibrinogen concentration, such that larger chest measurements were associated with higher fibrinogen levels. Sex contributed independently and significantly to neonatal plasma fibrinogen concentrations, female babies had higher fibrinogen levels than male babies.

5.4.1 - 5.4.4

The mechanisms which may influence the relationship between size at birth and neonatal fibrinogen were examined and it was found that maternal sociodemographic variables did not have any significant influence on either birthweight or neonatal fibrinogen levels. However, maternal birthweight, maternal height and the height of the maternal grandparents influenced birthweight and thus indirectly influenced neonatal fibrinogen levels. Likewise, other measurements of maternal size were significant influences on birthweight but not directly on neonatal fibrinogen. Smoking was associated with a significant reduction in birthweight but there was no independent influence on neonatal fibrinogen levels. Measures of maternal blood pressure and haematology were not statistically associated with either birthweight or neonatal fibrinogen levels. The health of the father and paternal grandparents were not associated with either birthweight or neonatal fibrinogen levels.

5.5.1 - 5.5.7

Of the perinatal variables that were analysed, prolonged labour was positively associated both with an increase in birthweight and a rise in neonatal fibrinogen levels. Infection in the neonate was also associated with a significant rise in fibrinogen.

5.6 - 5.6.3

In a subset, C-reactive protein (CRP) was measured as another but unrelated acute phase protein in addition to fibrinogen. CRP was significantly associated with plasma fibrinogen, infection and a long labour but not with chest circumference. It was found that the sex of the baby, its birthweight and the acute phase reaction each had a significant and independent association with plasma fibrinogen concentrations.

5.7.1 -5.7.3

The results section concluded by examining fibrinogen as measured by three different methods and illustrating the significant degree of association between them. The prolongation of thrombin time as measured by the Clauss method was not associated with growth retarded babies. CRP was positively associated with all three measurements. Size at birth was associated with neonatal plasma fibrinogen whatever method of measurement was used.

Table 15 (page 147) summarizes the variables that were significantly related to birthweight

Table 16 (page 148) summarizes the variables that were significantly related to neonatal fibrinogen

Table 15

Variables significantly related to birthweight

x variable	B	SE B	95% CI B	p value
Gestational age(days)	18.15	3.03	12.18 to 24.11	<0.0001
Placental weight (g)	2.07	0.15	1.77 to 2.37	<0.0001
Maternal B.weight (oz)	6.59	1.41	3.80 to 9.39	<0.0001
Maternal height (cm)	19.46	3.87	11.83 to 27.09	<0.0001
M's father's ht (in)	29.9	7.87	14.46 to 45.47	0.0002
M's mother's ht (in)	24.5	10.24	4.34 to 44.66	0.02
Mother's qual(>A level)	72.8	31.87	10.09 to 135.5	0.02
Pregnancy wt gain (lb)	3.59	1.83	0.07 to 7.20	0.05
*Smoking in pregnancy	-192.43	52.87	-296.5 to -88.45	0.0003
*Smoking 3rd trimester	-143.12	36.83	-215.5 to -70.73	0.0001

* Reference group = non-smokers

Table 16

Variables significantly related to neonatal fibrinogen

x variable	B	SE B	95% CI B	p value
Chest (cm)	10.30	2.41	5.55 to 15.05	<0.0001
Birthweight (g)	0.03	0.01	0.02 to 0.05	0.0002
Length (cm)	4.42	2.13	0.23 to 8.61	0.04
Placental weight (g)	0.07	0.03	0.011 to 0.13	0.02
*Females	21.95	7.96	6.30 to 37.6	0.006
Hours in labour	8.31	4.14	0.15 to 16.48	0.05
Labour >18hrs	58.32	17.7	23.48 to 93.17	0.001
Apgar at 1 min	14.64	5.74	3.34 to 25.93	0.01
# Suspected infection	44.97	16.11	13.26 to 76.68	0.006
Log C-reactive Protein	30.65	11.63	7.61 to 53.70	0.01

* Reference group is male

Reference group is infection not suspected.

6. Discussion

This is the first study to report evidence that neonatal fibrinogen levels are positively associated with size at birth. This is contrary to the hypothesis proposed. The selection criteria and the characteristics of the sample are discussed next, followed by a consideration of the results put in context with the published literature.

6.1 Discussion of Methods

6.1.1 Pilot Study

Three important prerequisites for the main study were considered. One of the main concerns of the author was that mothers would refuse unnecessary venepunctures on their newborn babies. However, this was not a problem and over 95% mothers gave their consent.

Secondly the feasibility of the transport arrangements was proved. This involved a dawn delivery of the frozen samples to the local airport for the first flight from the Isle of Man and their synchronized collection from Heathrow. It would have been possible to do the estimations locally but the MRC collaborators requested the samples so as to standardize the results and explore other aspects of neonatal fibrinogen. The transport arrangements were tested and found to be satisfactory.

Thirdly it has been shown that the normal range of fibrinogen levels in the full term healthy infant is indistinguishable from adult levels (Hathaway & Corrigan, 1991). Consequently after analysis of the serial measurements in the pilot study and mindful of the fact that fibrinogen is an acute phase protein, the most appropriate day for sampling in the main study was selected as Day 4.

6.1.2 Selection of the sample and possible bias

The study was restricted to primigravida mothers as birthweights rise with increasing parity, multiparous mothers stay in hospital only briefly after delivery and the acute phase response may be altered in those neonates who are born - often more easily - to multiparous women.

Selection was representative of the local population for two reasons. All primigravida mothers were delivered at this hospital as it is the only Maternity Unit on the Island. Secondly **all** the mothers who fulfilled the criteria of consent, primigravida status and timing over a defined two -year period were approached when the author was physically on the island (88% of the period). In addition, as the study was based on internal comparisons, the neonates not selected will not have biased the results unless non-selection was systematically related to the current measures of interest - size at birth and fibrinogen levels. Such an association is unlikely.

6.1.3 Generalizability

The results are likely to be generalizable to a rural British Isles Caucasian population of primigravidas. As expected in a normal population sample the mean of the neonatal measurements was on the 50th centile of the 1988 Gairdner - Pearson growth charts. In this sample only 2 babies were outside this range and weighed below 2.25 kg (see Figure 7, p.104).

The mean birthweight for this study was 3.39 kg, compared with a mean birthweight of 3.42 kg for Manx babies of all gestations and parity during the same two year period. The fetal growth of these babies is unlikely to be peculiar to the Isle of Man as the mean birth weight was the same as that of a similar sample of Caucasian neonates in Southampton also measured in 1991/92 whose mean birth weight was 3.39 kg. Further comparisons showed

that there was an increase in birthweights of both the Manx cohort and the Southampton cohort compared with their mothers' birthweights when the two groups were compared (Godfrey, personal communication, 1996).

In the Isle of Man the non-manual social classes were slightly under represented in this sample, with 45% neonates coming from a family headed by a man with a non - manual occupation, whereas the Isle of Man Census in 1991 showed that 47.5% men were in this group.This compares with 56.2% for the whole of the U.K. in the 1996 Census. In addition when the social class of the Island mothers was categorized, it was found that there was a very similar social class distribution in Island mothers compared with the distribution of social class in a cohort of 538 Southampton mothers who were being studied at about the same time (Godfrey et al,1996b).

6.1.4 Participation rate

The participation rate was reduced from 474 (100%) of the mothers eligible at interview to 322 (68%) mainly by either gross or microscopic clots in the blood specimens (Table 2, page 83). These would normally be overcome in cases of clinical need by further venepuncture but only one attempt was made for the purposes of this study. If the blood specimens were partially or completely clotted before leaving the ward the data sets were discarded, but in 64 cases the clots were microscopic and the data sets were completed in ignorance of the laboratory findings. Consequently these 64 data sets are complete apart from the fibrinogen results. The two groups of data were analyzed and compared to see if there was any bias - for instance whether the blood of the babies from smoking mothers was associated with more rapid clotting. However the smoking rate in the whole cohort was 31% and in the final sample 30%. The characteristics of the whole cohort of 390 mothers, fathers and neonates are described in the Appendices (page 198) and for the final sample of 322 in Tables 3, 4 and 5, pages 98,100 and 102. Statistically there is no significant difference between the two sets of 390 and the final 322 in these characteristics.

6.1.5 Maternal data

Maternal height was measured at four days by the author as described in 4.2.2, page 84. It is possible that there may have been a slight reduction in height due to the temporarily different posture of some mothers postnatally. Those who had had an instrumental delivery or sutures may be slightly shorter as they try to minimise discomfort by stooping. It is unlikely that this made any difference to the outcome measures as the mean height of the Island mothers was the same as the mean for a cohort of similar size of Caucasian Southampton mothers measured in 1991/1992, that is 1.63 m (Godfrey et al, 1996b).

Administration of the questionnaire and collection of the data from the notes was straightforward, however two points should be made. The year of the menarche was often imprecisely remembered and when two years were given the younger age was recorded. Secondly the number of attendances at the antenatal clinic may indicate poor health and/or a diligent primigravida mother and did not show any statistical association with any of the variables used in this study.

6.1.6 Neonatal measurements

6.1.6.1 Anthropometry

Birthweight was measured electronically within minutes of birth, whereas the placentae were weighed on similar scales but they were weighed with untrimmed membranes and with only an approximate 20 cm of cord attached. The results of the placental measurements may then be less exact than the birthweights. The correlation between the two was 0.6. The correlation between birthweights done by midwives and the lower chest circumference done by the author was even higher at 0.8 as these were more exactly measured than placental weights.

