INTERACTION BETWEEN FETAL AND NEONATAL GROWTH AND BLOOD PRESSURE DEVELOPMENT

Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

by

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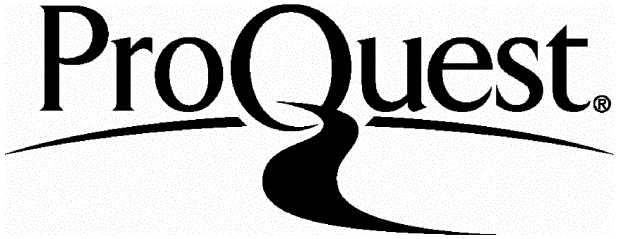


For Mum and Dad

and Q

EVOLUTION - A FABLE

The angel said " How could I have once been a man.

See I am non-material and omnipresent."

The man said "How could I have come from an ape.

See I have no tail. "

The ape said "How could I have ever come from a fish.

I climb trees and cannot breathe under water."

The fish said "How could a graceful flashing thing of speed

like me ever have evolved from a shapeless jelly fish."

The jelly fish said "and how could I a material thing have come

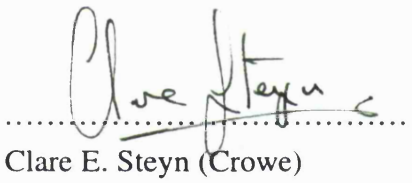
from an idea?"

The idea spoke "How could I who am everything come from nothing."

Percy William Crowe

Personal statement

Except as acknowledged on pages 3, 302, 404 and 405, the work presented in this thesis was performed solely by the candidate and is original.



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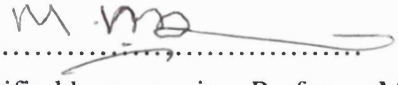

Certified by supervisor Professor M.A. Hanson

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ABSTRACT

There is much debate concerning the influence of nutrition *in utero* on fetal and neonatal growth and development. Recent epidemiological studies have suggested that nutritional influences acting during intrauterine and early postnatal life may have long term consequences for cardiovascular disease. More specifically, the suggestion has been made that small babies born with a large placenta are predisposed to hypertension in later life. Thus, there would appear to be important links between fetal nutrition, and thus growth, and cardiovascular disease in adult life.

The availability of nutrients is essential for fetal growth. Whilst there is obviously a relationship between maternal nutrition and nutrient supply to the fetus, the placenta is another major determinant of nutrient availability. The metabolic and endocrine interactions that occur between fetus, placenta and mother have important regulatory effects on fetal growth.

The work described in this thesis was undertaken with the aim of trying to establish more clearly the mechanisms by which fetal and neonatal nutrition and growth influence blood pressure development. The role of oxygen availability to the fetus was studied using two models, anaemia and repeated acute hyoxaemia. The role of nutrient availability was studied by altering maternal nutrition in early pregnancy. I have investigated the development of blood pressure and cardiovascular reflexes in relation to fetal and neonatal size and growth, placental size, endocrine status, and maternal nutritional plane.

The results of this work show that there is a link between postnatal blood pressure and maternal nutritional anaemia during pregnancy, and that there is also an important association with the rate of postnatal growth. Postnatal blood pressure may relate to blood pressure development during fetal life. So, it was interesting to find that mean arterial blood pressure (MAP) follows distinct trajectories in individual fetuses and fetal chemo- and baroreflexes are also altered. Chronic fetal hypoxaemia may be an important factor in the aetiology of adult hypertension, however, two weeks of repeated acute hypoxaemia during late gestation does not affect cardiovascular development and periconceptual maternal undernutrition does not result in hypoxaemia during late gestation. Nevertheless, fetal cardiovascular development during late gestation is perturbed by periconceptual undernutrition, an effect that may be the result of alterations in placental function.

These effects on fetal cardiovascular development in relation to oxygen, nutrient supply and maternal nutrition, and their interaction with fetal growth, have important implications for understanding the processes of cardiovascular development in health and disease.

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ABBREVIATIONS

ABP	arterial blood pressure
AC	abdominal circumference
ACE	angiotensin-converting enzyme
ACh	acetylcholine
ACTH	adrenocorticotropic hormone
AI	angiotensin I
AII	angiotensin II
AN	anaemic group (rats)
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
BE	base excess
BNC	binucleate cell
BNP	brain natriuretic peptide
bpm	beats per minute
BW	body weight
C	control group
Ca^{++}	calcium ion
ca.	*circa*
CaO_2	arterial oxygen content
CBG	corticosteroid-binding globulin
CNP	C-type natriuretic peptide
CNS	central nervous system
CO	cardiac output
CRH	corticotrophin releasing hormone
CRL	crown-rump length

CSF	colony stimulating factor
CVO	combined ventricular output
DBP	diastolic blood pressure
DNA	deoxyribonucleic acid
E	embryonic day (age)
ECG	electrocardiogram
ECoG	electrocorticogram
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EMG	electromyogram
et al.	and others
FGF	fibroblast growth factor
Fig.	figure
FiO_2	fraction of inspired oxygen
FSH	follicle stimulating hormone
GA	gestational age
GHV	growth hormone variant
GI	gastro-intestinal
GnRH	gonadotrophin-releasing hormone
H	Hypoxia group (sheep)
Hb	haemoglobin
hCG	human chorionic gonadotrophin
HCO_3^-	bicarbonate ion
HGF	hepatocyte growth factor
HPA	hypothalamic-pituitary-adrenal (axis)

hPL	human placental lactogen
HPLC	high performance liquid chromatography
i.m.	intra-muscular
i.v.	intra-venous
ID	inside diameter
IGF	insulin-like growth factor
IGF-BP	insulin-like growth factor binding protein
IGF-R	insulin-like growth factor receptor
IgG	immunoglobulin G
IL	interleukin
IMS	industrial methylated spirit
iu	international unit
IUGR	intra-uterine growth retardation
K^+	potassium ion
KL	kit-ligand
L-GLU	L-glutamate
LH	leutenizing hormone
MAP	mean arterial pressure
mCG	monkey chorionic gonadotrophin
mRNA	messenger ribonucleic acid
n/s	not statistically significant
Na^+	sodium ion
NAd	noradrenaline
NGF	nerve growth factor
NO	nitric oxide
NTS	nucleus tractus solitarius

OD	outside diameter
P	postnatal day (age)
P	probability
P20	postnatal age of 20 days (rats)
P40	postnatal age of 40 days (rats)
PaO_2	partial pressure of arterial oxygen
PD	pressure down group (sheep)
PDGF	platelet-derived growth factor
PGI_2	prostacyclin
PMSG	pregnant mare's serum gonadotrophin
POMC	proopiomelanocortin
PU	pressure up group (sheep)
RAS	renin-angiotensin system
REM	rapid eye movement
S.E.M.	standard error of the mean
SaO_2	arterial oxygen saturation
SBP	systolic blood pressure
SBP	systolic blood pressure
SHR	spontaneously hypertensive rat
T_3	triiodothyronine
T_4	thyroxine
TGF	transforming growth factor
TRH	thyrotrophin-releasing hormone
VEGF	vascular endothelial growth factor
vs.	*versus*

UNITS

CONCENTRATION

M	molar
fmol	fentomole
mmol	millimole
pmol	picomole
iu	international units

ELECTRICITY

nA	nanoamp
v	volt

ENERGY

kcal	kilocalories

INFORMATION TECHNOLOGY

Mb	megabyte

LENGTH

cm	centimetre
m	metre
mm	millimetre

MASS

g	gram
kg	kilogram
µg	microgram
ng	nanogram
pg	picogram

TEMPERATURE

°C	degrees Celsius

TIME

m	month
d	day
min	minute
s	second

VOLUME

l	litre
dl	decilitre
ml	millilitre

OVERVIEW

This thesis investigates the interaction between fetal and neonatal growth and blood pressure development.

The Introduction reviews current knowledge of placental structure, development and growth; and fetal growth and development, particularly cardiovascular development. The interaction between fetus and placenta is also discussed. Then, I go on to talk about maternal undernutrition and its effect on fetal and placental growth and development, and ways of investigating fetal responses to nutrient deprivation. Penultimately, I discuss work which suggests that maternal nutrition and its effect on fetal growth and development are important in the aetiology of disese in later life, before finally presenting the aims of the work that is discussed in this thesis.

The next chapter is Methods which describes in detail the protocols employed in the carrying out of the work for this thesis, and the statistical tests employed in analysing the data generated. The Methods section is detailed and therefore the methods section in each of the Results chapters is more properly considered to be experimental design and describes only those aspecrs of the method peculiar to that particular project.

The Results chapters, of which there are five in all, follow on from each other as the results of each suggested the next avenue of investigation. Each chapter gives a detailed description of the results of that particular project and discusses them in detail.

Finally, the Discussion chapter attempts to sum up and bring together the conclusions of each of the preceding Results chapters. So, the discussion is general and gives a clear picture as to the contribution of this thesis to our current understanding of the subject.

Chapter 1

INTRODUCTION

1.1 GENERAL

The subject of this thesis is the interaction between fetal and neonatal growth and blood pressure development. Normal fetal growth and development relies on the ample supply and suitable utilisation of nutrients. In the normal situation, where there is enough food available and no imposed stress, the primary source of fetal substrates is the maternal pool. However, before nutrients can be supplied to the fetus they must first be transported across the placenta from the maternal circulation. Thus, the placenta forms an interface between mother and fetus. Therefore, any discussion concerning fetal growth and development would not be complete without also considering the placenta. Thus, I shall first describe the formation and early growth and development of the placenta and embryo. I shall then go on to discuss the factors that are important for placental and fetal growth and describe the transport and endocrine functions of the placenta, which are important for fetal growth and development. The primary interest of this thesis is cardiovascular development, so I shall then go on to describe normal fetal cardiovascular development in early and late gestation. I shall not discuss the development of other systems, e.g., gastrointestinal, renal, etc., as they are beyond the remit of this thesis. Having thus described our current knowledge of the factors involved in fetal growth and cardiovascular development, I shall describe the epidemiological findings in humans that were the reason for the research carried out and described in this thesis. Finally, the hypotheses and aims of this thesis will be stated.

1.2 EMBRYONIC AND PLACENTAL DEVELOPMENT AND STRUCTURE

The information contained within this section is not central to the research that is the subject of this thesis. Nonetheless, an understanding of placental development and structure is important for the discussion of possible factors that may influence events later on in development. Therefore, I did not think it necessary for an in-depth review of the literature on this subject, and have chosen to draw information from four texts on placental physiology. Thus, unless stated otherwise, the information in this section is attributable to Steven (1975), Johnson & Everitt (1988), Wooding & Flint (1994) and Page (1993).

1.2.1 The Blastocyst

After fertilisation, whilst the conceptus is progressing down the oviduct into the uterus, it undergoes a series of cell-divisions during which time it derives essential materials for cleavage from the large volume of oocyte cytoplasm. On reaching the 16-cell stage it undergoes a process of compaction forming what is known as a morula. Following this, the morula undergoes further morphological change making the transition to blastocyst. The blastocyst is still free-living, i.e. unattached to the uterus, at this time and is bathed in the secretions of the uterus. In this period, prior to attachment and implantation, and also whilst invasion is taking place, the conceptus is obviously unable to derive nutrients from the maternal blood. It therefore derives nutrients from endogenous reserves, secretions of the genital tract, and exocrine secretions from uterine glandular tissue and the decidual tissue most adjacent to the invading conceptus. This is called histiotrophic support.

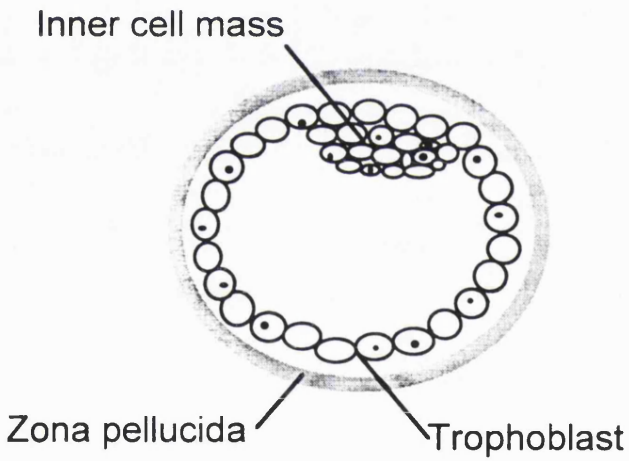

Figure 1.1. Early embryonic development. The blastocyst.

1.2.2 Attachment

Having entered the uterus the conceptus becomes positioned for implantation. First the process of attachment takes place. There is species variation in the time after conception at which attachment takes place. In the rat it takes place about 5 d *post coitus* and in man at about 6 d, whereas in the sheep (a species

where non-invasive implantation takes place - see later) attachment does not occur until about 16 d. Attachment requires rupture and/or dissolution of the *zona pellucida*, uterine 'closure' (development of uterine muscular tone so bringing the blastocyst and the uterine epithelium close together), and establishment of physical and chemical contact of the trophoblast (outer cell mass) with the uterine epithelium. The location of attachment varies from species to species, though there is evidence that whether monotochous (a single conceptus in the uterus) or ditochous (a conceptus in each uterine horn), there are preferred sites of attachment. Ruminants (e.g. sheep/cows) have distinct areas of projecting aglandular uterine mucosa, known as caruncles, which are the only sites at which attachment occurs. Intriguingly, it is almost always half way between the cervix and the uterotubal junction, for unknown reasons, as no unique characteristics of the caruncles at this site have been identified. Sheep and cow blastocysts anchor themselves to their preferred site of attachment by cellular outgrowths of the trophectoderm (papillae) down into the uterine glands. In polytochous (several conceptuses in one uterine horn) species there also seem to be preferred sites of attachment that are normally at evenly spaced sites along the uterine horn. Location and spacing of attachment is important in minimising physical and nutritional competition between conceptuses.

1.2.3 Implantation

Once attachment has occurred, implantation of the conceptus takes place. The maternal uterine epithelium is invaded by cytotrophoblasts (trophoblast cells leading the invasion process) and there is penetration into the endometrial stroma. The cytotrophoblasts are also commonly referred to as trophectoderm. Again, there is variation from species to species as to the type of implantation that takes place (Fig. 1.2).

1.2.3.i Interdigitation

Simple interdigitation takes place in the pig, where trophectodermal microvilli and uterine epithelial cells interdigitate, development occurring by an increase in the area of apposed epithelia and microvilli. There is no syncytial formation.