6.1.6.2 Haematology

There are several ways in which fibrinogen as measured in the epidemiological studies may cause thrombosis and heart disease, such as through increased blood viscosity or fibrin deposition for example, and these have been discussed in the literature review. However the vast majority, if not all, of the epidemiological studies involving large numbers have used a method of fibrinogen estimation based on thrombin time such as the Clauss method. This measures the functional capacity of the fibrinogen fraction in the blood, which is the primary concern of these studies in order to explore how fibrinogen functions in relation to pathological conditions such as cardiovascular disease. As the main purpose of this study was to find out if fibrinogen in the neonate has a similar relationship with size at birth as obtains between fibrinogen in the adult and measurements in early life, it was logical to use a similar method to measure functional fibrinogen as that used in the epidemiological studies whose results posed the question in the first place. The Clauss method is the one most commonly used and provides a rapid measure of functional fibrinogen in healthy subjects, and was therefore an appropriate choice for this study of a large cohort of neonates.

Fibrinogen by this method determines the polymerizing time to clot formation on addition of thrombin. This reaction is subject to interference from other sources such as sialic acid and the partially clottable X and Y fragments. Sialic acid may be an integral part of a fibrinogen/sialic acid complex which functions as a whole. Its role is discussed in more detail below, but no measurement of sialic acid was done as part of this study.

Other assays were done in order to ascertain
(i) whether or not the fibrinogen as measured by methods which were not based on thrombin time were consistent with the Clauss results.

It was found that the mean fibrinogens measured by the Laurell and Intact methods were higher (2.28 g/l and 2.24 g/l) than fibrinogen measured by the Clauss method (2.08 g/l). However on analysis the correlations were highly significant.

(ii) whether or not the association that has been found using the Clauss method was echoed using the Intact method. Measurements of Total fibrinogen and Intact fibrinogen were used and the results compared (Table 12, page 139). The associations between chest size and birthweight in a subset of 84 neonates and plasma fibrinogen levels using the Intact method were similar in magnitude and direction (Table 13, page 141). It was concluded that the relation of size at birth and fibrinogen was not dependent on the method of measuring the latter.

Fibrinogen levels using different methodology in normal adults

The coagulation laboratory in the Isle of Man has found that fibrinogen measured by the Clauss method in normal adults produces a reference range (180-400 mg/dl) that is slightly below the levels given using other methods. This is the most common experience of other laboratories in the U.K. However it is standard practice for each laboratory to establish its own reference range for normal fibrinogen values due to the variability of calibration material, reagent combinations and machines depending upon the systems in use (Woods, 2000). A further complication is that the heterogeneity of the fibrinogen molecule differs from one healthy person to the next, and may affect the apparent fibrinogen concentrations using different assays from the same blood sample (Nieuwenhuizen, 1994). It has also been found that the ratio between fibrinogen levels comparing Clauss and other methods was close to 1 unless hypo- or hyperfibrinogenemia was present when the Clauss method was more accurate and informative (Nieuwenhuizen,1995; Tseimakh et al,1997).

Fibrinogen degradation products

In a subset (54) of samples in this study the associations between fibrinogen as measured by Clauss, Laurell and Intact methods and fibrinogen degradation products were examined (Stirling et al, 1994). The investigations found that fibrinogen degradation products increase as fibrinogen decreases. They produced evidence that both fibrinogen and fibrin were being broken down by plasmin, as Fragment X from fibrinogen and D-dimer from fibrin increase together as fibrinogen decreases. It is possible that some of the fibrinogen degradation products may have arisen in vitro in the hour before separation and freezing of the samples since no fibrinolytic inhibitors were used, as noted in another study of blood samples from neonates (Chessells & Pitney, 1970). However these results are consistent with the prevailing view that fetal fibrinogen merely reflects an increased fibrino(geno)lysis in the neonate and not a separate entity (Stirling et al, 1994). They are also consistent with the results of this study in that the clottable fibrinogen as measured by Clauss was lower than the fibrinogen measured by the other methods in the same samples.

Haematocrit levels as a possible cause of bias

The range of haematocrit values for cord blood is usually between 0.45 and 0.51 (Foley et al,1978). A haematocrit reading of > 65 from a central vein is diagnostic of polycythaemia. It is known that plasma clotting factors become diluted by the fixed volume of anticoagulant at very high levels of haematocrit (Komp & Sparrow,1970), and therefore it is theoretically possible that a raised haematocrit might account for the findings in this study. This possibility was examined.

There are no published studies on the effect of haematocrit on coagulation factors in normal AGA (appropriate for gestational age) neonates (Ries, 1998). Some studies of clotting factors in fetuses, premature babies and neonates have been performed in citrated plasma with a fixed ratio of citrate: blood of 1:9

(Andrew, 1995; Reverdiau-Moalic et al, 1996) but reference values were established using volumes of citrate adjusted for haematocrit (Hathaway et al, 1991).

It is known that haematocrit levels rise in the first 2 hours after birth and return to cord blood levels by 18 hours in AGA babies. The incidence of polycythaemia at 6 hours in IUGR babies was 12-15%, in AGA babies 2- 4% and in macrosomic babies it was up to 8%. The polycythaemia in well term babies was transient and passes by four/five days but may cause hyperbilirubinaemia. (Ramamurthy & Brans, 1981; Ramamurthy & Berlanga, 1987). In this cohort although the haematocrit was not measured, it is likely that it was within a normal range for the following reasons:

(a) It has been shown that with early clamping the haematocrit decreased from 0.48 +/- 0.04 at birth to 0.43 +/- 0.6 after 24 hours, whereas in delayed clamping the haematocrit rose to 0.63 +/- 0.05 and was still raised to 0.59 +/- 0.5 at 24 hours (Linderkamp et al, 1992). Early cord clamping is practised in this maternity unit and so the extra placental transfusion of blood caused by delay in clamping the cord and often associated with polycythaemia would not have taken place.

(b) No reduction exchange transfusions have been needed in the past 20 years for polycythaemia in this Neonatal Unit, implying that polycythaemia is rarely a problem.

(c) This thesis is concerned with the normal variation that occurs in full term pregnancies that deliver between 37 and 42 weeks. Using Ramamurthy's figures to calculate the likely prevalence of polycythaemia at 6 hours, 21 (6%) of the neonates in this cohort had birthweights that were below the 3rd centile

and of these 3 (15%) may have been polycythaemic at 6 hours of age (Ramamurthy & Berlanga, 1987). 9 (3%) of the AGA neonates may have had early polycythaemia and 5 (1.5%) neonates that were over the 97th centile may have been likewise affected. In total this suggested that 17(5%) neonates may have had polycythaemia in the first 24 hours. If this polycythaemia was not resolved by the time of the venepunture on the 4th day there may have been an increased haematocrit. Another researcher described a fall in haematocrit by 4 days and a small rise in plasma fibrinogen concentration (Buonocore et al, 1991). The latter is consistent with the findings of the pilot study in this work and makes it unlikely that the haematocrit was still raised in the 5% of the cohort who may have had a raised haematocrit within the first 24 hours.

(d) Plasma fibrinogen concentrations were not significantly associated with jaundice in this sample, and the total bilirubin level only rose above 240 umol/l in 7 cases.

(e) The plasma fibrinogen concentrations were regressed against the lower to upper quartiles of birthweight in this cohort (Figure 15, p.158). This showed that the positive association of birthweight with plasma fibrinogen was strengthened by the removal of the lowest and highest quartiles of babies.This finding is consistent with the possibility that some part of the described association may have been due to a raised haematocrit in small for dates and very large babies.

However it was reasonable to assume that, due to the factors above, the variation in haematocrit values in this sample of neonates was unlikely to explain very much of the relationship between birthweight and fibrinogen. However any future studies of neonatal fibrinogen should include direct measures of haematocrit.