1.2.3.ii Displacement

Displacement implantation takes place in the mouse, rat and hamster (Fig. 1.2). The trophectoderm delaminates the uterine epithelial cells and phagocytoses them. The trophectoderm then invades intrusively (see section 1.2.3.iv).

1.2.3.iii Fusion

In rabbits, the cytotrophoblast cells fuse to form a trophectodermal syncytium with numerous processes. There is then fetomaternal fusion of the cytotrophoblast processes with the apposed uterine epithelial cells, and the trophectodermal syncytium goes on to invade the maternal stroma (Fig. 1.2).

The ruminant trophectoderm is unique in that it characterised by granulated binucleate cells (BNCs) that are first produced just before implantation. BNCs migrate and form a flat apposition with a uterine epithelial cell, with which they then fuse to form a trinucleate cell within the uterine epithelium (Fig. 1.2). Thus a fetomaternal syncytium is formed. However, in the sheep and cow, there are large areas of the definitive placenta where no fetomaternal syncytium is formed and fetomaternal contact is by apposition only. (Thus, they are sometimes classified as synepitheliochorial as opposed to just epitheliochorial, see section 1.2.5.ii).

1.2.3.iv Intrusion

The guinea pig and human are examples of species where implantation is by intrusion (Fig. 1.2). A trophectodermal syncytium forms at the site of attachment and protrusions are sent out that penetrate the uterine epithelium without destroying the uterine cells. It is thought that the fetal trophectoderm may form tight junctions with the maternal uterine epithelial cells. The entire conceptus penetrates into the endometrium after which the uterine epithelium closes over the site of implantation.

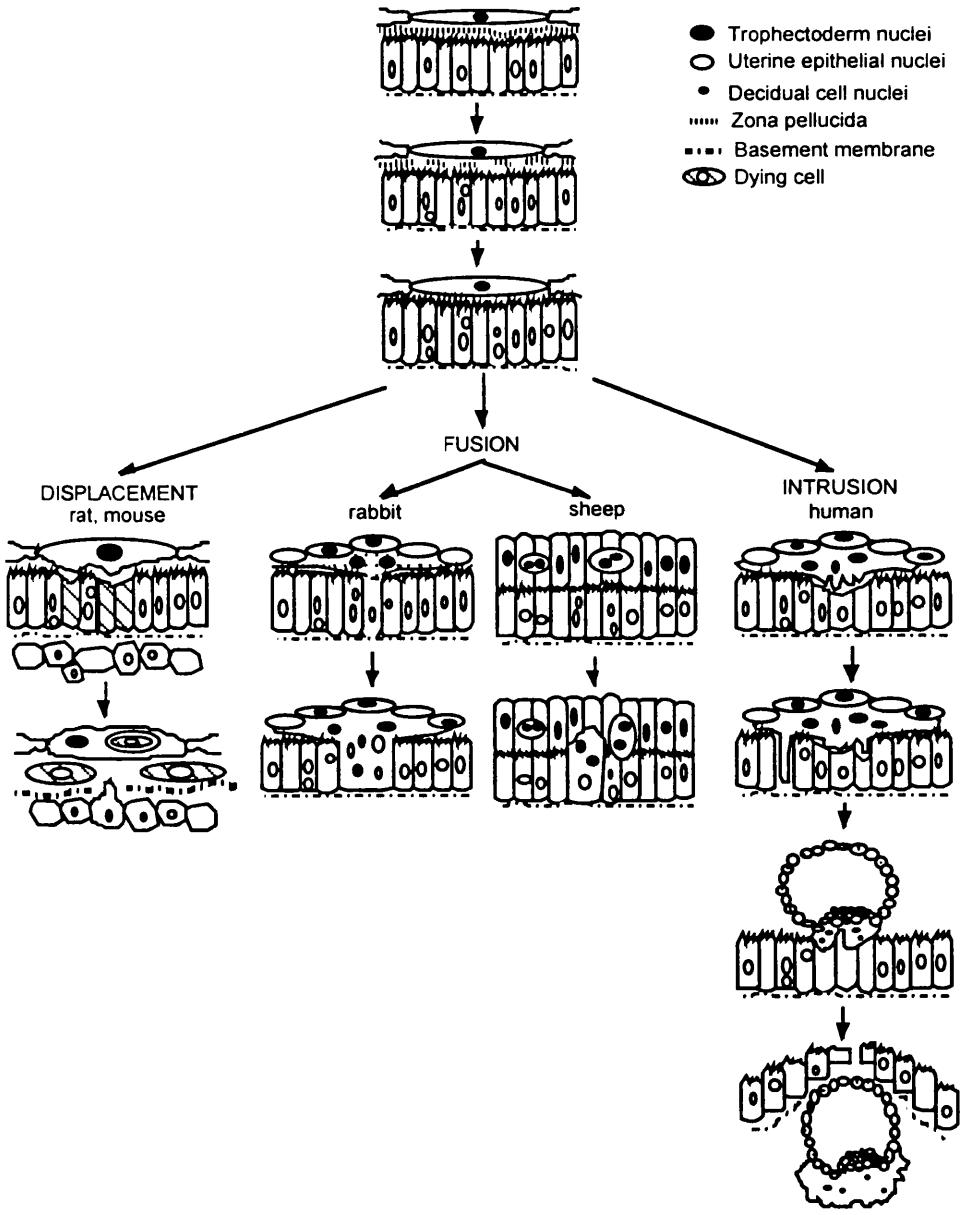

Figure 1.2. Classification of the different fetomaternal cellular interactions at implantation. (From Wooding & Flint, 1994).

1.2.4 Embryonic membranes

The extra embryonic membranes of vertebrates comprise the yolk sac, chorion, amnion and allantois. They are formed from the three germ layers of the embryo - ectoderm, mesoderm and endoderm (see Fig. 1.3).

The yolk sac is formed initially by enclosure of the yolk within a single cellular layer of ectoderm (unilaminar yolk sac). Subsequently, migration of a layer of endoderm forms a bilaminar yolk sac, and then growth of mesoderm between the ectoderm and endoderm forms a trilaminar yolk sac (Fig. 1.3).

Chorion, amnion and allantois form initially from the mesoderm of the partly trilaminar yolk sac, which splits to form the exocoelom (Fig. 1.3). The mesoderm may then develop in association with the endoderm to form the definitive yolk sac wall, or it may develop in association with the ectoderm to from the chorion. The chorion (ectoderm + mesoderm) folds and encloses the embryo in what becomes the fluid-filled amniotic sac. Meanwhile, an outgrowth of the endoderm from the hindgut region of the developing embryo forms the allantois (Fig. 1.3).

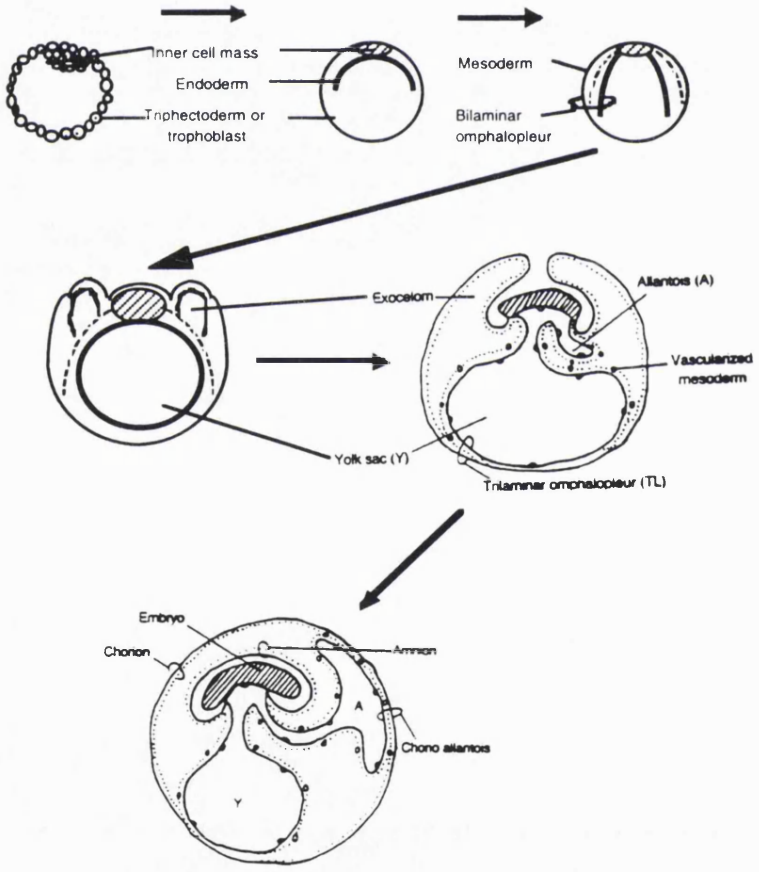

Figure 1.3. Development of the basic extraembryonic membranes. (From Wooding & Flint, 1994).

1.2.4.i Variations in membrane development

The functional placenta may be of two types, choriovitelline or chorioallantoic, depending on how much it specialises along the common scheme of extraembryonic membrane development described above.

Choriovitelline and chorioallantoic placentae

The choriovitelline placenta is homologous to the yolk sac in the eggs of birds, reptiles and monotremes. Also known as the yolk sac placenta, it is formed if the yolk sac and chorion come into contact with one another, and functions transiently in most mammals before the chorioallantoic placenta takes over. However, in some species (most marsupials) it functions alone throughout pregnancy, or secondary to the chorioallantoic placenta (e.g. rat, mouse, rabbit). The chorioallantoic placenta is formed as a result of the formation of a cavity known as the allantoic cavity (see Fig. 1.3). Man and other higher primates have a chorioallantoic placenta, although they do have a choriovitelline placenta that exists early on during embryonic life

1.2.5 Placental classification

As described above, the extent and shape of implantation varies from species to species, and this results in a variety of different placental morphologies.

1.2.5.i Classification by shape

The simplest classification of the placenta is by shape (Fig. 1.4). The placenta of man is described as discoid, as is that of the rat, and is characterised by a single attachment site to the uterine epithelium. Other types of placental morphologies are: bi-discoid, where there are two attachment sites (e.g. rhesus monkey); zonary, which looks like a belt around the conceptus (e.g. cat, dog, elephant); cotyledonary, where attachment is limited to uterine caruncles, which vary between about 90 and 150 (e.g. sheep, cow); and diffuse, where attachment occurs at multiple sites (e.g. horse, pig, whale).

The shape of the uterus is also characteristic of a species. Primates, including man, have a 'simplex' uterus in which the horns are very small. In rats and rabbits the horns are completely separate, forming what is known as a 'duplex' uterus. The ruminant placenta is 'bicornuate', there being partial fusion of the uterine horns.

Diffuse placenta

Chorionic villi are distributed over almost the entire surface of the chorionic sac e.g. horse, pig, also Cetacea, Lemuroidea, camel.

Cotyledonary placenta

Chorionic villi are normally restricted to circular or oval areas of the chorionic sac, e.g. ruminants. Numbers may vary from 90 or more in cattle and sheep, to 4 or 6 in deer.

Zonary placenta

Chorionic villi are restricted to an equatorial girdle, e.g. cat, dog

Incomplete zonary placenta

May resemble the single or double discoidal condition, but can usually be distinguished by the presence of central or marginal effusions of maternal blood e.g. mink, polar bear.

Discoid placenta

Chorionic villi are arranged in a circular plate e.g. man, rat, mouse, guinea-pig.

Double discoid

Found in certain monkeys and as an abnormality in man.

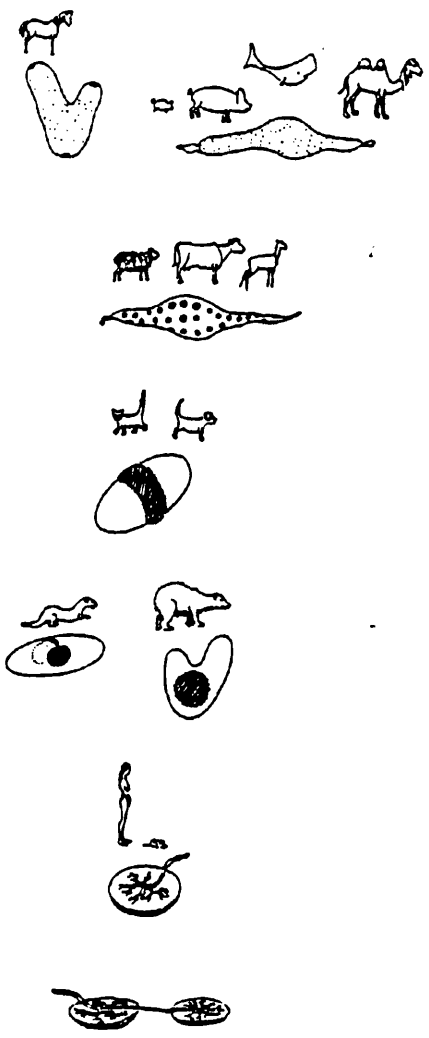

Figure 1.4. Placental classification by shape (from Steven, 1975).

1.2.5.ii Classification by structure

Histological structure is a more physiologically relevant form of classification, as it describes the organisation of the layers at the fetomaternal interface that separate fetal and maternal blood.

The different levels of invasion from species to species results in variation in the proximity between maternal and fetal circulations. Grosser first described (and categorised) the various degrees of proximity established between the conceptus and maternal circulations, based on how much integrity the maternal tissue retains (Fig. 1.5). Placentae that preserve maternal epithelia and endothelia are described as epitheliochorial (e.g. sheep, pig, cow, horse), those with maternal endothelium only are known as endotheliochorial (e.g. dog, cat), and those where the maternal blood comes into direct contact with the fetal villi are haemochorial. The haemochorial class is further subdivided according to the number of layers of trophoblast between the maternal blood and fetal endothelium. Thus, when there is only one such layer the placenta is classified as haemomonochorial (e.g. man), with two it is haemodichorial (e.g. rabbit) and with three it is haemotrichorial (e.g. rat).

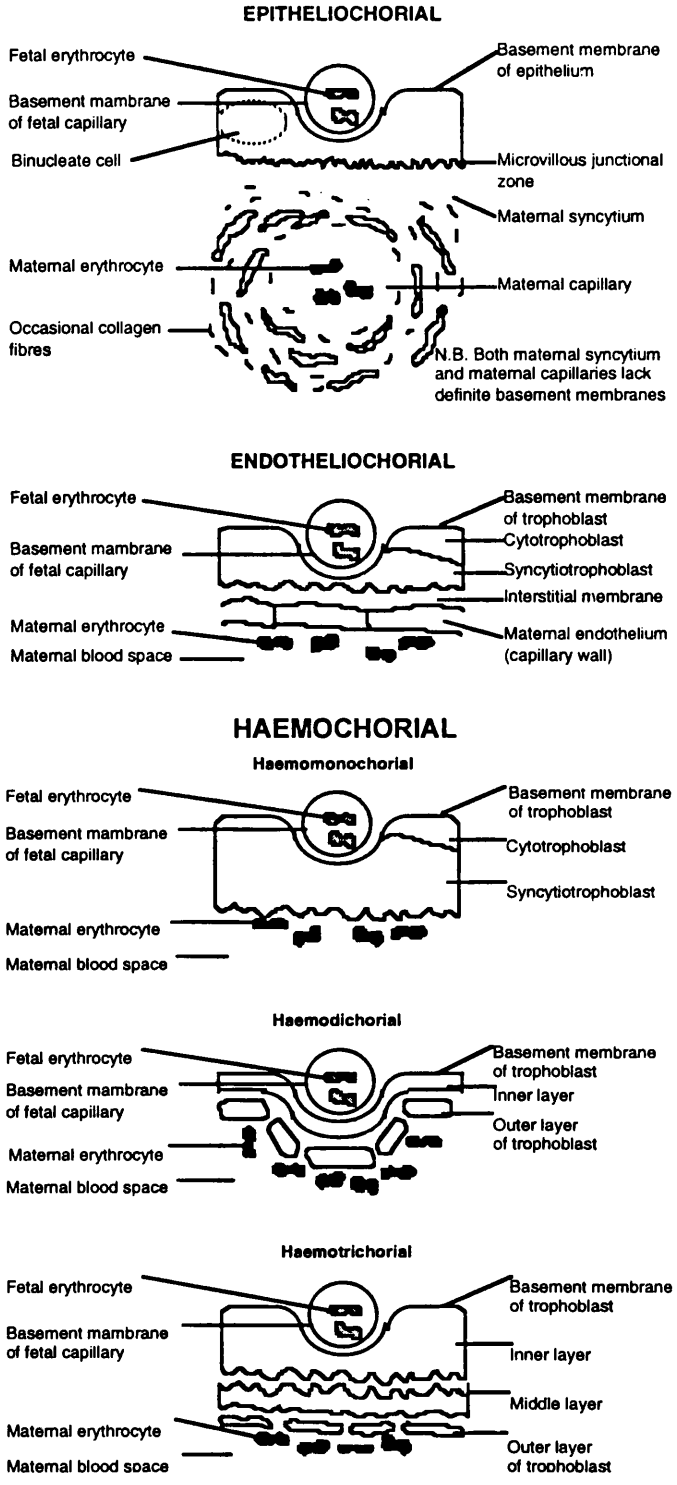

Figure 1.5. Schematic representation of the microstructure of the mature placental interface of various species according to the Grosser classification (adapted from Steven, 1975 & Johnson & Everitt, 1988).

Species	Shape of attachment	Grosser classification
Man	Discoid	Haemomonochorial
Rabbit	Discoid	Haemodichorial
Rat / mouse	Discoid	Haemotrichorial
Rhesus monkey	Bi-discoid	Haemomonochorial
Dog	Zonary	Endotheliochorial
Cat	Zonary	Endotheliochorial
Sheep	Cotyledonary	Epitheliochorial
Pig	Diffuse	Epitheliochorial
Cow	Cotyledonary	Epitheliochorial
Horse	Diffuse	Epitheliochorial

Table 1.1. Characteristics of different placental forms in various species.

1.2.6 Structure of the mature placenta

The placenta is a functional unit once the conceptus is implanted and development of the embryo and fetal membranes has taken place. The cytotrophoblast now has contact with maternal tissues and gives rise to chorionic villi. These penetrate and interdigitate with the maternal endometrial tissue which also becomes more highly vascularised. Thus, histiotrophic support is superseded by the more efficient haemotrophic support. This stage is reached after about 14-18 days post-conception in human pregnancies, about 24 days in the sheep, and about 6 days in the rat.

I will describe the placental structure of the human and the sheep. Other than the structural differences outlined below, it is interesting that the sheep has two umbilical veins which drain separate portions of the placenta and two umbilical arteries, unlike the human fetus which has only one umbilical vein and two umbilical arteries. It is possible that this variation is due to the different placental morphologies of the two species. A single umbilical vein would perhaps not be able to drain efficiently the cotyledonary sheep placenta.

1.2.6.i The human placenta

The human placenta reaches its final form by the fourth month of pregnancy (0.45 gestation). It consists of a network of villous trees which originate from the chorionic plate. These villi contain blood vessels and are anchored to the basal plate, through which maternal spiral arteries penetrate and venous openings form. The umbilical arteries and vein divide into secondary and then tertiary vessels. Paired tertiary arteries and veins devoid of any nerve supply, and with 3-5 layers of smooth muscle, accompanied by a variable number of smaller blood vessels, penetrate the chorionic plate and enter the main 200-400 μm stem villus (*truncus chorii*). The *truncus chorii* branch into secondary stem villi with diameters of about 150 μm, in which the arteries and veins transform into arterioles and venules with one or two layers of smooth muscle. The tertiary stem villi are 80-100 μm in diameter and contain a single arteriole and venule accompanied by about ten capillaries. The tertiary stem villi divide into 2-5 intermediate villi (*rami chorii*) which subdivide further into *ramuli chorii*. Some of the *ramuli chorii* terminate on the basal plate of the cytotrophoblast to form anchoring villi and the rest terminate in freely suspended branches within the cytotrophoblast forming what are known as the terminal villi. The arterioles and venules within the stem villi gradually transform into capillaries as they approach the terminal villi, which are the exchange site of material between maternal and fetal blood. The mature placenta contains between 60 and 70 stem villi and their associated villous trees. Each villous tree is known as a fetal cotyledon. Maternal blood from the spiral arteries, of which there are about one hundred in all, is ejected into the central region of each villous tree (fetal cotyledon) and perfuses centrifugally through the intervillous space. It then drains into the subchorial space between each villous tree and into some fifty or sixty venous openings in the basal plate (Fig. 1.6). It is presumed that the direction of maternal and fetal blood microflows are in a mixed or random relationship to each other.

The basal surface of the placenta has between 10 and 30 slightly raised areas, known as maternal cotyledons, that are partially separated from each other by projections of maternal tissue which form the placental septa. Several fetal cotyledons are associated with each maternal cotyledon and each such unit forms what is called a placentome.

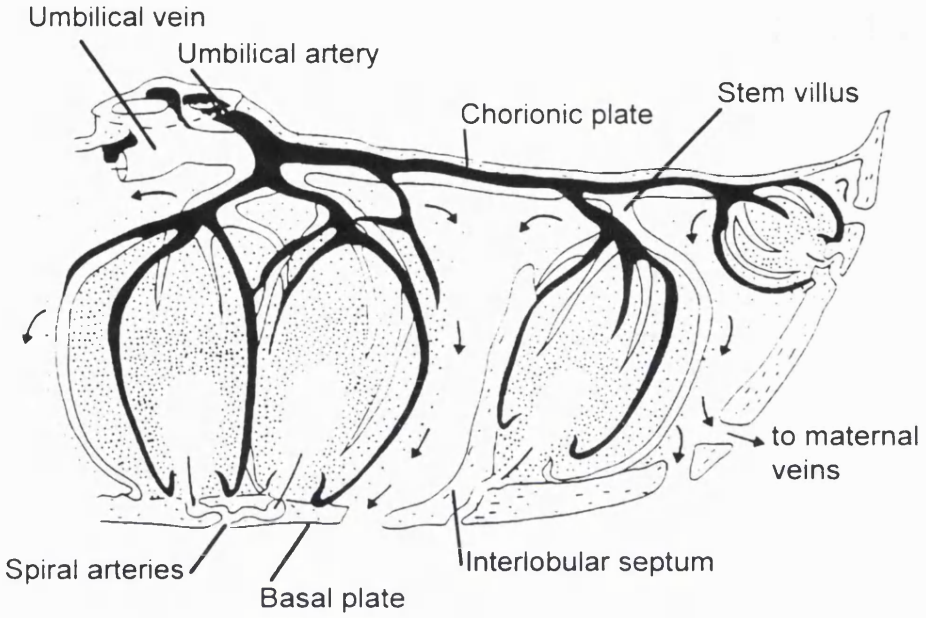

Figure 1.6. Structure of the mature human placenta. From Page (1993).

1.2.6.ii The ovine placenta

The sheep placenta is also made up of individual functional units known as placentomes, which must not be confused with the placentomes of the human placenta. As described earlier, attachment of the conceptus in the sheep occurs at specific sites within the uterus known as caruncles. The point where the chorion attaches to the caruncle specialises into a cotyledon. The placentome in the sheep is a functional unit consisting of fetal cotyledon and maternal caruncle. Generally, when referring to the ovine placenta, the terms placentome and cotyledon are used interchangeably. Strictly, however, as just described, the cotyledon is only a part of the composite structure that is the placentome. Most placentomes have a central depression so are convex in shape. There are, however, variations in shape and size (Vatnick *et al.*, 1989) and some placentomes are flat while others are concave. The sheep placenta has between 80 and 150 placentomes.

The main features of the mature sheep placenta are established by about 78 days gestation (0.5 gestation, term=147 d). Within the surface of the maternal

caruncle are depressions known as crypts which interdigitate with fetal chorionic villi (Fig. 1.7). Between one and three branches of the umbilical vessels enter the central depression of the cotyledon where they divide and ramify, sending their terminal branches into the villi. Each villus has a single central artery that penetrates to its tip and branches to supply an extensive capillary plexus that lies under the surface of the trophoblast.

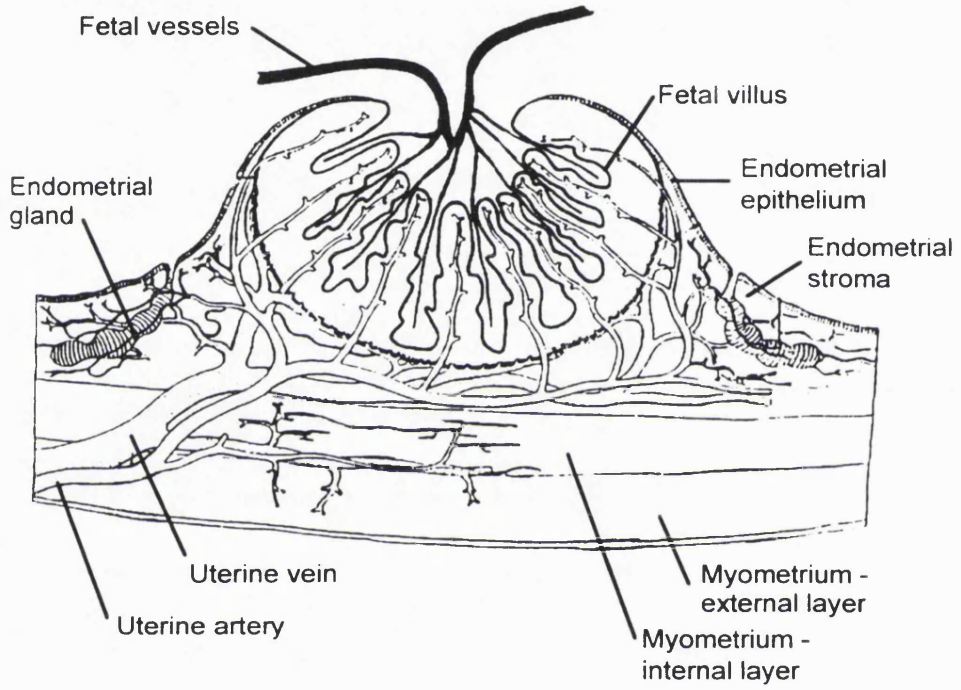

Figure 1.7. Vertical section through a mature ovine placentome. Adapted from Steven (1975) and Johnson & Everitt (1988).

The sheep placenta would appear to have a mixed cross- and counter-current blood flow system.

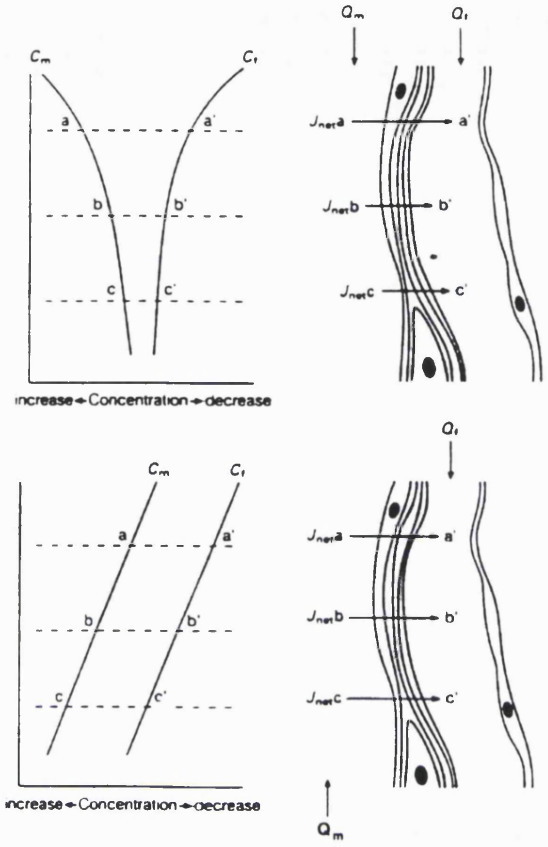

Figure 1.8. Illustration showing the relative efficiencies of concurrent (top) and countercurrent (bottom) flow systems, due to the directional relationships of maternal (Q_m) and fetal (Q_f) blood flows.

Concurrent:- *Left*: Maternal (C_m) and fetal (C_f) plasma concentration profiles are shown along the length of the exchange vessel at points a, b and c on the maternal side of the vessel and the corresponding points a', b' and c' within the lumen of the fetal vessel. *Right*: The net flux (J_{net}) decrease from point a-a', to point b-b', to point c-c' along the length of the exchange vessels. C_f increases along the length of the exchange vessel until the concentration difference that drives the net diffusion decreases to almost zero.