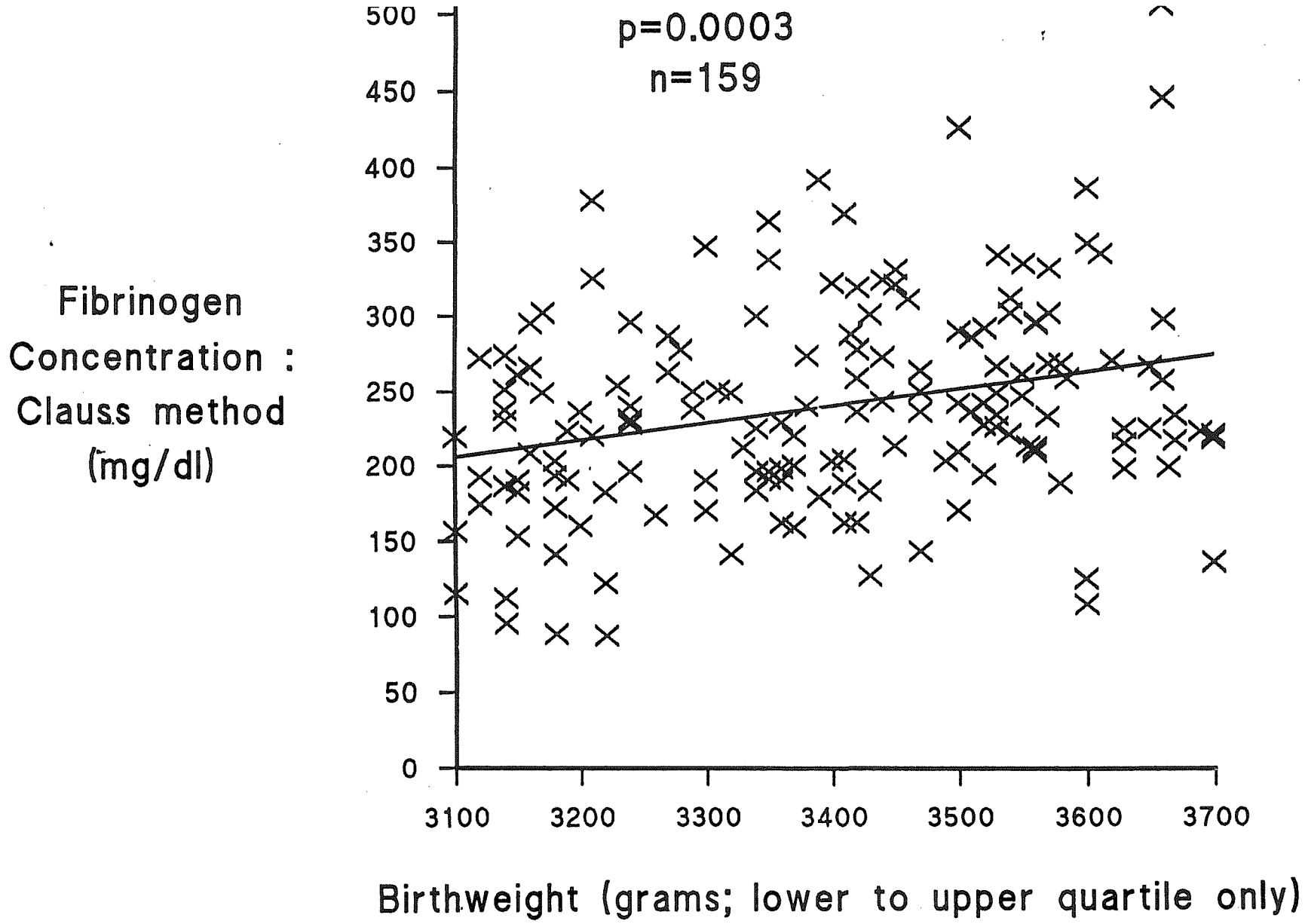


Figure 15 The relationship between fibrinogen concentration and birthweight

Sialic acid

In the neonate fibrinogen shows variable amounts of sialation. This is relevant as sialation leads to a prolongation of the time taken by fibrinogen to clot when exposed to thrombin in the neonate. The Clauss method of measuring plasma fibrinogen may therefore underestimate the amount of fibrinogen present. In this study plasma fibrinogen levels on the same samples were indeed lower than when measured by either Laurell or the Intact method, but the essential relationships with measurements of size at birth remained the same. However, in functional terms the degree of sialation may not matter much if at all. The vast majority of prospective studies showing the association between fibrinogen and coronary heart disease have used methods based on thrombin time and if the association is causal it must be a functional effect of the fibrinogen/sialic acid complex rather than due to sialic acid alone. Furthermore it is unlikely that changes in fibrinogen itself are exclusively responsible for changes in sialic acid concentrations. Serum and plasma sialic acid concentrations are the same, and no sialic acid-containing carbohydrate is released from fibrinogen during the formation of fibrin (Haberli, 1984).

Sialic acid may be involved in the pathology of cardiovascular disease, but several studies have shown that it does not behave in a way that is directly comparable to fibrinogen. For example, plasma fibrinogen is clearly increased in smokers, while any effect on sialic acid is much less apparent (Lindberg et al, 1991b; Kario & Matsuo,1993). It is also well known that fibrinogen rises at the menopause, - not so with sialic acid (Crook et al, 1998). The association between sialic acid and fibrinogen more probably reflects just one association of sialic acid with several other acute phase proteins, as reflected in the large number of pathological conditions in which a rise in sialic acid has been documented.

Sialic acid is also integral to fibrinolysis. The sialation of plasminogen and its variability in the neonatal period is similar to the relationship between sialic acid and fibrinogen (Ries, 1997). The widespread occurrence of sialic acid in

the physiology of clot formation and fibrinolysis (see page 47) suggests that the rise and fall of sialic acid may link the two processes in maintaining the stability of the circulation.

In this study the functional activity of the fibrinogen/sialic acid complex in full term healthy newborn babies was greater the higher the birthweight.
As function is almost certainly important in adult life, it is reasonable to have used a method which measures fibrinogen function without necessarily being able to apportion the association between fibrinogen itself and sialic acid. However, as this study produced unexpected results, it would be desirable to include a direct measure of sialic acid in any further work on plasma fibrinogen levels in early life.

In conclusion the results reached in this study were not affected by the choice of measurement, although they indicate the need for additional biochemical measures to be included in future studies.

6.2 Discussion of results

This is the first study to show an association between size at birth and neonatal fibrinogen concentrations. These findings are in the opposite direction to the association found between size at birth and adult fibrinogen levels in males.

6.2.1 Relationship between size at birth and neonatal fibrinogen

Some weight loss is usual in a baby's first few days of life and the calculations done in this study were based on the birthweight rather than the weight at four days, whereas length, head and chest measurements were all made at between 84-108 hours after birth. It is possible that a small drop in weight by four days may alter the association between birthweight and plasma fibrinogen concentration at four days. However breast feeding babies are known to lose more weight initially and feeding methods did not have any effect upon these associations. In addition the main association of plasma fibrinogen was with lower chest circumference and the small and variable amount of body weight

loss in the first four days is unlikely to alter the strength of the association with these measurements.

It has been shown that birthweight is significantly associated with neonatal fibrinogen concentration, whereas placental weight, head circumference and length are not. The lower chest circumference which contains the bulk of the neonatal liver has an even stronger association, showing a strongly graded increase with fibrinogen levels (Table 7, page 115). This is consistent with literature on liver growth and function. In rats the fetal liver is the organ affected earliest and to the greatest extent by intrauterine growth retardation, especially in the second half of pregnancy (Winick, 1971).

When the supply of nutrients by the placenta is decreased there is a reduction in fetal growth, particularly of the liver, accompanied by a reversed flux of non - essential amino acids back to the placenta. This results not just in a smaller liver but in wasting and a marked reduction in the synthesis of plasma proteins as observed in the human growth retarded infant (Cetin et al, 1988). In this study the strong association between lower chest circumference and plasma fibrinogen is consistent with liver growth and its dependence upon the supply of amino-acids in order to produce such plasma proteins as fibrinogen.

Liver growth is compromised selectively when nutrition is inadequate in order to protect the brain's development. Brain growth at the end of pregnancy is rapid and involves DNA synthesis, myelination, dendritic growth and establishment of synaptic connections (Dobbing, 1974; Herschkowitz, 1974). If the nutritional supply to the fetus of the sheep is reduced in the last half of gestation, brain weight is maintained relative to body weight, with a disproportionate fall in liver weight (Owens et al, 1989). In this study the head circumference is the only birth measurement where a unit rise was associated with a fall in the plasma fibrinogen concentration produced by the liver, and this was not significant (5.3.5.4).

What is the mechanism which selectively allows head circumference and hence brain size to be protected and the liver and its functions to be so diminished? Part of the reason may be that the brain is an obligate user of glucose whereas the liver needs amino acids to synthesize proteins, including fibrinogen. It has been shown that prolonged growth retardation in the rat produces a large decrease in protein synthesis and a small fetal liver whereas the brain escapes relatively unscathed (Johnson & Dunham, 1988). In sheep when growth retardation was produced experimentally by a reduction of nutrients, there was an increase in the rate of fetal glucose utilisation per kg of fetus as well as a disproportionate fall in liver size (Bozzeti et al, 1988).

This mechanism may be due to hormonal control by the fetus which is autonomous in endocrine terms, as the placenta is impermeable to many maternal hormones including growth factors. Fetal hormones regulate growth in the well fed state and during restricted nutrition. In healthy sheep insulin is the dominant hormone in fetal endocrine balance and glucose is available to the cells and used preferentially (Fowden, 1989). This allows superfluous nutrients to be deposited in fuel reserves in the fetus. When nutrients are limited fetal insulin level falls, altering the fetal metabolic balance in favour of catabolism. In the malnourished guinea pig the cellular uptake of nutrients by insulin sensitive tissues such as the liver was reduced leaving available nutrients to be used by tissues which were insensitive to insulin - such as the brain (Jones, 1980). In humans growth hormone levels are normal in the growth retarded fetus but the insulin - like growth factor (IGF-1) is reduced. It is thought that hypoglycaemia and hypoinsulinaemia may retard fetal growth by inhibiting the production of IGF-1 (D'Ercole, 1987).

6.2.2 Fibrinogen and sex

Female neonates have higher fibrinogen levels than male neonates (5.3.5.6, page 116). Sex-related differences are known to exist in the haemostatic variables of both young and old adults (Meade et al, 1979; Balleisen et al, 1985; Folsom, 1992) showing consistently higher values in women.

In this study the relationship between sex and neonatal fibrinogen at four days is similar to levels in the non-smoking young adult, that is females have 8.7% higher levels than males. Females between 25-34 yrs have fibrinogen levels 6.4% higher than males and this increases to 13% after the menopause (Lowe et al, 1988). Fibrinogen has been found to be consistently higher in adult females than in males in the age groups up to 29 years, 40-59 years, 45-64 years (Balleisen et al, 1985; Lee et al, 1990; Folsom,1992). However no difference in fibrinogen concentrations between the sexes was found in cord blood of neonates although there were sex-related differences in Factor VII and Factor VIIIc both being greater in female neonates (van der Salm et al, 1994a). It is speculated that sex-related differences in haemostatic variables are related to hormonal effects. Male neonates have higher testosterone levels than female neonates and there is an inverse relationship between testosterone and Factor VIIc in adult males but no association with fibrinogen concentrations (Heller et al, 1984) and the work of van der Salm and his colleagues is consistent with these findings. Female infants experience higher oestrogen levels in the first week of life than males and have higher Factor VII levels (Weinberg et al, 1992) and as this study has shown, higher plasma fibrinogen concentrations. As these other haemostatic variables have been shown to be influenced by hormones, it is therefore likely that there is a hormonal influence between fibrinogen and the sex of the neonate as yet not elucidated.

A follow up study of men born between 1920-30 demonstrated that reduced fetal growth as expressed by low birthweight was associated with raised plasma fibrinogen concentrations (Barker et al, 1992b). However, females did

not show the same associations between plasma fibrinogen concentrations in middle life and low birthweight as males (Martyn et al, 1995a). Low birthweight combined with above average weight at one year and/or high body mass index in middle life significantly increases the risk of cardiovascular disease, lower HDL cholesterol and impaired glucose metabolism in females, but no association could be established between plasma fibrinogen concentrations in middle life and indices of reduced early growth with or without excessive weight gain later. It is possible that protection offered by female hormones is amplified over time, masking the relationship between reduced early growth and raised fibrinogen levels in middle - aged females and only falls after the menopause, when plasma fibrinogen concentrations and risk of cardiovascular disease both rise sharply. The rapid onset of a measurable sex difference in plasma fibrinogen concentration at four days after no difference in cord blood lends credence to this suggestion.

Thus there were two remarkable findings in this study relating to sex. The first is the fact as early as four days old female neonates had significantly higher plasma fibrinogen concentrations than the males. although previous workers have shown that cord blood showed no difference between plasma fibrinogen concentrations and sex (van der Salm et al, 1994c). Secondly the association with sex was strong enough to be independently significant when in multiple regression with birthweight and plasma fibrinogen concentrations. Female neonates are lighter than males and, as this work has shown, birthweight and plasma fibrinogen concentrations are significantly and positively associated. Despite the influence of lower birthweight in females, the influence of sex is so strong that female neonates have higher plasma fibrinogen concentrations than males.

6.2.3 Variables which may influence the relationship between size at birth and neonatal fibrinogen

6.2.3.1 Maternal circumstances, size and age

In this community the social circumstances of the family did not significantly affect either size at birth or fibrinogen levels, in spite of clustering of poor education, low social class and poor social support. Poverty alone sufficient to have an effect upon birthweight is rare in the Isle of Man. It has been shown that the effect of poverty **alone** on birthweight is marginal (Galbraith et al, 1979).

Maternal size - particularly height - is known to influence neonatal birthweight (Walton & Hammond, 1938; Klebanoff et al, 1984; Alberman et al, 1992; Emanuel et al, 1992). Moreover the birthweights of mothers have a profound effect upon the size of their offspring (Ounsted, 1986; Lumey, 1992). However none of the variables measuring maternal health or size in this study confounded the relationship between birthweight with neonatal fibrinogen. The only distinct maternal variable that could be identified that had an independent effect upon neonatal plasma fibrinogen concentration was increasing maternal age and this was only weakly associated ($p = 0.06$). Increasing age is known to be associated with increasing plasma fibrinogen concentrations (Meade & North, 1977) and the literature on fetal growth shows that the intrauterine environment is the predominant influence on birthweight (Penrose, 1954; Morton, 1955; Polani, 1974; McCance & Widdowson, 1974). This work has shown that maternal circumstances and size only influence plasma fibrinogen concentration via their influence - if any - on birthweight.

6.2.3.2 Smoking

The possibility that smoking may be a confounding variable affecting both birthweight and fibrinogen levels was examined. It has been shown repeatedly that birthweight is reduced in the babies of smoking mothers (Haddon et al, 1961; Miller & Hassanein, 1964; Scott Russell et al, 1966; Godfrey et al, 1991), and that fibrinogen levels are significantly raised in adults who smoke (Meade et al, 1979; Kannel et al, 1987a; Ernst et al, 1987). The present study showed the same effect of maternal smoking associated with low birthweight but maternal smoking was associated with slightly lower neonatal fibrinogen concentrations.

Carbon monoxide, nicotine and cyanide are all products of cigarette smoking and all cross the placenta. The separate effect of these smoke constituents on the human fetus is not known, but it is known that smoking reduces the oxygen carrying capacity of both fetal and maternal blood. This effect is seen not only in babies of smoking mothers but in babies born at high altitudes which are smaller than those born at sea - level (Grahn & Kratchman, 1963). Nicotine is a potent vasoconstrictor but there may be compensatory mechanisms in place to avoid a reduced placental flow. However inhaled carbon monoxide results in a fall in arterial oxygen tension as well as combining with haemoglobin to produce carboxyhaemoglobin and also causing a reduction in the oxygen delivering capacity of the remaining haemoglobin by a 'shift to the left' of the haemoglobin dissociation curve. The resultant tissue hypoxia is likely to contribute to lower birthweight (Cole et al, 1972). A Dutch study looked at haemostatic factors in the cord blood of the neonates of smoking and non-smoking mothers. It demonstrated that, although there was a reduction in birthweight and a rise in the placental/birthweight ratio in the babies of smoking mothers the clotting factors were the same in both groups (van der Salm et al, 1994a). Similarly the results presented in this work show no significance in the relationship between the smoking habit and neonatal plasma fibrinogen concentrations.

Tissue hypoxia is a reasonable explanation for smoking mothers to have low birthweight babies, but why should neonatal plasma fibrinogen concentrations be unaffected? In adults, smoke constituents may have a direct effect upon the endothelium of pulmonary blood vessels, or there may be a persistent stimulation of lung macrophages to produce interleukin-6 which induces the production of fibrinogen, whereas in fetal life the unexpanded lungs are not exposed to the direct effect of smoke.

6.2.3.3 Maternal blood pressure, haematology and perinatal history

Maternal blood pressure was analyz ed alongside birthweight and fibrinogen levels but no separate or confounding effect could be identified. As pre-eclampsia is known to cause a reduction in birthweight (Tervila, 1973; MacGillivray, 1983) it must be assumed that the mothers in this gestational age range with pre-eclampsia (6%) were too few to produce reliable data. The haematology data did not provide any significant associations with either birthweight and/or fibrinogen levels.

Analysis of the perinatal history showed that a labour longer than 18 hours was significantly associated with a raised plasma fibrinogen concentration at four days but was not associated with the birthweight of the neonate (see page 131). This relationship is in keeping with the known function of fibrinogen as an acute phase reactant. The reason for an unduly long labour of more than 18 hours may well be nothing to do with the birthweight of the neonate but may cause distress to the unborn baby with the measurable effect of a raised plasma fibrinogen concentration at four days.

A low Apgar score at one minute may be fleeting and not produce an acute phase reaction in the neonate, particularly if the condition of the neonate is reversed rapidly such that the Apgar score at five minutes is much higher. The higher Apgar scores in this cohort were positively associated with higher plasma fibrinogen levels (see page 132).

6.2.3.4 The acute phase reaction in the neonate

Is fibrinogen primarily an acute phase reactant in the neonate?
This possibility was examined in a sub-set which contained levels of fibrinogen and C-reactive protein. Although both are acute phase proteins they behave rather differently. C-reactive protein is very short-lived compared with fibrinogen. It is very sensitive and has a very short half-life of 4-6 hours compared with the half-life of fibrinogen which is 4 days (Whicher & Dieppe, 1985; Wang & Fuller,1991). It is often raised in cord blood before an infection can be detected by any other means (Sabel & Wadsworth, 1979). A single measurement is not recommended as a reliable indicator of infection in the neonate as conspicuous rises often occur in uninfected infants due to other stressful events (Ainbender et al, 1982). However serial measurements of C-reactive protein are a sensitive indicator of infection in the neonate as it rises and falls so quickly (Russell et al, 1992).

These properties made it possible to distinguish between birth stress and infection at four days by measuring both CRP and fibrinogen in the subset. The babies infected at four days had significant rises in both CRP and plasma fibrinogen levels which were independent of birthweight and lower chest circumference but did not negate the association with size or sex. There was a significant positive association of the CRP level with birthweight but unlike fibrinogen, not with a long labour or a low Apgar score. It is possible that the larger baby did indeed have perinatal stress but the measurement of CRP at four days did not detect the fleeting rise.

The results of this study indicate that although neonatal plasma fibrinogen was significantly and independently associated with size at birth and sex, it also acts independently as an acute phase protein when infection is present.

6.2.4 The relationship between size at birth and neonatal fibrinogen levels with early growth and adult fibrinogen levels

6.2.4.1 Birthweight, weight at one year and adult fibrinogen levels

Several studies have found that reduced growth in fetal life and infancy is associated with high plasma fibrinogen concentrations in adult life. In Preston fibrinogen was not associated with birthweight, but fell progressively as the ratio of placenta/birthweight decreased. In Hertfordshire the plasma fibrinogen concentrations in middle-aged men fell with increasing weight at one year but were not associated with birthweight (Barker et al, 1992b). In Sheffield it was found that middle-aged men and women had low density lipoprotein cholesterol concentrations which fell with increasing birthweight and abdominal circumference at birth (Barker et al, 1993c; 1995). A further study in Sheffield showed that plasma fibrinogen concentrations fell with increasing birthweight and increasing neonatal abdominal circumference. It was suggested by the authors that the process linking reduced early growth with increased concentrations of both LDL cholesterol and fibrinogen in later life involves impaired liver development at a critical period in early life (Martyn et al, 1995a). This suggestion may well be true but this study has shown that a small liver in a small full term neonate is associated with a lower plasma fibrinogen concentration than the level associated with a larger liver in a larger neonate. If reduced production is measurable by four days it is difficult to understand why this small impaired organ is able to produce more than average fibrinogen in adult life, particularly as this work shows the opposite relationship at four days.