Countercurrent:- *Left*: C_m declines along the length of the exchange vessel and C_f increases. The maternal-fetal concentration difference is constant along the exchange vessel. *Right*: J_{net} remains uniform along the length of the capillary.

(From Sibley, 1995)

1.3 PLACENTAL FUNCTION

It is clear that there is considerable variation between species in terms of the structure and shape of the placenta, however, its functions are common to all. The primary role of the placenta is one of transport, of nutrients to, and waste products away from, the fetus. It also has important endocrine functions that are essential for maintaining pregnancy, and for the growth and development of the fetus. These functions are essential for fetal survival. The placenta is also a metabolic organ, undergoing an array of metabolic activities for both its own growth and that of the fetus. The placenta is devoid of any nerve supply, therefore its function is determined by the supply of substrates, and by hormonal and paracrine action.

1.3.1 Transport functions

Substances that need to be transported across the placenta include respiratory gases, carbohydrates, lipids, amino-acids, water, electrolytes, proteins, vitamins and minerals.

1.3.1.i Respiratory gases

Oxygen

Oxygen crosses the placental membranes by diffusion, and there are a number of factors that determine its transfer.

Vascular geometry is important (Comline & Silver, 1975; Carter, 1989; Boyd & Kudo, 1994; Doughty & Sibley, 1995) because it determines the relative directions of fetal and maternal blood flows, which is important for solute exchange between capillaries. The most efficient system is countercurrent flow, where, theoretically, 100% transfer is possible. In a concurrent system only a maximum of 50% transfer is possible, and in a cross-current system transfer efficiency depends on the number of capillaries that cross each other.

Uterine and umbilical blood flows also affect transplacental transfer of oxygen (Comline & Silver, 1975; Wilkening *et al.*, 1989; Carter, 1989; Page, 1993; Doughty & Sibley, 1995) and there is a significant correlation between maternal and fetal placental blood flows, although mismatching of flows is important in maintaining a PO_2 gradient (Carter, 1989). Oxygen transfer across the placenta is not limited by placental permeability, which means that

it is not diffusion limited, but is to a large part flow limited (Comline & Silver, 1975; Doughty & Sibley, 1995).

Oxygen binding capacity of maternal and fetal bloods is another factor that determines placental transfer of oxygen (Battaglia *et al.*, 1969; Comline & Silver, 1975; Soothill *et al.*, 1988; Carter, 1989; Page, 1993; Rurak, 1994; Doughty & Sibley, 1995). This depends on the concentration of haemoglobin (determined by haematocrit) and haemoglobin affinity for oxygen. There is a higher concentration of haemoglobin in fetal (ca. 12 g dl^{-1}) blood than maternal (ca. 9 g dl^{-1}). However, of more significance is the greater affinity of fetal haemoglobin for oxygen. This is seen as a shift to the left of the fetal oxygen dissociation curve (Fig. 1.9). The Bohr effect also facilitates transfer of oxygen from mother (pH 7.47) to fetus (pH 7.37) (Comline & Silver, 1975; Page, 1993), despite the fact that the affinity of oxygen for haemoglobin is reduced as the blood becomes more acidic. The pH of the blood is closely related to PCO_2, therefore when placental gas exchange takes place there is an increase in maternal PCO_2 and a decrease in fetal PCO_2, i.e pH of fetal blood increases and that of maternal blood decreases, so a double influence of the Bohr effect.

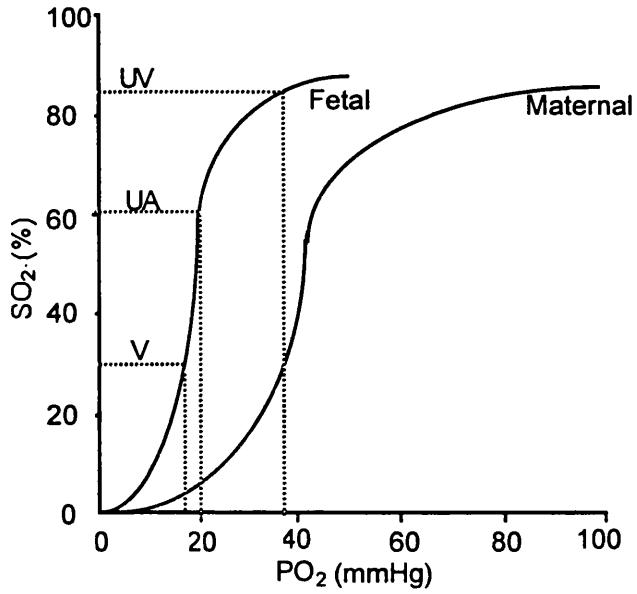

Figure 1.9. Oxygen dissociation curves for fetal and maternal blood in sheep. UV - umbilical vein, UA - umbilical artery, and V - systemic vein. (From Rurak, 1994).

The surface area at the maternofetal interface also determines oxygen exchange across the placenta (Rurak, 1994). With increasing gestation, as the oxygen demands of the fetus increase, there is an increase in villous growth (Wooding & Flint, 1994). Similarly, the surface area and density of capillaries in the terminal villi increase in response to hypobaric hypoxia (Reshetnikova *et al.*, 1993), and anaerobic conditions are associated with increased growth of placental fibroblasts (Wheeler *et al.*, 1995).

The driving force for oxygen diffusion is determined by the difference between maternal and fetal oxygen partial pressures (PO_2) (Comline & Silver, 1975; Rurak, 1994; Doughty & Sibley, 1995). Thus, the rate of diffusion can be expressed in terms of Fick's equation:

$$M = D\,(A/h)\,\Delta PO_2$$

where M is the diffusive flux, i.e. the amount of substance that diffuses through a layer with area A and thickness h per unit time, and D is the diffusion coefficient which depends on the diffusion medium and temperature. ΔPO_2 is the difference between maternal and fetal circulations in the partial pressure of the gas.

Finally oxygen consumption by the placenta itself limits transfer to the fetus. In the sheep, about 40% of the oxygen delivered in the uterine arteries is consumed by the placenta.

With increasing gestational age there is an increase in the materno-fetal oxygen transfer across the placenta (Battaglia *et al.*, 1965). This is facilitated by the increase in uterine blood flow (Mostello *et al.*, 1991).

Carbon dioxide

Transport of carbon dioxide relies on many of the same principles as apply for oxygen. However, most of the carbon dioxide in the blood is carried as bicarbonate:

$$CO_2 + H_20 \xleftrightarrow[\text{anhydrase}]{\text{carbonic}} H_2CO_3 \leftrightarrow HCO^{3-} + H^+$$

Therefore, it may cross the placenta in this anionic form. It has been shown, however, that the amount crossing the placenta in the form of the bicarbonate ion is insignificant, due to the small concentration difference between

maternal and fetal circulations and the fact that bicarbonate ion (negatively charged and lipophobic) has a low permeability across the placental membranes (Page, 1993; Rurak, 1994). Thus, most diffusion of carbon dioxide is in the gaseous form. The fetal haemoglobin dissociation curve for carbon dioxide is to the right of the maternal curve i.e. maternal haemoglobin has a higher affinity for carbon dioxide than does fetal (Haldane effect), which augments the transfer from fetus to mother. The placenta also contains carbonic anhydrase which speeds the conversion of HCO_3^- to CO_2.

1.3.1.ii Carbohydrates

Glucose

The main carbohydrate to be transported by the placenta is glucose and this occurs by a process of facilitated diffusion, by way of membrane transport proteins (Simmons *et al.*, 1979; Battaglia & Meschia, 1988; Page, 1993; Doughty & Sibley, 1995). The glucose concentration in maternal blood is greater than in fetal blood, so transfer can occur without input of energy because the driving force is the concentration gradient (Battaglia & Meschia, 1988; Doughty & Sibley, 1995). Thus, as well as the transport characteristics (surface area, direction of blood flow, etc.) of the placenta that were discussed above in relation to oxygen, transfer of glucose across the placenta depends on maternal and fetal blood glucose concentrations, which are determined by the magnitude of the gradient, placental blood flow, and glucose transporters.

To date, 5 different facilitative glucose transporters have been characterised, which are GLUT 1 -5, named in order of their cloning (Zhou & Bondy, 1993; Boyd & Kudo, 1994). Uptake and transfer of glucose by the placenta is mediated by GLUT-1 and GLUT-3 (Zhou & Bondy, 1993; Hay, 1996). Both GLUT-1 and GLUT-3 increase over the second half of gestation , when there is also an increase in placental glucose transfer (Hay, 1996), suggesting that glucose transfer is limited to some extent by transporter number. It is also interesting to note that elevated maternal IGF-I levels result in altered placental expression of GLUT-1 (Gluckman, 1995).

Transfer of glucose across the placenta is slower in sheep than in man. This may be due to differences in placental morphology (Page, 1993), the sheep having an epitheliochorial placenta and man a haemomonochorial placenta i.e. the sheep has a greater number of tissue layers between maternal and fetal

bloodstreams. Insulin does not appear to affect directly glucose transfer across the placenta (Battaglia & Meschia, 1988; Doughty & Sibley, 1995).

Lactate

Lactate, the second most important carbohydrate supplied to the fetus after glucose, is produced in large amounts by the placenta (Burd *et al.*, 1975). It is released into both maternal and fetal circulations, with 65-75% going to the fetus. Lactate concentrations are greater in fetal than maternal blood which means that the kinetics governing its transport across the placenta are not just simple diffusion. Moll *et al.* (1980) suggested that lactate transport across the placenta occurs by facilitated diffusion and is coupled with proton transfer. More recently it has been postulated that there may be a specific lactate carrier system, but little is known about how it functions. (Fowden, 1994).

Moll *et al.* (1980) found no effect of placental lactate metabolism on its transfer across the placenta.

1.3.1.iii Lipids

Lipids cannot cross the placental barrier, however they are catalysed by lipoprotein lipase into free fatty acids and glycerol, all of which are lipophilic molecules and cross the placenta by simple diffusion (Battaglia & Meschia, 1988; Page, 1993). There is only a limited amount of fatty acids transported across the sheep placenta (Elphick *et al.*, 1979). Generally, those species whose placentae are relatively impermeable to free fatty acids give birth to offspring with relatively little body fat (e.g. pig, sheep). But, it does not necessarily follow that those species whose placentae are relatively more permeable to fatty acids give birth to comparatively fat offspring. There are those species that do have newborns with alot of fat (e.g. human, guinea pig) and some do not (e.g. rat, rabbit). Generally, epitheliochorial placentae are less permeable to free fatty acids than haemochorial placentae (Battaglia & Meschia, 1988).Maternal supply is an important source of fatty acids to the fetus, though in those species studied (sheep, pig, human) there is a significant degree of fatty acid synthesis by the placenta. (Page, 1993).

1.3.1.iv Proteins

The human placenta has a low permeability to proteins, thus they cross at a very slow rate. They are transported by receptor mediated endocytosis, either bound to specific receptors in the endocytotic vesicle membrane, or non-selectively in the fluid within the vesicle (Wooding & Flint, 1994; Page, 1993; Doughty & Sibley, 1995). The most important protein to cross the placenta from mother to fetus is immunoglobulin G (IgG) which is important for immunoprotection of the human fetus. Most IgG transport occurs during the last trimester of pregnancy and at term maternal and fetal concentrations are roughly equal. (Doughty & Sibley, 1995; Page, 1993).

1.3.1.v Amino acids

The concentration of amino acids is higher in fetal than in maternal plasma and higher in placental tissues than in either fetal or maternal blood (Doughty & Sibley, 1995; Page, 1993). This is because they are 'actively' concentrated in the cytosol of the trophoblast (Hay, 1996). Amino acids are conveyed across the placenta by active transport using a number of different transport systems. Thus amino acid transfer across the placenta is affected by blood flow rates as for other substrates, transporters, energy supply, fetal and maternal concentrations, and placental metabolism

The transporter proteins are located in the microvillous membrane (Hay 1996). Some are sodium-dependent, e.g. systems A, ASC, X_{AG}, N, β, but there are others, e.g. systems L and y^+, that are not (Page, 1993; Hay 1996). The amino acid transporter proteins are not specific, so any one amino acid may be carried by a number of different transporters (Boyd & Kudo, 1994), thus there is competition for transporters. Transporter affinity and capacity also determine the rate of transfer (Hay, 1996).

The placenta itself requires amino acids for growth. It metabolises amino acids by oxidation e.g. glutamate, transamination, deamination and protein synthesis (Page, 1993; Boyd & Kudo, 1994, Hay 1996), both for its own use and for the fetus.

1.3.1.vi Water

A large proportion of the weight gain by fetus and placenta throughout gestation is due to the acquisition of water from the mother, the net flux of

water to the fetus being greater than that of any other substance. However, little is known of either the route or the forces by which water crosses the placenta. It is likely that diffusion is the main process, thus osmotic pressure gradients will provide the force (Boyd & Kudo, 1994; Doughty & Sibley, 1995). Transplacental fluid flow is about 30 ml/day in the late-gestation fetal sheep (Brace, 1996).

Water is taken up by the fetal placenta from the amniotic fluid also, so-called intramembranous flow. In the late-gestation fetal sheep it has been estimated that about 300 ml/day of amniotic fluid is taken up into the fetal blood by this way. However, nothing is known of the regulatory processes involved. The fetus also swallows a large amount of amniotic fluid - 1000 ml/day (Brace, 1996).

1.3.1.vii Electrolytes

In the human, fetal plasma concentrations of sodium, potassium, chloride, calcium, magnesium and phosphate are higher than in maternal blood. Such concentration gradients may be due to electrical potential differences across the placenta or to active transport mechanisms. Different views exist, however, as to the maternofetal potential difference across the placenta, so the contribution of the fetomaternal ionic concentration gradient to diffusion of electrolytes is not certain. Evidence does exist for the active transport of sodium, potassium, calcium, magnesium, phosphate and chloride, though it is thought that a large proportion of transplacental sodium transfer occurs by diffusion (Doughty & Sibley, 1995).

1.3.1.viii Vitamins

Transport of the vitamins depends upon whether the vitamin is water soluble or not. Generally, there is a higher maternal plasma concentration of the water insoluble vitamins and a higher fetal plasma concentration of those that are water soluble. Vitamins A, D, E and K are insoluble in water and cross the placenta by simple diffusion bound to proteins. Of the water soluble vitamins, vitamin B6, folate and biotin cross by simple diffusion, though folate crosses in association with specific binding proteins. Transfer of vitamin B6 and folate is asymmetrical with materno-fetal transfer being higher than in the opposite direction. B12 is protein bound and is actively transported by receptor mediated endocytosis involving specialised carrier proteins. Vitamin

C transfer is by active transport and is probably sodium dependent. (Page, 1993).