A possible biological explanation might be that of 'catch-up' growth, especially as the liver is able to regenerate throughout life. This would explain levels in adult life reaching the normal range but not the significant association between poor early growth and raised plasma fibrinogen concentrations in adults that is so well documented.

It is perhaps more probable that the small liver continues to produce a lower amount of fibrinogen, LDL cholesterol and glucose just as in the neonatal period **but** the rate of use of these substances is controlled elsewhere.

6.2.4.2 Placental size

Work in animals has shown that placental size is sensitive to nutritional supply and the placenta may hypertrophy in anaemia (Beischer et al, 1970; Owens et al, 1989; McCrabb et al, 1992). In humans a large placental weight and a high placenta/birthweight ratio were shown to have an association with low maternal haemoglobin and a fall in maternal Mean Corpuscular Volume (Godfrey et al, 1991). The results in this study showed a positive association between birthweight and placental weight and showed that the greater the fall in haemoglobin the heavier the placenta, but the relationship between the fall in MCV and placental weight was not significant. A high placental/birthweight ratio had no significance with neonatal fibrinogen concentrations and the effect of placental size on fibrinogen was found to be mediated through birthweight on multiple regression. These relationships are the opposite of those found in male adults.

6.2.4.3 Females

Like low birthweight in men, low birthweight in women is associated with an increased risk of death from cardiovascular disease. Women who had below average birthweight followed by above average weight at one year had the highest death rate which is also the same as in men (Osmond et al, 1993). In Sheffield middle-aged women had higher fibrinogen concentrations than the men, but despite this, did not show any significant association between fibrinogen and birthweight or abdominal circumferences (Martyn et al, 1995a).

In Hertfordshire low birthweight in both men and women was associated with increased insulin resistance, raised blood pressure, high LDL cholesterol and high serum triglyceride but there was not any association between fibrinogen levels in middle-aged women and reduced early growth in contrast with the strong associations in men (Fall et al, 1995b). The underlying reasons for these differences are not yet known, however some observations have been made.

Male babies have a faster intrauterine growth rate than female babies and are generally heavier, the findings in this sample are consistent with this fact (5.3.3, page 103). Male babies may therefore be more susceptible to any diminution of nutrition in the last three months of pregnancy (Burgoyne, 1993). The effects of this may be to enhance any association between low birthweight and adverse health problems in middle life.

Significant associations between low birthweight and insulin resistance, hypertension, high LDL cholesterol as well as cardiovascular death have been found in both sexes. The association between low birthweight and raised fibrinogen concentrations in adult males has also been proved, but not in adult females. It has also been shown that fit postmenopausal women have an increase in fibrinogen concentrations (Meade et al, 1983; Lee et al, 1993a).

This work has shown that the similar associations occur in both sexes between low birthweight and low plasma fibrinogen concentrations at four days (5.3.5.1, page 111). However no differences in fibrinogen levels were found between the sexes in cord blood (van der Salm, 1994b), so that within this short time the sex significance has risen from zero to a highly significant level, powerful enough to produce an independent and highly significant association (5.3.5.6, page 116). It is therefore feasible that such a factor may continue to increase its significance throughout life until the menopause, thereby masking the association between reduced early growth and plasma fibrinogen concentrations in the female adult. It would be interesting to study non-smoking women who have been postmenopausal for some time and not taking hormone replacement therapy to see if an association between their plasma fibrinogen concentrations and birthweight could be established after the oestrogen supply is cut off.

6.2.4.4 Size at birth and fibrinogen levels in childhood

The normal range of fibrinogen levels in children has been established and it is known that fibrinogen breakdown products are higher in normal healthy children than in adults (Aballi & De Lamerens, 1962; Uttley et al, 1969). A recent study in 10-11 year old children showed no relations between fibrinogen levels and measures of fetal growth or social class (Cook et al, 1999).

6.2.4.5 A parallel between size at birth and blood pressure

A parallel can be drawn between fibrinogen metabolism and size at birth and blood pressure and size at birth. In both instances the relationship with birthweight is initially a positive one which changes to become negative, so that low birthweight is associated with raised plasma fibrinogen concentrations and raised blood pressure in the adult male. This work has shown that plasma fibrinogen concentration is positively associated with birthweight and other studies have found that neonatal blood pressure is also positively associated with birthweight (Contis & Lind, 1963; Versmold et al, 1981; Lee et al, 1976; Hulman et al, 1990).

The changing relationship between blood pressure and early growth from negative to positive during childhood has been documented in many studies. The positive relationship continues until later in childhood - up to 13-25 weeks (Launer et al, 1993) and up to two years (Law et al, 1993a) when it becomes a negative one. This was also found in Farnborough where four-year old children had systolic blood pressures which decreased by 2.8 mm Hg for every kg increase in their birthweight (Law et al, 1993a). Several studies from different countries agree that the negative association of blood pressure with birthweight is consistent as already described in studies spanning thirty years and 66,000 people from birth to 71 years as recently reviewed (Law & Shiell, 1996). Blood pressure in children after infancy and up to nine years is inversely associated with birthweight which is illustrated particularly well in those studies with repeated measurements (Law et al, 1991; Law et al, 1993a; Launer et al, 1993; Whincup et al, 1989, 1992; Williams et al, 1992). It may be possible to show a similar change to a negative association in plasma fibrinogen concentrations in a follow up study of this work in prepubescent children. However, a recent study on 10-11 year old children did not find any association between plasma fibrinogen concentrations and birthweight (Cook et al, 1999).

The findings of Launer in Rotterdam described a 'U' shaped relationship of birthweight and blood pressure at 4 years, with low and high birthweight infants showing higher blood pressure (Launer et al, 1993). These findings are similar to those presented more recently for childhood onset insulin dependent diabetes in 4 year old children in Sweden (Dahlquist et al, 1996).Tracking correlations measure the relationship between two measurements obtained at two points in time from the same individuals and were strongly positive for blood pressure in children up to ten years in the Brompton Study (de Swiet et al, 1975; 1980; 1984; 1989; 1992).

The relationship of the tracking of blood pressure in adolescence is less clear but in adult life the association is re-established and is amplified with age. (Law et al, 1993a; Godfrey et al, 1994; Wadsworth et al, 1985). In Hertfordshire systolic blood pressure of both men and women showed increasing amplification with age, such that systolic blood pressure decreased by 5.2 mm Hg for every kg increase in birthweight and similar findings were reported from Preston data (Law et al, 1993b).The possibility of tracking in plasma fibrinogen concentrations through childhood and adolescence has not yet been examined but similar amplification occurs in plasma fibrinogen concentration with age in both men and women (Meade & North, 1977).

6.2.4.6 A parallel between size at birth and gluconeogenesis

A parallel between size at birth and fibrinogenesis and between size at birth and gluconeogenesis may be drawn. A growth retarded neonate frequently suffers from hypoglycaemia and yet in middle life the high plasma glucose levels of diabetes are strongly associated with low birthweight. This compares with the results of this study in which low birthweight and a small lower chest circumference is associated with a low plasma fibrinogen concentration and yet the evidence from other work is that low birthweight is associated with a raised plasma fibrinogen concentration (Barker et al, 1992b).

Growth retarded infants have impaired pancreatic function (Snoeck et al, 1990; Van Assche & Aerts, 1979). Clinically impaired glucose tolerance has been documented in children who had low birthweight in Salisbury (Law et al, 1995) and India (Yajnik et al; 1995). In adults the association is even stronger, with plasma glucose inversely related to birthweight (Robinson et al, 1992) with impaired glucose tolerance and Type 2 diabetes associated with low birthweight in both Hertfordshire and Preston (Phipps et al, 1993; Barker et al, 1993a; Phillips et al, 1994) and Sweden (Lithell et al, 1996).

The association between fetal growth and glucose metabolism is now proved beyond all reasonable doubt, as well as the physiological fact that the liver is the organ of glucogenesis but the pancreas is the organ of control. ***Perhaps in a similar way the liver is the organ of fibrinogenesis but its regulation is likewise extrahepatic and is impaired by growth retardation in utero.***

6.2.5 Size at birth, fibrinogen and cardiovascular risk

Reduced size at birth and impaired early growth are proven cardiovascular risk factors and, in addition, a raised plasma fibrinogen concentration in adult life is acknowledged as a further independent risk factor for cardiovascular disease and stroke (Meade et al, 1986; Wilhelmsen et al, 1984; Stone & Thorp, 1985; Heinrich et al, 1994; Cremer et al, 1992). All these studies are consistent in showing that the third of the population with the highest fibrinogen levels had an increased relative risk of a cardiovascular event 2.3 times that of those in the lowest third (Ernst & Resch, 1993). The retrospective association of raised fibrinogen concentration in middle and old age with low birthweight is therefore a significant link in the epidemiology of cardiovascular disease.

6.2.5.1. The effect of intergenerational poverty

Poverty in a community is associated with poor growth in infancy and raised cholesterol levels and cardiovascular disease in adults in Scandanavia (Notkola, 1985; Forsdahl, 1977; 1978; Arnesen & Forsdahl, 1985). Similar studies in Britain showed the same trends, with the poverty of the 1920's being strongly associated with cardiovascular disease in the 1970's in the same communities (Barker & Osmond, 1986; 1987).

Other work has identified poor growth in the first year of life as a critical factor in the development of increased risk for cardiovascular disease (Fall et al, 1992; 1995a). Height is largely predicted by growth in the first year of life and an inverse relationship between height and cardiovascular disease has been found (Tanner et al, 1956; Marmot et al, 1984; Waaler, 1984; Notkola, 1985;

Barker et al, 1990). Maternal height is to some extent a measure of a mother's nutrition in her first year of life.

In this study the relationship between grandparents' health and birthweight was examined and positive associations were found between mother's birthweight and that of her baby, and maternal grandparental height and birthweight (Table 9, page 122). No association was found between maternal grandparental cardiovascular health and birthweight.

Intergenerational poverty is associated with low birthweight, and low birthweight is associated with raised fibrinogen in later life. It is possible that intergenerational socio-economic circumstances may confound the relation between birthweight and adult fibrinogen. High fibrinogen levels in adult life may be a consequence of poverty *per se,* and be independent of birthweight. Raised plasma fibrinogen concentrations in adult life may be caused by smoking or a chronic infection such as periodontal disease, both more common amongst the poorer sections of the community. It is difficult to separate out the effects of persistent intergenerational poverty over generations and the effects of intrauterine malnutrition in an individual. For example, the correlations between infant mortality between 1895-1908 and adult mortality in 1963-73 were much diminished by controlling for socioeconomic circumstances (Ben-Shlomo & Davey Smith, 1991). Other workers have shown different associations (Barker & Osmond, 1986). Further studies are needed following children through childhood and into adult life to elucidate these matters.

The findings in a recent study of 10-11 year old children did not support the view that fibrinogen concentrations were determined by either fetal growth **or** social factors. Instead they found that adiposity and physical exercise were more important in determining fibrinogen concentrations in this age group (Cook et al, 1999). This finding is consistent with a study of these relationships in adolescence which found a significant increase in plasma fibrinogen concentrations with rising ponderal index (Bao et al, 1993).

Wherever the epidemiological truth lies, there is likely to be a biological explanation for the associations between poor early growth and raised plasma fibrinogen concentrations and cardiovascular disease in later life which has not yet been understood. One possibility is that **vascular damage** sustained by either smoking or intrauterine programming may only become manifest after some years of adult life and may have a part to play in the regulation of plasma fibrinogen concentrations.

6.2.5.2 Is there a common cause for these associations?

In men both low birthweight and low weight at one year are predictive of increased risk of cardiovascular disease (Osmond et al, 1993). In Sheffield there were similar findings with increasing cardiovascular death rates in thin babies who had low birthweights and small heads (Barker et al, 1993b). The cluster of conditions known as Syndrome X (hypertension, hyperlipidaemia and Type 2 diabetes) are now known to be associated with reduced early growth (Barker et al, 1993a). It is possible that these conditions have a common cause which - it is suggested - may be impaired function of the vasculature due to reduced fetal growth at a critical time.

This study has shown that the effects of reduced fetal growth are not only apparent in reduced birthweight and liver size but have a measurable effect upon the levels of a major clotting factor as early as four days after birth which is at first a positive relationship and then, according to the literature, becomes a strongly negative one in adult males. The importance of this finding is discussed firstly in the context of fibrinogenesis/fibrinolysis and secondly in respect of the concept of programming.

6.2.5.3 Fibrinogenesis and fibrinolysis

Fibrinogenesis and fibrinolysis have been discussed already (pages 40 and 48). Fibrinolysis is an important means by which excessive thrombus formation is prevented in normal conditions. It is also a means of regulating the concentration of plasma fibrinogen in circulation and is brought about by a

complex enzyme cascade that generates localized proteolysis. Central to this action is the action of plasmin which is converted from plasminogen by the action of tissue plasminogen activator (t-PA). This substance splits the fibrinogen molecule to produce fibrinogen degradation products thereby reducing the plasma fibrinogen concentration and so the likelihood of clot formation.

Tissue plasminogen activator (t-PA) is released from the endothelium of blood vessels and its action is limited by its inhibitor called tissue plasminogen inhibitor (PAI-1) which is also released from the endothelium. The release of these substances from the vessel wall is prompted by substances called endothelins. Three of these have been identified from the vascular wall in tissue culture (van Hinsbergh, 1988; Kruithoff et al, 1984). Measurement techniques for these substances were not available when raised fibrinogen levels were linked to ischaemic heart disease in women in the Northwick Park Heart Study (Meade et al, 1977).

In the context of considering the fate of circulating fibrinogen it is also known that t-PA leads to an increase in fibrinolytic activity (Lidbury et al, 1990). Since then it has been shown that low fibrinolytic activity in women is linked to death from ischaemic heart disease (Meade et al, 1996). It has also been shown that altered levels of plasma fibrinogen and endothelial products are associated with peripheral arterial disease and atherosclerosis (Woodburn & Lowe, 1997).

If vasculature development is impaired during the last three months of pregnancy there may be a reduction in endothelial products or functions which will hinder normal fibrinolysis. This idea offers an explanation for the main finding in this study whereby a poorly grown neonate with a low plasma fibrinogen concentration may also have an impaired fibrinolysis system which in time allows an accumulation of fibrinogen to abnormal levels. This is compatible with present knowledge that high plasma fibrinogen concentrations in the adult can be linked with a history of reduced early growth.

6.2.5.4 The concept of programming

'Programming' is a term applied to a process whereby a stimulus or insult operating at a critical or sensitive period of development results in a lasting or life-long effect on the structure or function of the organism (Lucas, 1991). The animal studies that have been reviewed prove that this concept is biologically sound and can be reproduced experimentally (2.1.15, page 33). The concept of 'programming' has been applied to humans and is known as 'the Barker hypothesis' (Barker & Osmond, 1986).The results found in this study do not support this hypothesis, although it is possible that a cross-over later in life might occur, such that it is high rather than low fibrinogen levels that are associated with low birthweight and with heart disease. Fibrinogen would not be unique in this respect. Blood pressure is an example of a crossing over of the relationship such that low blood pressure is positively associated with low birthweight in early life which then reverses as the individual ages and high blood pressure becomes associated with low birthweight. In other words, the effect of a programmed change in an organ's function may not be apparent immediately. In addition, as animal models have shown the vasculature may be affected by diet and hypertension can be artificially induced (pages 33-34). Animal experiments lend extra credence to the suggestion that a programmed change in vascular function caused by reduced early growth is a reasonable possibility and fits with the facts as at present understood.

6.3 Conclusion

The hypothesis that reduced size at birth - as indicated by low birthweight and low chest circumference - is associated with high neonatal fibrinogen levels has been disproved. Reduced fetal growth is associated with low neonatal fibrinogen levels.

6.3.1 Implications for further research

Why should babies with low fibrinogen levels, low birthweight and small chests eventually grow into adults with high fibrinogen levels and have an increased risk of cardiovascular disease? It has been suggested in the literature that liver impairment is the likely cause of raised fibrinogen levels in adult life following poor intrauterine growth (Meade et al, 1980, 1986; Barker et al, 1995; Martyn et al, 1995a). In view of the findings in this study, this does not seem very likely.

Intrauterine growth is programmed to produce a normal baby. When this process is disrupted by inadequate supplies of nutrients at sensitive times the programming shifts to accomodate available resources (Phillips & Barker, 1993). This reprogramming is likely to affect function as well as size.

If the liver was functioning less well in adult life one might expect a lower plasma fibrinogen level rather than a raised one. A small liver may be one of the markers of poor fetal growth in which both the supply of plasma proteins and their subsequent degradation by fibrinolysis are reduced.

6.3.2 Speculation

The author speculates that the endothelium of the vascular tree may contribute to raised fibrinogen levels by a reduction in fibrinolysis. This function may be programmed in utero.

The endothelium is a complex structure which has variable functions in different organs and recent research into the molecular biology of vascular development suggests that this notion is not unreasonable (Risau et al, 1997). It has already been suggested that growth retardation has a role in endothelial dysfunction which may lead to microalbuminuria and insulin resistance (Yudkin, 1997). If one of the functions of the vascular endothelium is the regulation of fibrinogen levels - even indirectly - it may define a biological framework linking the findings in this study with previous work which found that low birthweight is associated with high fibrinogen levels in middle life. A compromised

endothelium, possibly programmed in utero, may lead to inadequate fibrinolysis which would allow even a diminished production of fibrinogen to rise to abnormal levels over time.

The vascular endothelium may be of major importance in the epidemiology of cardiovascular disease. It links many of the findings that have been separately identified such as raised blood pressure, raised plasma fibrinogen concentrations and reduced arterial compliance (Martyn et al, 1995b). This idea may explain the fact that there is an association between fibrinogen genotype and peripheral atherosclerosis rather than fibrinogen genotype and plasma fibrinogen concentrations (Fowkes et al, 1992). The raised fibrinogen concentration may be secondary to inadequate breakdown of fibrinogen rather than overproducton.

The endothelium is now recognised not merely as an inert lining to blood vessels but a highly specialised metabolically active interface between blood and underlying tissues, maintaining vascular tone and thromboresistance. Its diverse functions have a common intracellular control mechanism involving the activation of transcription factors as nuclear factor kB. This control mechanism can be triggered by a variety of agents such as interleukin 1 from platelets, circulating oxidised low density lipoprotein or from lipopolysaccharide/endotoxin from a bacterial cell wall. When the endothelium is activated in this way there is a graded response which may occur locally as in transplant rejection or systemically as in the acute phase reaction. It has been suggested that vascular diabetic complications may be due to such chronic endothelial activation (Hunt & Jurd, 1998).

When the endothelium is activated there are measurable effects which include leucocytes adhering to its surface, platelets are more likely to aggregate, a reduction in fibrinolysis due to enhanced plasminogen activator inhibitor type 1 release, the release of nitric oxide, and a loss of surface anticoagulant molecules such as heparan sulphate and thrombomodulin.