1.3.1.ix Minerals

Knowledge regarding the transport of minerals across the placenta is somewhat limited. Most is known about iron and zinc and very little about the other trace elements (Page, 1993).

The levels of iron in its ionic form are very low in both maternal and fetal plasma, most being incorporated in the proteins haemoglobin, myoglobin or ferritin. In the human plasma iron concentration is higher in fetal than maternal blood, so transfer across the placenta occurs against an uphill gradient. Placental uptake occurs by binding to the protein transferrin. First, the maternal transferrin binds to receptors on the maternal side of the placenta, the iron is released into the cell, and the transferrrin is recycled to the maternal plasma (McArdle *et al.*, 1985). The iron may then be compartmentalised within the placenta itself or be released into the fetal circulation. The materno-fetal transfer of iron increases with increasing gestational age (Douglas *et al.*, 1971), though the placental uptake of iron seems to be under intrinsic control as, in the absence of a fetus iron is not returned to the maternal circulation (McArdle *et al.*, 1985). The increasing uptake with gestation may be due to a number of different factors such as increasing numbers of receptors per unit weight of protein, the increase in placental blood flow, increasing syncytiotrophoblast surface area and decreasing thickness (Page, 1993).

As with iron, there is an uphill maternal to fetal concentration gradient of zinc and in its free ionic form it is found in only very small concentrations, most being bound to proteins and a little to amino acids. Zinc, bound to α-2-macroglobulin or albumin, may be taken up by the placenta, in a process that is thought to be dependent on metabolism. Transfer from placenta to fetus is thought to occur by diffusion (Page, 1993).

Current opinion is that the transport of iodine across the placenta occurs by a process dependent on metabolism. Selenate, chromate and molybdate anions have been shown to inhibit sulphate uptake by the placenta in a competitive fashion, so it is thought that they may be transported by the sulphate transport system. (Page, 1993).

1.3.2 Hormonal functions

The placenta synthesises an array of different hormones, proteins and various growth factors that are important not only for the growth and development of the placenta itself, but for the maintenance of pregnancy and the growth and development of the fetus. The definitive physiological functions of many of these substances are not fully understood, however there is currently much research being directed at this area of physiology.

1.3.2.i Steroid hormones

Progesterone and the oestrogens are secreted by the placenta, as well as by various other maternal and fetal organs. There is much inter-species variation in terms of the time during pregnancy that they are produced and the amounts produced. (Conley & Mason, 1994).

Progesterone

Progesterone is synthesised from pregnenolone within the cellular endoplasmic reticulum. It is a 2-step process that involves oxidation and isomerisation.

Cholesterol -> Pregnenolone -> Progesterone

The actions of progesterone during pregnancy are important from the time that fertilisation occurs. Promotion of nutritive secretions from glands in the endometrium before implantation of the conceptus is induced by progesterone (and the oestrogens), and it is also necessary for keeping the uterus quiescent and non-contractile during pregnancy. Progesterone is important for the growth of the mammary glands during pregnancy, and it is thought that it may be important for protecting the fetus from immunological rejection by the mother (Page, 1993). Progesterone is also metabolised by the fetal adrenal to cortisol and aldosterone.

Placental synthesis of progesterone is detectable from as early as 5-6 weeks gestation in the human (Page, 1993) and is produced in sufficient quantities to be independent of the ovaries by 50 d GA in the sheep (Wooding & Flint, 1994). The binucleate cells in the materno-fetal syncytium are a site of progesterone synthesis in the sheep, but it is not known what proportion of total progesterone output by the placenta they represent. It is thought that the syncytial plaques may also be an important site of placental progesterone

production (Wango *et al.*, 1992). Products of arachidonic acid metabolism (e.g. PGE_2) may modulate the synthesis of progesterone in the placenta (Wango *et al.*, 1992).

Oestrogen

The pathways of placental oestrogen synthesis vary between species. The precursors to oestrogens in ruminants are pregnenolone and progesterone, in a pathway that involves the enzymes 17α-hydroxylase and C-17, 20-lyase. Humans, however, lack these enzymes, so produce oestrogens by aromatisation of androgens secreted by the fetal adrenals (Wooding & Flint, 1994; Conley & Mason, 1994).

SHEEP: Progesterone -> 17α-hydroxyprogesterone -> oestrogens

HUMAN: Androstenedione -> oestrogens

Oestrogens are important during the pre-implantation period, as they (with progesterone) influence the secretion of various uterine proteins and secretions, that are important for the nutrition of the blastocyst. They may, however, be regarded in general terms as growth hormones for the female reproductive organs - the fallopian tubes, cervix, vagina, breasts and uterus being target organs. They are also important mediators of pelvic and cervical relaxation, and for the induction of myometrial oxytocin receptors.

Oestrogens are synthesised by the placenta from the seventh week of pregnancy, in the human, and by 9 weeks the placenta is the principal source of these hormones. (Page, 1993). In the sheep, the rise in cortisol that occurs towards the end of gestation causes activation of 17α-hydroxylase and C-17, 20-lyase and thus increased oestrogen levels, which are important in the initiation of parturition.

11-β-hydroxysteroid dehydrogenase

Placental 11-β-hydroxysteroid dehydrogenase (11β-HSD) is of interest in the context of this thesis because of its implication in the aetiology of hypertension (Edwards *et al.*, 1993; Oka *et al.*, 1993; Seckl *et al.*, 1995).

11β-HSD catalyses the reversible conversion of cortisol to the inactive cortisone. (Rats have corticosterone not cortisol). Two forms of the enzyme have been identified, 11β-HSD1 and 11β-HSD2 (Seckl *et al.*, 1995; Burton *et al.*, 1996). 11β-HSD1 has a capacity for both 11-oxoreductase and 11β-

dehydrogenase activity, but 11β-HSD2 exhibits only 11β-dehydrogenase activity. 11β-HSD2 is thought to be the predominant isoform present in the placenta, but this is not certain (Burton *et al.*, 1996).

11β-HSD activity is not detectable in human cytotrophoblast cells *in vitro* after 4 hours (Shepherd, *et al.*, 1996), but is detectable after 3 days by which time the cells have started to form a syncytium (Shepherd & McGarrigle, personal communication). This finding is supported by Krozowski (1995) who has identified 11β-HSD only in the syncytiotrophoblasts of the human placenta. In the rat placenta it has been shown that 11β-HSD1 is present in the basal zone and that its expression does not change significantly in late gestation. It is also present in the labyrinthine zone, but at barely detectable levels at 16 d GA (term=21 d), after which it increases dramatically to term. The expression of 11β-HSD2 is present in the basal zone in moderate amounts and shows a large increase towards term, whereas in the labyrinthine zone it is present in large quantities and activity falls dramatically towards the end of gestation (Burton *et al.*, 1996). 11β-HSD expression has not been localised in the sheep placenta, though it is known to be expressed (Kim *et al.*, 1995). Its activity does not change significantly between 80 d and 140 d GA in the sheep (term=147 d), though there is the suggestion that it may decrease (Burton *et al.*, 1996).

11β-HSD activity is stimulated by oestrogens and inhibited by progesterone and excess ACTH.

1.3.2.ii Protein hormones

The protein hormones of pregnancy include chorionic gonadotrophin (CG) and the placental lactogens (PL).

Chorionic gonadotrophins

The chorionic gonadotrophins that have been identified include human chorionic gonadotrophin (hCG), monkey chorionic gonadotrophin and equine chorionic gonadotrophin, also known as pregnant mare's serum gonadotrophin (PMSG).

hCG is a member of the gonadotrophic group of hormones that includes leutenising hormone (LH), follicle-stimulating hormone (FSH) and thyroid stimulating hormone (TSH). The rising levels of oestrogens and progesterone during pregnancy suppress the secretion of LH and FSH, so hCG takes over

from them. hCG is important for prolonging the life of the corpus luteum during the first few weeks of pregnancy (and it has also been postulated to influence sexual differentiation of the male fetus (Conley & Mason, 1994; Wooding & Flint, 1994). It is secreted by the trophoblast layer of the developing blastocyst and may be detected from 10 days after conception (Allen, 1975).

PMSG is not produced by the blastocyst, but by endometrial cups, structures that are unique to the horse (Allen, 1975; Conley & Mason, 1994).

Placental lactogens

Placental lactogens have been characterised in the human (hPL), in rodents and in sheep, cattle and goats, and are found in both fetal and maternal circulations. hPL is synthesised by the trophoblast throughout pregnancy and released predominantly into the maternal circulation. Its main actions are that it increases lipolysis and inhibits glucose uptake in the mother, therefore increasing the supply of glucose to the fetus (Conley & Mason, 1994; Page, 1993). PL levels in the mouse have been found to be influenced by fasting (Conley & Mason, 1994). Data from the sheep suggests that PL may be a modulator of fetal growth, by partitioning carbohydrates between mother and fetus (Oliver *et al.*, 1992). It is interesting that in the human (Page, 1993), the mouse and in ruminants (Conley & Mason, 1994) PL production shows a positive correlation with placental tissue mass.

Inhibin and Activin

Recently inhibin and activin have been found to be synthesised in the placenta, in both the cytotrophoblasts and syncytiotrophoblasts. Most that is produced is secreted into the maternal circulation and there is an increase throughout pregnancy, particularly during the third trimester. Inhibin, together with oestradiol and progesterone, suppresses maternal FSH secretion, and it is thought that inhibin may be involved in parturition. It has been suggested that activin may be a signalling protein and growth factor during embryogenesis. Both inhibin and activin regulate placental synthesis of gonadotrophin-releasing hormone (GnRH), hCG, progesterone, and perhaps other hormones too. (France, 1996).

1.3.2.iii Neurohormones

Most of the hypothalamic peptide hormones are produced by the placenta. These include GnRH, thyrotrophin-releasing hormone (TRH), somatostatin, GH-releasing hormone, CRH, and the opioid peptides. They are produced by the cytotrophoblast cells and are active in the syncytiotrophoblast cells, where they regulate synthesis of the protein hormones and chorionic ACTH. (France, 1996).

1.3.2.iv Growth factors

Insulin-like growth factors

(The IGFs are described in greater detail in section 1.6.2.v).

IGF-I and IGF-II are both synthesised by the placenta as are IGF-1R and IGF-2R (Page, 1993). IGF-II mRNA can be detected in human cytotrophoblasts soon after implantation and IGF-I mRNA is detectable in the placenta in the second trimester (Chard, 1994). IGF-I mRNA has been shown to be present in the ovine uterus in early pregnancy, mainly in the stromal cells of the endometrium and the muscle cells of the myometrium. IGF-II mRNA was found mainly in the caruncles and in the stroma of the endometrium (Stevenson *et al.*, 1994). Placental growth seems to be dependent only on IGF-II, and it has recently been shown that IGF-II may stimulate proliferation and/or metabolism in the ovine placenta by binding to an, as yet, uncharacterised receptor (Reynolds *et al.*, 1996). IGF-I has also been seen to accelerate placental structural maturation as well as influencing the synthesis of human placental lactogen (hPL), oestrogen and progesterone, and glucose metabolism (Page, 1993).

Other growth factors

Receptors for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) have also been identified in the placenta, as has transforming growth factor β (TGFβ) in the bovine placenta (Munson *et al.*, 1996). Vascular endothelial growth factor (VEGF), which promotes angiogenesis, has been identified in the human placenta (Anthony *et al.*, 1994). Hepatocyte growth factor (HGF), also known as hepatopoietin A and Scatter factor, has been found in significant concentrations in the placenta (Hernandez *et al.*, 1992). HGF primarily acts on epithelial cells where it has a range of effects, from enhancement of cell proliferation and cell growth, to stimulation of cell

migration and motility, promotion of angiogenesis and morphogenesis, to inhibition of tumour cell growth. 100% of HGF-induced DNA synthesis and 25% of HGF-induced protein synthesis are inhibited by TGFβ1, and HGF and EGF interact in an additive manner (Hernandez *et al.*, 1992). In the rat, HGF activity in the placenta (when expressed per gram of tissue) has been shown to increase rapidly up to 9 d GA, after which it levels off (Jin *et al.*, 1993). HGF is essential for placental organogenesis. It has been demonstrated in mice that when the HGF gene is disrupted, placentas are severely impaired with a substantial reduction in the number of labyrinthine trophoblast cells, and the fetuses die before birth (Uehara *et al.*, 1995). Furthermore, Uehara *et al.* (1995) showed *in vitro* that the growth of trophoblast cells is stimulated by HGF, and its actions are believed to be paracrine, because HGF mRNA is not expressed in cytotrophoblasts but the protein *c-met*, which is a receptor for HGF is found in cytotrophoblasts (Saito *et al.*, 1995).

The colony stimulating factors (CSF) have been detected in the placenta and CSF-1 is thought to induce proliferation and differentiation of the trophoblast, and to regulate macrophages (Robinson *et al.*, 1995; Sharkey *et al.*, 1994). Kit-ligand (KL) is another cytokine that has been identified in the trophoblast of both human and mouse and seems to be involved in regulating components of the immune system (Sharkey *et al.*, 1994). Other cytokines that have been detected in the placenta include various interleukins (IL) that are essential for implantation (Robinson *et al.*, 1995) and may be involved in regulating trophoblast development (Sharkey *et al.*, 1994).

(Growth factors are discussed further in relation to fetal growth in section 1.6.2.vi).

1.3.2.v Cytokines

The placenta also produces cytokines which are growth factors that regulate movement within and between cells. They have been suggested to play a role in the development of the embryo and fetus (Robinson *et al.*, 1995).

1.4 PLACENTAL GROWTH RATE

The normal placenta grows and gains in weight throughout gestation, though the cellular events occurring during this growth and the rate of growth are not uniform throughout. Over the course of pregnancy in the rat (term 21 days) its placenta shows three phases of growth, hyperplasia, hyperplasia and hypertrophy, and hypertrophy alone. Placental growth in the human (term about 40 weeks - 280 days) has been shown to be similar. Between 25 and 36 weeks gestation it has been demonstrated that hyperplasia is occurring, then for the last month of pregnancy, after about 36 weeks, placental growth is by hypertrophy alone (Winick *et al.*, 1967). The most rapid period of placental growth is during the first half of pregnancy (Fig. 1.10).