Thrombomodulin is a receptor for thrombin in the endothelium and is present on the vascular surface of the endothelial cells of arteries, veins and capillaries. When thrombin is bound to this receptor it loses its ability to coagulate fibrinogen and cannot activate factors V, VIII and XIII. The plasma soluble thrombomodulin level is a measure of endothelial damage. A study of thrombomodulin in very low birthweight and asphyxiated full term infants showed raised levels in both these groups, and found that birthweight was significantly associated with plasma thrombomodulin concentration (Nako et al, 1997).

Nitric oxide has been studied extensively since it was recognised as being one and the same as the endothelium derived relaxing factor (Ignarro et al, 1996; Palmer et al, 1998). The results of its activity and inhibition is not uniform throughout the body, but it is tissue and species specific. For example, although relaxation in the thoracic aorta is completely blocked by Nitric oxide inhibitors, the abdominal aorta and the carotid and iliac arteries partially relax and are not inhibited in the same way (Cowan et al, 1993). Similar observations have been found in the behaviour of nitric oxide and its inhibitors in different studies. The nitric oxide produced by endothelial cells can diffuse towards the vessel wall where it acts on smooth muscle cells to modulate vascular tone or into the vessel lumen where it enters platelets and reduces clotting.
It is therefore feasible that the endothelium may influence plasma fibrinogen levels in the adult by regulating fibrinolysis and that this function may be programmed in early life.

6.3.3 A follow-up study is desirable

A follow-up study is desirable to explore the following aspects of this subject:

1. The relationship between size at birth and plasma fibrinogen concentration may change from a positive one to a negative one during childhood in the same way as blood pressure.

2. In addition to anthropometry and plasma fibrinogen levels, more detailed haematology should include measurements of sialic acid, haematocrit, C-reactive protein and fibrinogen degradation products in the protocol.

3. In order to assess whether or not the endothelium has a major role to play in the regulation of fibrinogen levels some index of endothelial function such as the measurement of nitric oxide should be included (The Griess Reaction could be used for NO estimation, which specifically measures cellular nitric oxide activity and is based on a visible colour change (Hattori and Gross, 1993). Fetal distress or low birthweight is associated with endothelial damage which could be measured by plasma thrombomodulin levels (Nako et al, 1997).

In conclusion a follow-up study in children is desirable in order to examine why small babies with low neonatal fibrinogen concentrations grow into adults with raised plasma fibrinogen concentrations and an increased risk of cardiovascular disease.

7. Appendices

7.1 Appendix 1: Data sheets

DETERMINANTS OF PLASMA FIBRINOGEN CONCENTRATIONS IN THE NEONATE

IDENTIFICATION SHEET

MOTHER'S NAME.....

MOTHER'S MAIDEN NAME.....

ADDRESS.....

.....

.....

STUDY NUMBER.....

HOSPITAL NUMBER.....

CHILD'S NAME.....

GENERAL PRACTITIONER.....

ADDRESS.....

.....

.....

Measurements

ROOM TEMPERATURE.....

TIME.....

DATE.....

STUDY NO.....

MOTHER

1. HEIGHT

1.....cms

2.....cms

3.....cms

2. PELVIMETRY

1.....cms

2.....cms

3.....cms

INFANT

A. MEAN ARTERIAL PRESSURE .....

PULSE RATE .....

SYSTOLIC BLOOD PRESSURE .....

DIASTOLIC BLOOD PRESSURE

B. MEAN ARTERIAL PRESSURE .....

PULSE RATE .....

SYSTOLIC BLOOD PRESSURE .....

DIASTOLIC BLOOD PRESSURE

.....

C. MEAN ARTERIAL PRESSURE .....

PULSE RATE .....

SYSTOLIC BLOOD PRESSURE

DIASTOLIC BLOOD PRESSURE .....

(READINGS WILL BE TAKEN FROM THE LEFT ARM AT ONE MINUTE INTERVALS)

CUFF SIZE

1. NEONATE 2. NEONATE 3. NEONATE 4. NEONATE

STATE OF INFANT

1. AWAKE & CRYING

2. AWAKE & QUIET

3. ASLEEP

4. HEAD CIRCUMFERENCE

1.....cms

2.....cms

5. LOWER THORACIC CIRCUMFERENCE

1.....cms

2.....cms

3.....cms

6. LENGTH

1.....cms

2.....cms

3.....cms

WHAT TIME WAS THE LAST FEED? .....

WHAT WAS THE LAST FEED?

1. BREAST
2. BOTTLE

QUESTIONNAIRE STUDY NO.....

I WOULD LIKE TO ASK SOME GENERAL INFORMATION ABOUT YOU AND YOUR FAMILY

WHERE WERE YOU BORN?

1. 1SLE OF MAN...
2. U.K.....
3. OTHER.....

DO YOU KNOW HOW MUCH YOU WEIGHED WHEN YOU WERE BORN?

.....LBS.....OZ

ETHNIC ORIGIN (INTERVIEWER'S ASSESSMENT)

1. WHITE
2. AFRICAN
3. ASIAN
4. MONGOLOID

DO YOU SHARE YOUR HOME WITH A PARTNER OR ARE YOU A SINGLE PARENT?

1. SINGLE
2. TWO PARENTS

DO YOU WORK OUTSIDE THE HOME? 1. YES 0. NO

IF SO WHAT IS YOUR OCCUPATION?

DID YOU PASS ANY PUBLIC EXAMINATIONS BEFORE YOU LEFT SCHOOL?

CSE	1. YES/NO
GCSE OR O-LEVEL	1. YES/NO
A-LEVEL	1. YES/NO

DID YOU HAVE ANY TRAINING OR PASS ANY EXAMS AFTER LEAVING SCHOOL?

(IF NO, PROMPT - FOR INSTANCE, HAIRDRESSING, CITY & GUILDS ETC)

QUALIFICATIONS IN SHORTHAND/TYPING	1. YES/ 0.NO
OTHER e.g. HAIRDRESSING APPRENTICESHIP	1. YES/ 0.NO
STATE ENROLLED NURSE	1. YES/ 0.NO
STATE REGISTERED NURSE	1. YES/ 0.NO
CITY AND GUILDS	1. YES/ 0.NO
IF YES - INTERMEDIATE TECHNICAL	1. YES/ 0.NO
FINAL TECHNICAL	1. YES/ 0.NO
FULL TECHNICAL	1. YES/ 0.NO
TEACHING QUALIFICATION	1. YES/ 0.NO
OTHER.....	1. YES/ 0.NO
QUALIFICATION NOT KNOWN	1. YES/ 0.NO

HOW OLD WERE YOU WHEN YOU STARTED YOUR MENSTRUAL PERIODS?.....YRS

WHAT WAS YOUR USUAL WEIGHT BEFORE YOU BECAME PREGNANT?

...STS...LBS

DID YOU SMOKE DURING THIS PREGNANCY? 1.YES 0.NO

IF YES, HOW MANY CIGARETTES DID YOU SMOKE A DAY

1. IN THE FIRST 3 MONTHS? .....
2. IN THE SECOND 3 MONTHS? .....
3. IN THE THIRD 3 MONTHS? .....

LATER ON, I AM GOING TO MEASURE YOUR OWN HEIGHT, BUT CAN YOU REMEMBER HOW TALL YOUR PARENTS ARE?

HOW TALL IS YOUR MOTHER? ...FT...INCH

HOW TALL IS YOUR FATHER? ...FT...INCH

DOES/DID YOUR MOTHER HAVE ANY OF THESE CONDITIONS?

1. HIGH BLOOD PRESSURE 1.YES 0.NO
2. STROKE 1.YES 0.NO
3. ANGINA/HEART DISEASE/HEART ATTACK 1.YES 0.NO

DOES/DID YOUR FATHER HAVE ANY OF THESE CONDITIONS?

1. HIGH BLOOD PRESSURE 1.YES 0.NO
2. STROKE 1.YES 0.NO
3. ANGINA/HEART DISEASE/HEART ATTACK 1.YES 0.NO

I WOULD NOW LIKE TO ASK A FEW VERY GENERAL QUESTIONS ABOUT THE BABY'S FATHER.

HOW OLD IS HE? .....YEARS

WHERE WAS HE BORN? 1.ISLE OF MAN
2.U.K.
3.OTHER.....

IS HE EMPLOYED AT PRESENT? 1.YES 0.NO

WHAT IS HIS PRESENT OCCUPATION? .....

DID HE PASS ANY PUBLIC EXAMINATIONS BEFORE HE LEFT SCHOOL?

CSE	1.YES 0.NO
GCSE OR O-LEVEL	1.YES 0.NO
A-LEVEL	1.YES 0.NO

DID HE HAVE ANY TRAINING OR PASS ANY EXAMS AFTER LEAVING SCHOOL?
(IF NO, PROMPT- FOR INSTANCE CITY & GUILDS, BANKING ETC).

QUALIFICATIONS IN SHORTHAND/TYPING 1.YES 0.NO

OTHER e.g. HAIRDRESSING APPRENTICESHIP 1.YES 0.NO

	STATE ENROLLED NURSE	1.YES 0.NO
	STATE REGISTERED NURSE	1.YES 0.NO
	CITY AND GUILDS	1.YES 0.NO
IF YES -	INTERMEDIATE TECHNICAL	1.YES 0.NO
	FINAL TECHNICAL	1.YES 0.NO
	FULL TECHNICAL	1.YES 0.NO
	TEACHING QUALIFICATION	1.YES 0.NO
	UNIVERSITY DEGREE	1.YES 0.NO
	OTHER.....	1.YES 0.NO
	QUALIFICATION NOT KNOWN	1.YES 0.NO

HOW TALL IS HE? ...FT...INS

DOES HE SUFFER FROM ANY OF THESE CONDITIONS?

HIGH BLOOD PRESSURE	1.YES 0.NO
STROKE	1.YES 0.NO
ANGINA/HEART DISEASE/HEART ATTACK	1.YES 0.NO

DATA EXTRACTION SHEET : MOTHER

DATE.....

MOTHERS DATE OF BIRTH.....

HOSPITAL NUMBER.....

STUDY NO.....

HISTORY OF

1. DIABETES
2. HYPERTENSION
3. OTHER CHRONIC ILLNESSES
4. NONE OF THESE

PARITY	.....
GRAVIDITY	.....
DATE OF LAST MENSTRUAL PERIOD	.....
SURE OF LMP	1.YES 0.NO
FIRST BLOOD PRESSURE IN SECOND TRIMESTER	SYSTOLIC
	DIASTOLIC
	DATE.....
FIRST BLOOD PRESSURE BY HOSPITAL	SYSTOLIC
	DIASTOLIC
	DATE.....
MAXIMUM BLOOD PRESSURE BEFORE LABOUR	SYSTOLIC
	DIASTOLIC
	DATE.....

NO. OF BLOOD PRESSURE READINGS DURING PREGNANCY.....

WEIGHT

1. ON BOOKING	....ST....LBS
DATE	.....
2. HEAVIEST DURING PREGNANCY	....ST....LBS
DATE	.....

HOURS IN LABOUR	1. NONE 2. <6 HOURS 3. 6-12 HOURS 5. 19-24 HOURS 6. >24 HOURS
METHOD OF DELIVERY	1. NORMAL 2. FORCEPS/VENTOUSE 3. BREECH 4. ELECTIVE LSCS 5. EMERGENCY LSCS
ESTIMATED BLOOD LOSS	.....ML
PLACENTA	1. COMPLETE 2. INCOMPLETE
PLACENTAL WEIGHT	.....GM
HAEMOGLOBIN	1. DATE..... FIRST HB..... MCV..... MCH..... MCHC..... HCT..... 2. DATE..... HIGHEST HB.... MCV..... MCH..... MCHC..... HCT..... 3. DATE..... LOWEST HB..... MCV..... MCH..... MCHC..... HCT.....
E.D.D. BY ULTRASOUND SCAN	.....

DATA EXTRACTION SHEET : INFANT

DATE.....

DATE OF BIRTH.....

HOSPITAL NUMBER.....

STUDY NO.....

SEX	1. MALE 2. FEMALE
BIRTH WEIGHT	.....GMS
APGAR SCORE	1. AT 1 MIN
	2. AT 5 MIN
NUMBER	1. SINGLETON
	2. TWIN
	3. TRIPLET
FEEDING	1. BREAST
	2. BOTTLE
	3. BOTH
	4. OTHER
IF BOTTLE FED	1. FARLEY'S OSTERMILK
	2. WYETH SMA GOLD CAP
	3. COW & GATE PREMIUM
	4.COW&GATE
	5.PREMATALAC
	6.. MILUPA MILUMIL
	7.. MILUPA APTAMIL
	8. OTHER
TIME OF BIRTH	.....
OXYGEN THERAPY	1. NONE
	2. 1-6 HRS
	3. 7-24 HRS
	4. 25-48 HRS
	5. 2-7 DAYS
	6. >7 DAYS

MAXIMUM OXYGEN PERCENTAGE GIVEN	.....%
JAUNDICE	1. YES 0. NO
TIME OF ONSET	1.<24 HRS
	2. 24-47 HRS
	3. 48-71 HRS
	4. > 72 HRS
HIGHEST RECORDED LEVEL	O. LEVEL NOT RECORDED
	1. <169 UMOL/L
	2. 170-239 UMOL/L
	3. 240-339 UMOL/L
	4. >340 UMOL/L
CAUSE	1. PHYSIOLOGICAL
	2. ABO INCOMPATABILITY
	3. RH INCOMPATABILITY
	4. SEPSIS
	5. G-6-PD INSUFFICIENCY
	6. NOT KNOWN
	7. OTHER (SPECIFY)
PHOTOTHERAPY	1. YES 0.NO
EXCHANGE TRANSFUSION	1. YES 0.NO
HYPOGLYCAEMIA (<2 MMOL/L)	1. YES 0.NO
TEMPERATURE HIGHER THAN 39 C	1. YES 0.NO
LOWER THAN 35 C	1. YES 0.NO
INFECTION SUSPECTED	1. YES 0.NO
SEPTIC SCREEN	1. YES 0.NO
POSITIVE CULTURES	1. YES 0.NO
TOPICAL ANTIBIOTICS	1. YES 0.N0
SYSTEMIC ANTIBIOTICS	1. YES 0.NO

CHECK LIST	DATE
1. QUESTIONNAIRE COMPLETED	.....
2. MEASUREMENT SHEET COMPLETED	.....
3. BLOOD SAMPLES DELIVERED TO LAB	.....
4. EXTRACTION SHEET COMPLETED	.....
5. BLOOD SAMPLES SENT TO NORTHWICK PARK	.....
6. BLOOD SAMPLES RECEIVED BY NORTHWICK PARK	.....
7. FIBRINOGEN RESULTS RECEIVED	.....

7.2 Quality control of the measurements

Stadiometer

02.01.91	01.07.91	03.01.92	01.06.92
81.5 cm	81.5 cm	81.4 cm	81.5 cm
81.5 cm	81.5 cm	81.5 cm	81.5 cm
81.5 cm	81.5 cm	81.5 cm	81.5 cm

Neonatometer

02.01.91	01.07.91	03.01.92	01.06.92
40.4 cm	40.4 cm	40.3 cm	40.5 cm
40.4 cm	40.4 cm	40.4 cm	40.5 cm
40.5 cm	40.4 cm	40.4 cm	40.4 cm

7.2 Explanation of laboratory quality control table on next page

Column 1

The batch number. Each batch contained approximately fifty samples as sent from the Isle of Man.

Column 2

The date on which the samples were assayed.

Column 3

The laboratory uses a commercially produced Fibrinogen Standard from Immuno of Vienna. This column states the Batch number of the Standard.

Column 4

The Fibrinogen Standard potency is measured using the Clauss method and is expressed in mg/dl.

Column 5

This column states the date of the internal reference plasma that was being used when the outside samples were being assayed.

Column 6

The laboratory takes six donated plasma packs from the blood transfusion centre, pool them and put them into 0.5 ml aliquots. A Clauss estimation is done on each of these and the mean of all the samples is then calculated as the local reference plasma and this column expresses 1 standard deviation of the mean in mg/dl.

Column 7

This column expresses the reference plasma value obtained in mg/dl for each batch.

Quality Control For Isle Of Man Neonatal Fibrinogen

1	2	3	4	5	6	7	8
Batch no.	Date assayed	Fibrinogen Standard (Immuno)	Fibrinogen Standard potency mg/dl	Reference plasma	Ref. Plasma 1s.d. of mean mg/dl	Ref. Plasma 2 s.d. of mean mg/dl	Ref. Plasma value obtained mg/dl
1	09.07.91	KN27	232	Oct 1990	205-235	190-250	223
2	21.10.91	KN27	232	Sept 1991	170-190	160-200	189
3	28.10.91	EO1C	241	Sept 1991	198-221	187-232	175
4	22.01.92	1EOA	254	Oct 1990	192-226	175-243	214
5	11.05.92	1EIA	254	Oct 1990	192-226	175-243	214
6	11.05.92	1EIA	238	Nov 1992	208-241	191-258	210
7	03.02.93	1EIA	238	Nov 1992	208-241	191-258	239

7.3 Characteristics of the whole cohort

Characteristics of mothers in whole cohort (390)

Mothers	Mean (SD)
Age (yrs)	26.3 (5.0)
Height (cm)	1.63 (6.1)
Booking wt (lb)	139.7 (22.8)
Own birth weight (oz) known in 304 mothers	114 (20.2)
	Number (%)
Living with baby's father	314 (86)
Social class I, II, IIIN*	149 (38)
Social class IIIM, IV, V*	165 (42)
Single mothers living alone with no occupation	42 (11)
Single mothers living alone with an occupation	34 (9)
Qualifications: none	52 (13)
CSE,O-level,Sec/cler	231 (60)
A-level, RGN, SRN, Tech	79 (20)
Teaching, Degree	28 (7)
Maternal smoking at beginning of pregnancy	121 (31)

*Social class is characterised by occupation of the head of household.

221 mothers were not economically active.

Characteristics of fathers in the whole cohort (390)

Fathers	Mean (SD)	Number (%)
Height (ins)	69.5 (2.8)	387
Age (years)	28.9 (6.4)	389
Social class I, II, IIIN*		162 (42)
Social class IIIM, IV, V*		205 (53)
With no occupation		23 (5)
Qualifications: none		100 (26)
CSE, O-level, Sec/cler		179 (46)
A-level, RGN, SRN, Tech		68 (17)
Teaching, Degree		38 (10)
Not known		5 (1)

Characteristics of neonates in the whole cohort (390)

	Males (190)	Females (200)
	Mean (sd)	Mean (sd)
Birthweight (kg)	3.4 (4.65)	3.4 (4.35)
Length (cm)	50.6 (1.9)	49.9 (1.7)
Chest (cm)	32.8 (1.7)	32.6 (1.6)
Head circ (cm)	34.9 (1.2)	34.5 (1.1)
Gestational age (wks)	39.9 (8.1)	39.9 (7.6)
	Number (%)	
Social class I, II, IIIN*	73 (19)	93 (24)
Social class IIIM, IV, V*	109 (28)	97 (25)
Social class missing		18 (4)
Breast fed	89 (23)	118 (30)
Infection suspected	12 (3)	12 (3)
Jaundice	67 (17)	71 (18)
Hypoglycaemia	15 (4)	15 (4)

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