Placental growth is not easy to measure, particularly in animals such as the sheep, which has a cotyledonary placenta. Usually placental size is determined either at delivery, in humans, or at post-mortem, in experimental animals, by weighing. Placental growth is assessed by killing animals at different gestational ages. However, measurements of placental volume have been made during mid-pregnancy in the human using ultrasound (Howe *et al.*, 1994). In the sheep measurements of placentome diameter have been made between 45 d and 141 d GA using ultrasound (Kelly *et al.*, 1987). But, the technique can only give mean placentome diameter and it does need further validation.

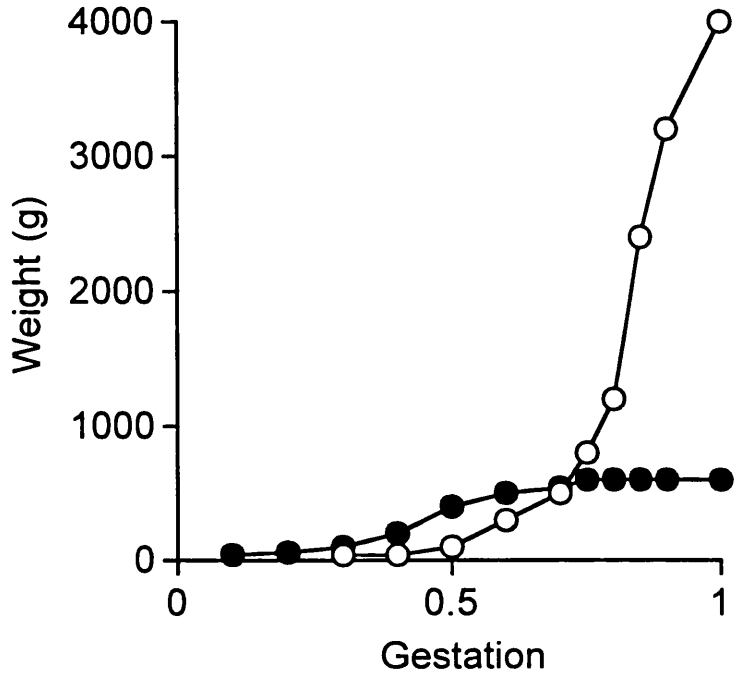

Figure 1.10. Ontogeny of fetal (o) and placental (●) growth. Adapted from Page (1993) - human, and Owens (1995) - sheep.

1.5 FETAL GROWTH RATE

Conversely to the placenta, the period of maximal fetal growth is during the second half of pregnancy, particularly late gestation (Owens *et al.*, 1995). (Fig. 1.10)

Technological limitations mean that it is difficult to measure fetal growth in early gestation, due to the small size of the fetus. In experimental animals fetal growth has been measured by killing animals at different gestational ages. The limitation of this method is that longitudinal measurements of growth within individual animals are obviously not possible. Mellor and Matheson (1979) developed a technique for measuring fetal growth during late gestation using what they called a CRL measuring device. This consisted of a nylon monofilament threaded through a sleeve of transparent polyethylene tubing which was sealed at one end. The open end of the device was attached at the rump and the monofilament was tunnelled sub-cutaneously along the spine of the fetus to the crown where it was secured, ensuring that the nylon thread

was fully inserted into the tubing, which was then exteriorised through the ewe's flank. Growth of the fetus caused the nylon to be drawn out of the polyethylene tube. Measurement of the distance between the end of the sealed tube and the nylon added to CRL measured at surgery gave the CRL on any given day. Using this device curves of growth in the fetal sheep between 100 and 140 days gestation were obtained. This technique has also been implemented more recently to look at the effect of various factors (e.g. cortisol) on fetal growth during late gestation (Fowden, 1995). Longitudinal measurements of fetal growth are also available for the human fetus, growth being assessed by serial ultrasound measurements (Robson & Chang, 1995). It is possible to measure CRL by ultrasound in the human from 6 weeks gestation. Recently Anjari *et al.* (1996) have successfully measured growth of alpaca fetuses from as early as 33 d GA (term=345 d) using ultrasound.

1.6 DETERMINANTS OF FETAL GROWTH

Nutrient supply, endocrine status, genetic make-up, and various environmental factors all influence fetal growth. It is difficult to quantify the extent to which these different factors determine the growth of the fetus, however it is possible to indicate their relative importances. For example, without oxygen the fetus would die, therefore oxygen is essential for growth. However, adrenalectomised fetuses are able to survive (Fowden *et al.*, 1990), therefore cortisol is not essential for growth and survival of the fetus. Likewise, thyroidectomised fetuses survive but are growth retarded (Fowden & Silver, 1995), which suggests that the thyroid hormones are necessary for normal growth, but are not essential.

Requirements for fetal growth	Importance
Oxygen	↑↑↑
Glucose	↑↑↑
Lactate	↑↑↑
Fructose	↑
Amino acids	↑↑↑
Lipids	↑↑↑
Insulin	↑↑↑
Thyroid hormones	↑↑
Cortisol	↑
Growth hormone	↑
IGFs	↑↑↑
Other growth factors	↑↑
Genetic	↑
Maternal size	↑

Table 1.2. Relative importance of the various factors that determine fetal growth.

1.6.1 Nutrition

Nutrient supply to the fetus is the major regulator of fetal growth. There are three different sources from which the fetus may receive nutrients: the maternal circulation, substances synthesised by the placenta, endogenous production by the fetal tissues. Under normal circumstances the maternal pool is the primary source of nutrients for the fetus (Fowden, 1995). The main nutrients required for fetal growth are oxygen, glucose, lactate, amino acids and lipids.

1.6.1.i Oxygen

PaO_2 values in the mother are higher (aorta: 95 mmHg) than those of the fetus (umbilical vein: 35 mmHg), which facilitates diffusion of oxygen from

mother to fetus. The rate of oxygen uptake by the placenta is about 50% greater, per gram of tissue, than that of the fetus, which significantly reduces the amount of oxygen that eventually reaches the fetus (Fowden, 1994 & 1995). Oxygen affinity is higher in the fetal than in the maternal blood and tissue perfusion is relatively high, so adequate supply of oxygen to the fetal tissues is ensured. Oxygen consumption by the fetal carcass (skin, bone, muscle) is highest, about 50% of total oxygen consumption, and the heart, brain and liver together make up 35-40% of total oxygen consumption, the heart consuming almost double that of the other two organs on a weight-specific basis (Fowden, 1994).

1.6.1.ii Carbohydrates

Glucose and lactate are the major carbohydrates metabolised by the fetus, though some species (ruminants, pig, horse) also utilise fructose (Fowden, 1994).

Glucose

Glucose is the main substrate for oxidation *in utero*. Maternal and fetal blood glucose concentrations and the ratio between the two vary from species to species. Typical values measured in the sheep are 2.25-3 mmol/l in the mother and 0.75-1.25 mmol/l in the fetus. The maternal to fetal concentration gradient means that facilitated diffusion of glucose across the placenta occurs. As with oxygen, a large amount of the glucose leaving the maternal circulation is consumed by the placenta, 60-75% of the total uterine glucose uptake in late gestation (Fowden, 1995). The glucose taken up by the placenta may either be oxidised or may be used for lipogenesis, glycogenesis and conversion to lactate, fructose and amino acids, some of which may be released into the umbilical circulation. The fetus can also produce glucose endogenously by glucogenesis in the liver and kidneys, though this usually only occurs in adverse conditions. The carcass utilises most of the glucose supplied to the fetus, though the brain also consumes fairly large amounts (15%). (Fowden, 1994 & 1995).

Lactate

The second most important carbohydrate fuel in the fetus is lactate. It is produced in large quantities by the placenta as a result of the breakdown of glucose (both maternal and fetal in origin) and fructose (fetal), and is released

into both maternal and fetal circulations. Its production is affected by fetal, but not placental, metabolism. There is also endogenous production of lactate by the fetal tissues at almost double the rate of that produced by the placenta. Most endogenous lactate is derived from glucose, though some may be formed from fructose and amino acids. 70% of the lactate consumed by the fetus is oxidised to CO_2 and the rest contributes to the fetal carbon pool. A large proportion (30%) of the lactate taken up by the fetal organs is utilised by the heart and liver. Although some lactate is produced anaerobically, generally it does not imply anaerobic metabolism in the fetus. In stressful conditions, lactate may be a substrate for glucogenesis by the liver (Fowden, 1994 & 1995).

Fructose

Fructose is not an important carbohydrate for metabolism in the fetus. It is produced at a very low rate from glucose in the placenta of the sheep. Only very small amounts are oxidised and it may be converted to lactate. (Fowden, 1994).

1.6.1.iii Amino acids

In the fetus amino acids are essential for oxidation, protein accretion, and as a source of carbon and nitrogen. Concentrations are higher in the fetal than in the maternal circulation and higher in the placenta than in either, therefore transport is an active process. The essential amino acids must come from the mother, whilst non-essential amino acids may be synthesised either by the placenta or endogenously by the fetus. Therefore, the supply of some amino acids to the fetus is dependent on placental metabolism as well as tranplacental transport (Battaglia, 1992; Fowden, 1994 & 1995).

1.6.1.iv Lipids

Lipids are essential for fetal growth and they may play a minor role in oxidative metabolism. The source of free fatty acids may be maternal, *de novo* synthesis by the feto-placental tissues, or the breakdown of triglycerides and phospholipids. It is thought that the triglycerides are produced endogenously. (Fowden, 1994 & 1995).

1.6.2 Endocrine and paracrine control of fetal growth

Fetal growth involves not only supply of substrates to the fetus, but is a complex interaction between nutrition and various endocrine and paracrine regulators.

Growth during childhood is known to be dependent on a wide variety of hormones, in particular GH and the IGFs, thyroid hormone, insulin, glucocorticoids, and the sex steroids.. However, the endocrine regulation of growth in the fetus is much less clearly defined. Maternal hormones that are involved in the regulation of growth, e.g. GH, thyroid hormones, insulin, and steroid hormones do not cross the placenta in concentrations that are enough to influence fetal growth. Therefore, the hormones regulating fetal growth seem to be contained within the fetus itself. (Fowden, 1995; Han & Hill, 1994; Han & Fowden, 1994).

1.6.2.i Insulin

Insulin is essential for fetal growth, and is first detected at 11 weeks GA in the human fetus, 42 d GA in the sheep, and 18 d GA in the rat (Parkes, 1988). The action of insulin in allowing the cellular uptake of glucose and amino acids in part explains the role of insulin in fetal growth. However, it is also thought to modulate growth by its actions on the IGFs (the IGFs and fetal growth are discussed in section 1.6.2.iii). It enhances IGF-I secretion and at high concentrations can bind to IGF-1R, as well has having an inhibitory effect on IGFBP-1 production. Thus, it is likely that insulin does not have a direct effect on fetal growth, but regulates other factors that do. (Han & Fowden, 1994; Han & Hill, 1994; Bassett, 1995; Milner & Gluckman, 1996).

1.6.2.ii Thyroid hormones

The hypothalamic-pituitary-thyroid axis is active from 10 weeks GA in the human fetus (Milner & Gluckman, 1996), 50 d GA in the sheep and 19 d GA in the rat (Parkes, 1988). Triiodothyronine (T_3), the active form of thyroid hormone, promotes fetal growth by increasing protein synthesis, and by its synergistic action with GHRH in the secretion of GH and the IGFs. It has been suggested that increased thyroxine (T_4) levels may cause an increase in IGF-1 and asymmetrical growth retardation is observed in hypothyroid sheep (Han & Fowden, 1994). Fetal thyroid function is significantly affected by

nutrient availability. Reduced substrate supply, e.g. due to maternal starvation, uterine artery occlusion, etc., leads to a decrease in plasma T_4 concentrations and IUGR (Symonds, 1995).

1.6.2.iii Cortisol

Cortisol concentrations are low (about 10 ng ml^{-1}) for most of gestation in fetal sheep, then start to rise gradually 10-15 days before birth (up to about 20 ng ml^{-1}), with a final surge 3-5 days before birth (up to about 70 ng ml^{-1}) (Fowden, 1995). Cortisol is maternal and placental in origin, as well as from the fetal adrenal. Cortisol is thought to be a modulator of fetal growth because there is a decline in fetal growth concomitant with the rise in cortisol towards the end of gestation, and adrenalectomised fetuses are heavier than intact fetuses. However, the mechanisms by which cortisol may affect growth are not known. Postnatally, cortisol has an antagonistic effect on insulin and down-regulates IGF gene expression.

Cortisol is known to be involved in the maturation of various organs perinatally. In the lung it is involved in the manufacture of pulmonary surfactant (Han & Fowden, 1995; Milner & Gluckman, 1996). In the gut it is important for villi proliferation and the induction of digestive enzymes (Fowden, 1995). In the liver it is involved in the maturation of glycogen synthetase which is needed for the accumulation of hepatic glycogen during the last trimester, and it also induces β receptors and hepatic gluconeogenic activities, which are important for the supply of glucose to the neonate after birth (Fowden *et al.*, 1990; Fowden *et al.*, 1992; Fowden *et al.*, 1995; Fowden, 1995). These effects on tissue maturation may in part be due to the effects of cortisol on IGF production. In the ovine fetus, liver and adrenal gene expression of IGF-II is depressed, expression of IGF-I is enhanced in the liver, and hepatic GH receptor mRNA is increased (Fowden 1995).

1.6.2.iv Pituitary hormones

Growth hormone

GH is found in the fetal circulation from at least 12 weeks gestation in the human. It reaches levels of around 100 ng ml^{-1} during mid-gestation, which is higher than at any other time during life (Han & Fowden, 1994; Milner & Gluckman, 1996). Until recently it was thought that GH was not a major determinant of fetal growth. However, it is now thought to be involved in the

control of fetal growth through its actions on the IGFs. GH deficiency is accompanied by a decrease in IGF-I levels, and GH is thought to be responsible for the production of IGFBP-3 during late gestation (Milner & Gluckman, 1996).

1.6.2.v Insulin-like growth factors (IGFs)

The IGFs, IGF-I and - II, are polypeptide hormones which structurally and functionally are similar to insulin. They have both anabolic and mitogenic effects by which they promote growth. The primary source of IGF-I is the liver, but all tissues produce both IGF-I and -II. (Gluckman, 1995; Blum & Gluckman, 1996). Their actions are modulated through IGF-binding proteins (IGFBP) to which they are bound in the plasma, and of which six have so far been identified, IGFBP-1 - 6. IGF-II binds specifically to IGFBP-2 and IGFBP-6, and the other IGFBPs show similar affinity for either IGF-I or -II (Blum & Gluckman, 1996). The IGFs also have specific cell membrane receptors, type 1 IGF receptors (IGF-1R) and type 2 IGF receptors (IGF-2R). IGF-I binds to IGF-1R, and IGF-II can bind to both IGF-1R and -2R. The IGFs also interact with 2 other receptor types, the so-called 'hybrid receptor' and the insulin receptor (Gluckman, 1995). The principal sites of IGF-I synthesis are muscle and liver, however, all tissues express mRNA for both IGF-I and -II.

Regulation of the IGFs is complex. The most potent regulator of IGF-I is growth hormone (GH), though prolactin, TSH, LH (or hCG) and FSH have also been seen to stimulate IGF-I production. Low doses of oestrogens cause an increase in plasma IGF-I, whereas pharmacological doses cause a decrease, except in the uterus where IGF-I expression is stimulated by high oestrogen doses. The glucocorticoids also have an effect on IGF-I. They do not affect IGF-I expression, but seem to inhibit its effects. There are a number of other hormones that influence IGF-I synthesis. Parathyroid hormone causes an increase in IGF-I concentration in bone, but not in plasma, and insulin has a permissive effect on GH-stimulated IGF-I production. platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor α (TGFα), and basic fibroblast growth factor (bFGF) stimulate local IGF-I production, whilst TGFβ is both stimulatory and inhibitory. After GH, nutritional status is the second most important regulator of IGF-I concentration. Both protein and calorie intake have an influence, and a decrease in intake results in a decrease in IGF-I.

Less is known about the regulation of IGF-II stimulation than about IGF-I. However, it has been observed that GH causes an increase in brain, skeletal and cardiac muscle, but not hepatic levels. ACTH and FSH have also been seen to stimulate IGF-II production (Blum & Gluckman. 1996). Reduced nutrient intake causes a decrease in IGF-II, but to a lesser degree than IGF-I (Gluckman, 1995).

It appears that early during fetal development IGF-II is critical for the regulation of growth, but in later gestation IGF-I is important (Gluckman, 1995). "Knockout" models have provided evidence for the roles played by the IGFs in fetal growth. Mice that have had the IGF-I gene knocked out by homologous recombination have severe growth retardation, but growth retardation does not become evident until 13.5 d GA, supporting the idea that IGF-I is important for growth only in late gestation. IGF-II knockout mice display growth retardation (and reduced placental growth) from 11 d GA and resume a normal growth rate after 18 d GA, which suggests that IGF-II plays a role in fetal growth early on in gestation but that IGF-I takes over later (Gluckman, 1995; Milner & Gluckman, 1996). Clearly then the IGFs have a substantial impact on placental and fetal growth and development, however their regulation of growth is complex and is not fully understood. The growth-promoting actions of both IGF-I and -II are thought to be mediated via the IGF-1R, however it appears that the actions of IGF-II are also mediated by another as yet unknown receptor type. That IGF-1R is important in mediating the actions of IGF-I and IGF-II is demonstrated by IGF-1R knockout mice, which are more growth retarded than IGF-I or IGF-II knockouts. IGFBP-1 is associated with reduced fetal growth, perhaps because it reduces the availability of IGF-I. The actions of the IGFs are somatogenic, anabolic and anti-catabolic, and generally IGF-I is more potent than IGF-II. Their roles during fetal development are multiple, including the regulation of embryogenesis, placental growth, fetal and placental metabolism, and organ growth and differentiation (Gluckman, 1995). The actions of the IGFs are both paracrine and endocrine.

1.6.2.vi Other growth factors

Most growth factors act in an autocrine or paracrine fashion, though some may be endocrine, intracrine or juxtacrine (Han & Fowden, 1994).

Apart from the IGFs there are a plethora of other growth factors that are involved in the regulation of fetal growth. Epidermal growth factor (EGF), transforming growth factor α (TGFα) and platelet-derived growth factor (PDGF) are mitogenic in mesenchymal cells, and EGF also has proliferative effects on epithelia. There are various fibroblast growth factors (FGF), the most abundant of which is basic FGF. Basic FGF is a potent mitogen in connective tissue and is extremely angiogenic. TGFβ exists in various isomeric forms. It inhibits epithelial cell proliferation, but is also involved in the formation of basal membranes where it causes fibroblasts to increase their production of fibronectin and collagen. Nerve growth factor (NGF) promotes axonal growth, differentiation and survival of sympathetic ganglia (Engström & Heat, 1988; Han & Fowden, 1994; Hill & Han, 1996). Together these factors promote cellular proliferation, differentiation, induction, migration, aggregation, maintenance, regeneration and apoptosis (programmed cell death).

1.6.3 Other influences on fetal growth

1.6.3.i Genetic determination

Certainly growth is in some part genetically determined, which is evident from the fact that within particular species there is a similar birthweight and similar adult height, e.g. in man birthweight is about 3500 g and adult height is about 1.7 m. However, there is variation from one individual to another which is in part genetic variation, but also reflects environmental influences (Yates, 1988). There is interaction between maternal and paternal genetic contribution, which is necessary for normal fetal growth. For example, it is essential that the IGF-II gene is paternal and the IGF-2R gene maternal. Over- and under-expression of the IGF-II gene result in overgrowth and dwarfism, respectively (Milner & Gluckman, 1996).

1.6.3.ii Environmental factors

Maternal size

During life *in utero*, growth is strongly influenced by the mother, and paternal genotype is relatively unimportant, so long as expression is normal (as described above in section 1.6.3.i). After birth, the genetic makeup of the offspring is more important than environmental influences. The influence of maternal size was very elegantly demonstrated by the experiments of Walton

and Hammond in 1938, where they crossed Shire horses with Shetland ponies. They found that when either a Shire or a Shetland stallion was crossed with a Shire mare the newborn foals were of similar size and were quite large. When the mare was a Shetland, again the newborn foals were similar in size, regardless of whether Shire or Shetland stallion, but were small (Yates, 1988).

Maternal nutrition

Maternal nutrient intake primarily determines the nutrient supply to the fetus. It follows, therefore, that if maternal nutrient supply is restricted it will result in fetal nutrient restriction also. The effect of maternal undernutrition on fetal growth depends on the specific nutrients that are in short supply, timing, duration, and severity. The fetus requires 95 kcal $(kg.day)^{-1}$, of which 40 are required for growth and 55 are oxidised (Milner & Gluckman, 1996). The specific effects of maternal undernutrition on fetal growth are described and discussed later in this chapter, section 1.7.2.

1.6.3.iii Placenta

The placenta is the interface between mother and fetus, and is thus the route by which nutrients are supplied to the fetus and waste products removed, as well as the various endocrine functions it performs that are essential for fetal growth. I have described in some detail the importance and the influence of the placenta for fetal growth (section 1.3).

1.7. INTRAUTERINE GROWTH RETARDATION (IUGR)

There are a variety of different measurements by which fetal growth outcome is described. In the human infant, commonly used measures include birth weight, length, head circumference, and body proportions derived therefrom e.g. ponderal index (weight (kg) x 100 / length $(m)^3$), birth weight to length ratio. In animals the same measurements may be made, though it is also possible to kill the animal and record organ weights.

1.7.1 Categories of IUGR

IUGR has been described by many different terms by different people e.g. Type 1, Type II; proportionate, disproportionate; chronic fetal distress,

subacute fetal distress. However, despite the extensive number of different names that have been used to describe the phenotype and outcome of IUGR, it is apparent that there are two main types: primary IUGR and secondary IUGR (Fay & Ellwood, 1993). Fay & Ellwood (1993) reviewed the human literature between 1977 and 1991 in order to distinguish these two types of growth retardation.

1.7.1.i Primary IUGR

Primary IUGR infants are small for gestational age (below the 10th centile) but with proportional anthropometric measurements for their weights. So they are symmetrically growth retarded. It was suggested that the cause of IUGR in these babies is inherent to the fetus (metabolic) as they seem to be well nourished and generally there is no evidence of asphyxia. It may also be that they are genetically growth restricted (Fay & Ellwood, 1993). Primary IUGR is not associated with increased perinatal morbidity and mortality, though mental development scores are significantly lower than those of appropriately grown infants and there is the suggestion that these infants are at greater risk of spastic cerebral palsy (Fay & Ellwood, 1993; Owens *et al.*, 1995). Up to 4 years of age primary IUGR infants show no evidence of catch-up growth, remaining smaller and lighter than their appropriately grown counterparts (Fay & Ellwood, 1993).

1.7.1.ii Secondary IUGR

Secondary IUGR infants show a pattern of asymmetrical growth. Their birth weights may be normal, but they are relatively thin (wasted) and long, so have a low ponderal index. Pregnancies resulting in secondary IUGR have been associated with small or abnormal placentae, high resistance umbilical and uterine artery Doppler waveforms, and hypoxaemia. Fetal distress *in utero* is common and there is a high rate of perinatal asphyxia. Secondary IUGR is associated with higher rates of perinatal morbidity and mortality, infants appear malnourished, being thin with little subcutaneous fat, and their livers, spleens, adrenals and thymuses are reduced in size, whilst their brains are relatively large (Fay & Ellwood, 1993). Infants with secondary IUGR exhibit catch-up growth, but have higher incidences of mental retardation and learning disabilities (Fay & Ellwood, 1993).

1.7.2 Causes of IUGR

Despite this dichotomous classification of body proportionality, the way in which fetal growth is defined has not been so easy to determine. Pregnancy outcome is a matter that has always interested man and there have been a large number of studies carried out in an attempt to improve the prognosis for the unborn child. McCance & Widdowson (1974) and Osofsky (1975) reviewed data from the preceding 50 years or so in an attempt to gain insight into the factors that are important for growth and development of the fetus and child. Whilst they both concluded that the factors determining growth are complex and there is much work to be done in unravelling the multifactorial elements concerned, it was clear that growth retardation depends upon not only the type of insult, but also on its severity, duration, and the time of exposure during development.

1.7.2.i Timing of insult

McCance & Widdowson (1974) made the statement that " ..there is a metabolic clock in every animal which determines when each organ shall begin and end its growth or its function, how the animal will behave at every age and when its life is getting near its end". It therefore follows that the outcome of an interruption in growth and/or function is determined by exactly when during the development of the animal that interruption occurs. The effect on an organ or system will be dependent upon the stage of development of the organ/system. This is defined by what has come to be known as 'critical' periods of development.

It has been suggested that primary growth restriction is the result of factors acting early in gestation, and that secondary growth restriction is a consequence of events later on in development (Barker, 1994; Owens *et al.*, 1995).

Studies of human pregnancies (Osofsky, 1975; Stein & Susser, 1975b; Kramer *et al.*, 1990) are extremely inconsistent. One observation was made of babies born to mothers who were subjected to near starvation by the Germans during the siege of Leningrad, which took place during World War

II from August 1941 to January 1943 (Antonov, 1947). This study showed no effect of early nutritional deprivation on fetal growth but a decline in birth weight with undernutrition in late gestation. Similarly, Stein & Susser (1975b) reported that early nutritional deprivation was not associated with impaired fetal growth, whereas nutritional deprivation in late gestation resulted in secondary growth retardation. Contrary to this, however, is the report that maternal weight gain prior to 28 weeks gestation affects anthropometric measurements at birth (Osofsky, 1975). To add to the discrepancies already seen, some studies carried out in the 1940s related maternal nutritional supplementation during pregnancy to improved fetal growth whilst others did not (Osofsky, 1975). It may be possible to account for the disagreements between studies on the basis of geography. Those studies that saw a benefit from supplementation were carried out in Toronto, England, Wales and Glasgow, and those that did not were carried out in Chicago, Philadelphia and Nashville. Another complicating factor is the food supplements that were used. In the study carried out in England and Wales a multivitamin milk supplement was used. The study in Chicago gave a protein supplement, whilst that in Philadelphia split the women into 3 groups, some receiving vitamin supplement, some protein, and some vitamin and protein. Another factor which may be significant for all these studies is that the women recorded their own food intake. Further observations have been carried out in Santiago, Chile on infants whose mothers were severely undernourished during pregnancy, resulting in fetal or neonatal death from malnutrition (Winick, 1969). Severe IUGR resulted, and there was up to a 40% reduction in brain cell number, which was also associated with reduced head circumference. Kramer (1990) challenged the view that early onset of growth retardation leads to symmetrical growth retardation, and related asymmetrical growth retardation to those babies with taller mothers, whose mothers had severe pregnancy-related hypertension, and to males.

McCance & Widdowson (1974) described studies conducted in neonatal rats from which it was concluded that undernutrition for a brief period earlier, rather than later, in neonatal life retards growth and also the subsequent rate of growth, despite unlimited amounts of food later on. Body proportionality was not affected. Osofsky (1975) agreed with the idea that growth was more profoundly and irreversibly affected the earlier following birth at which nutritional deprivation occurred. He also cited studies in pregnant rats which showed that both protein and caloric restriction resulted in fetal growth

retardation with decreases in brain weight (brain to body weight ratios were not given so it is not clear whether growth was symmetrical or not). Furthermore, and somewhat contrary to the data in neonatal animals, he described effects of a protein-free diet during pregnancy where the effects on brain growth were more severe the later in pregnancy deprivation occurred; presumably body weights were affected similarly.

There are a number of more recent studies in rats (Tanaka *et al.*, 1994; Desai *et al.*, 1995), guinea pigs (Detmer *et al.*, 1991) and sheep (Creasy *et al.*, 1972; Owens *et al.*, 1986; Jacobs *et al.*, 1988a; Block *et al.*, 1990; Kamitomo *et al.*, 1993; Harding & Johnston, 1995; Harding, 1995; Bauer, 1995; Murotsuki *et al.*, 1996) that describe the effects of substrate deprivation at certain times during pregnancy on fetal body proportionality (Table 1.3). DeBarro *et al.* (1992) restricted maternal nutritional intake in sheep during early pregnancy by altering grazing stock rates in pastures, and they found that there was a reduction in fetal growth. They did not specify the type of growth restriction observed - symmetrical or asymmetrical. Undernutrition in mid-gestation (food intake regulated to achieve a loss of 8 kg liveweight between 30 d and 96 d GA) has been reported not to result in growth restriction (McCrabb *et al.*, 1991). Some workers have investigated the effects of nutrient deprivation throughout mid and late gestation (Detmer *et al.*, 1991; Bauer *et al.*, 1995). Detmer *et al.* (1991), working in the guinea pig found that uterine artery ligation, which tended to result in decreased CaO_2, produced fetuses that were asymmetrically growth retarded; whilst Bauer *et al.* (1995) found that a mid through to late gestation nutritional insult (25% of the recommended energy and protein requirements) caused growth restriction which was symmetrical. Nutrient deprivation in late gestation whether in the sheep (Creasy *et al.*, 1972; Jacobs *et al.*, 1988a; Harding, 1995; Harding & Johnston, 1995; Murotsuki *et al.*, 1996) or rat (Tanaka *et al.*, 1994), and whether due to undernutrition (Harding, 1995; Harding & Johnston, 1995), placental embolization (Creasy *et al.*, 1972; Murotsuki *et al.*, 1996), hypobaric hypoxaemia (Jacobs *et al.*, 1988a), or uterine artery ligation (Tanaka *et al.*, 1994) always resulted in asymmetrical growth retardation. There was however an exception: Block *et al.* (1990) produced symmetrical growth retardation in fetuses where placental embolization had been carried out. This inconsistency, so far as fetal growth is concerned, may arise because they only embolized for 9 days as opposed to 21 days or more in the other embolization studies (Creasy *et al.*, 1972; Murotsuki *et al.*, 1996).

Finally, restricted nutrient supply for the whole of or most of gestation always caused secondary IUGR, regardless of species or method of nutrient restriction employed (Table 1.3).

The complexities surrounding the issue of timing are further emphasised by recent experiments in sheep (Harding & Johnston, 1995; Harding, 1995). It was found that periconceptual undernutrition caused a slowing of fetal growth that was not affected by a further nutritional insult in late gestation, although the added insult in late gestation did produce asymmetrical growth (small brains, big hearts and kidneys). These findings suggest that the early nutritional insult increased susceptibility to a second nutritional insult, and that there may have been an alteration in growth sequence or organ function (Harding & Johnston, 1995).

It is clear that the timing of a reduction in substrate supply is critical in influencing fetal body and organ growth. However, there is definitely no clear cut pattern from which we can say that an insult at a particular time will result in a particular pattern of fetal and placental growth. Perhaps the evidence so far is clouded by differences in the duration and severity of exposure.

1.7.2.ii Duration/severity

The work described by McCance & Widdowson (1974), showed studies in pigs where severe prolonged undernutrition in the early postnatal period resulted in severe asymmetrical growth retardation, whether the diet was one deficient in energy or protein. Rehabilitation onto a normal diet resulted in spectacular catch-up growth, though full size was never attained. They also quote similar results in undernourished rats. Osofsky (1975) cited a study where the decrease in brain weight was greater the longer the duration of protein deprivation. Thus, it would appear that the longer the period of stress, the greater the degree of IUGR, though it is unclear as to whether longer duration produces symmetrical or asymmetrical growth.

Undernutrition in early pregnancy for 20% of gestation in sheep did not affect fetal growth in one study (Harding & Johnston, 1995) but caused reduced growth in another (DeBarro *et al.*, 1992). The discrepancy between these two studies may be because DeBarro *et al.* (1992) recorded fetal weight at 90 d GA, whereas Harding & Johnston (1995) measured fetal body and organ weights at 125 d GA. Undernutrition for 30% of gestation in the human

(Stein & Susser, 1975b) did not affect fetal growth. Similarly, in the sheep, undernutrition for 40% gestation did not produce growth restriction of the fetus (McCrabb *et al.*, 1991). Nutrient restriction for a period during both mid and late gestation resulted in primary IUGR when imposed for 20% of gestation (Bauer *et al.*, 1995) and secondary IUGR when it lasted for 50% of gestation (Detmer *et al.*, 1991). This pattern is in keeping with the suggestion that the longer the duration of insult the more severe the outcome. However, the fact that the 20% duration was in the sheep and the 50% duration in the guinea pig, means that any differences may be simply due to species differences rather than duration *per se*. In late gestation growth retardation resulted irrespective of duration of insult or species (Table 1.3). Secondary IUGR resulted in all but one study where the insult lasted for 10% of gestation (Block *et al.*, 1990). This finding may be related to severity of insult. Perhaps the degree of hypoxaemia (PaO_2 about 17 mmHg), produced for a period of only 9 days, was not sufficient to cause secondary IUGR. Nutrient deprivation throughout pregnancy always produced growth retarded fetuses that were asymmetrically growth retarded (where growth pattern was reported). It seems, therefore, that greater duration of deprivation does indeed have more profound effects on growth.

1.7.2.iii Type of insult

The particular nutrient that the fetus is deprived of is also an important factor in determining body and organ growth. McCance and Widdowson (1974) found that pigs kept on an energy deficient diet were asymmetrically growth retarded and skinny, whilst protein-deficient animals were also asymmetrically growth retarded but tended to become fat. Osofsky (1975) described the decreased brain weight as a consequence of protein deprivation as being more severe than if diet was purely restricted in calories. He also described another study where it was found that permanent growth stunting resulted in offspring when dietary intake was restricted during pregnancy and especially when protein intake was restricted. He indicated that brain growth and development is particularly affected by protein deficiency.

During both early and mid gestation, decreased calorific intake has either no effect on fetal growth (Stein & Susser, 1975b; Harding and Johnston, 1995; McCrabb *et al.*, 1991) or causes a reduction (DeBarro *et al.*, 1992). Placental weight was either unaffected (Stein & Susser, 1975b) or increased (Harding and Johnston, 1995; McCrabb *et al.*, 1991). A mid to late gestational

deprivation of calories causes primary IUGR (Block *et al.*, 1990) and also reduced placental weight, but placental to birthweight ratio is increased. However, uterine artery ligation over a similar period of gestation which resulted in decreased CaO_2 evoked secondary IUGR and reduced placental growth. But the ratio of placental to fetal weight remained unchanged from controls (Detmer *et al.*, 1991). Undernutrition in late gestation results in asymmetrical growth (Stein & Susser, 1975b; Harding & Johnston, 1995; Harding, 1995) and appears to have little effect on placental growth (Stein & Susser, 1975b). Oxygen deprivation in late gestation caused either secondary (Creasy *et al.*, 1972; Murotsuki *et al.*, 1996, Jacobs *et al.*, 1988a; Tanaka *et al.*, 1994) or primary (Block *et al.*, 1991) growth restriction and placental weight was reduced also, so placental weight to fetal body weight ratio was unaffected (Jacobs *et al.*, 1988a; Tanaka *et al.*, 1994). Block *et al.* (1990) found that placental weight was reduced, but this is not surprising as they were embolizing the placenta. Reduced oxygen supply for most of pregnancy caused growth retardation (Krüger & Arias-Stella, 1970; Beischer *et al.*, 1970; Bacon *et al.*, 1984; Jacobs *et al.*, 1988a; Godfrey *et al.*, 1991) but had varying effects on placental growth, there being either a decrease (Bacon *et al.*, 1984; Jacobs *et al.*, 1988), so no effect on placental to body weight ratio, or an increase (Krüger & Arias-Stella, 1970; Beischer *et al.*, 1970; Godfrey *et al.*, 1991) such that placental ratio also increased.

The data in Table 1.3 below is drawn from: [a] Stein & Susser, 1975b; [b] Harding & Johnston, 1995; [c] Creasy *et al.*, 1972; Murotsuki *et al.*, 1996; [d] Jacobs *et al.*, 1988a; [e] Detmer *et al.*, 1991; [f] Kamitomo *et al.*, 1993; [g] Desai *et al.*, 1995; [h] DeBarro *et al.*, 1992; [i] Tanaka *et al.*, 1994; [j] Block *et al.*, 1990; [k] Bauer *et al.*, 1995; [l] Beischer *et al.*, 1970; Godfrey *et al.*, 1991; [m] Krüger & Arias-Stella, 1970; [n] Bacon *et al.*, 1984; [o] McCrabb *et al.*, 1991; [p] Mellor & Matheson, 1979; Mellor & Murray, 1981; [q] Owens *et al.*, 1986; [r] Harding, 1995.

	Early	Mid	Mid and late	Late	Throughout
IUGR	none [ab]	none [o]	primary [k] secondary [e]	primary [j] secondary [abcdir]	secondary [dfgq]
Growth	↓ [h]			↓ [p]	↓ [lmn]
Species	human [a] sheep [bh]	sheep [o]	sheep [k] guinea pig [e]	human [a] rat [i] sheep [bcdjpr]	human [lm] rat [g] sheep [dfq] guinea pig [n]
Pl. : BW	⇔ [a] ↑ [h]	↑ [o]	⇔ [e] ↑ [k]	⇔ [adi] ↓ [j]	⇔ [dn] ↑ [lm] ↓ [q]
Duration - absolute	30 d [bh] 3 m [a]	66 d [o]	24 d [k] 30 d [e]	60 min [i] 9 d [j] 10 d [br] 21 d [d] 28 d [c] 3 m [a]	
Duration - % gestn	20 [bh] 30 [a]	40 [o]	20 [k] 50 [e]	0.2 [i] 10 [bdjr] 20 [c] 30 [a]	
Insult	undernutrition [abh]	undernutrition [o]	undernutrition [k] uterine artery ligation [e]	undernutrition [abr] uterine artery ligation [i] embolization [jc] hypobaric hypoxia [d]	hypobaric hypoxia [dn] caruncleclectomy [q] high altitude [m] anaemia [l]

Table 1.3. Summary of the growth patterns observed in different species as a result of various insults.

1.8 THE FETAL CIRCULATION

Having looked at the factors that facilitate and control fetal growth I shall now go on to look at development of the cardiovascular system, with relation to the ontogeny of structure, function and control.

1.8.1 Development

Embryonic cardiac growth and development is split into four developmental periods: precardiogenesis (before 16 days gestation in the human), fusion (21 days gestation in the human), looping (25-30 days gestation in the human) and septation (after 30 days gestation in the human). Precardiogenesis is characterised by folding, at the cephalic end of the early embryo, of two lateral areas of mesoderm, each containing endocardial tubes and cells that will become the heart. As the endocardial tubes grow closer together, the cavity that becomes the primitive foregut is formed. Fusion of the endocardial tubes then occurs and a single tubular heart is formed in what is the fusion period. Soon after its formation this primitive heart becomes functional, i.e. starts beating. Following fusion the heart continues to grow and various changes in shape take place during what is the looping period. The middle of the tube bulges to the right to form a C-shaped loop which then becomes S-shaped and is further transformed until the primitive atria are adjacent to the primitive ventricles, at which time the looping process is complete. The heart then becomes partitioned by the formation of septa, in what is the septation period, so that four-chambers are formed. By 55 d GA (human) the heart is basically formed, and the remainder of gestation is a process of growth and maturation of the heart. (Thornburg & Morton, 1993 & 1994; Bristow, 1996). There are species differences with regard to heart development, however the various stages of development are applicable to both avian and mammalian hearts (Thornburg & Morton, 1994).

Concurrent with the formation of the heart is the development of the circulatory system. The early embryonic circulation, which is bilaterally symmetrical, undergoes extensive and complicated rearrangement resulting in the formation of the mature fetal circulation.

1.8.2 Structure

The fetal circulation has some structural features which differ from that of the adult (Fig. 1.11). These are the *ductus venosus*, *foramen ovale*, *ductus arteriosus* and the umbilical circulation. After birth the *ductus venosus*, *foramen ovale*, *ductus arteriosus* close and the placenta is removed. It is these structures that enable the fetal circulation to function as a parallel system where right and left ventricular outputs mix. This is unlike the adult circulation where the heart may be regarded as 2 pumps in series supplying two separate circulatory systems, pulmonary and systemic.

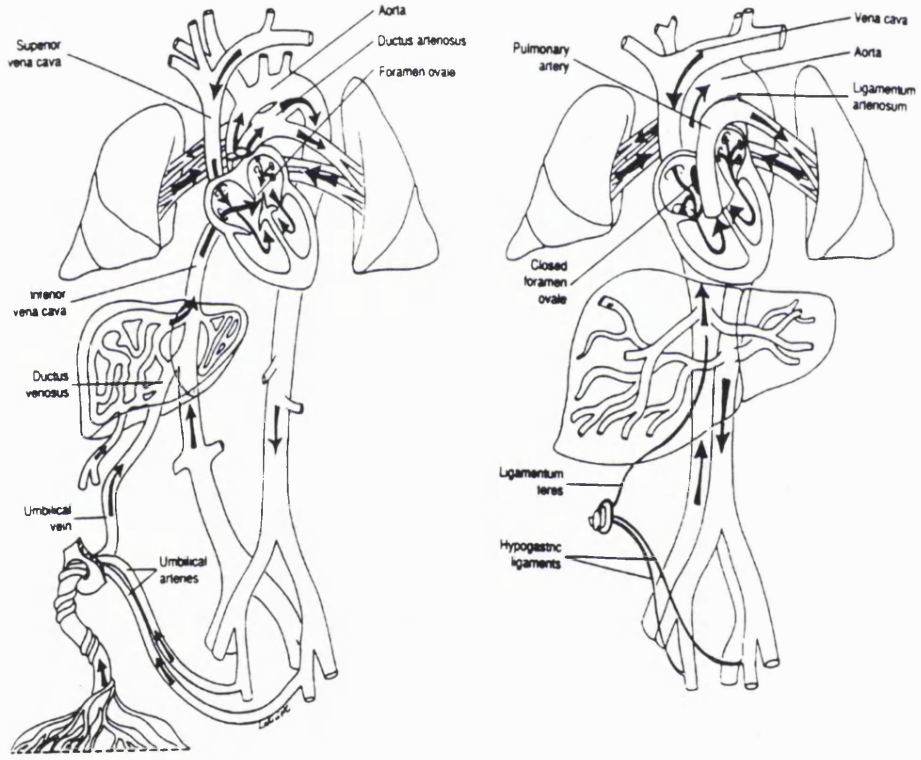

Figure 1.11. Circulation in the fetus and newborn (from Coustan *et al.*, 1995).

1.9 FETAL CARDIOVASCULAR FUNCTION

1.9.1 Combined ventricular output

As described above, the pulmonary artery and aorta are joined by the *ductus arteriosus.* This means that the fetal circulation is not arranged with the right heart and pulmonary circulation in series to the left heart and systemic circulation, with left and right ventricular outputs being similar in volume. Rather, in the fetus, left and right ventricles pump blood into the arterial circulation in parallel. Thus, the term 'combined ventricular output' (CVO) is applied in the fetus. CVO in the late-gestation fetal sheep is about 480 ml min^{-1} kg^{-1} (Jensen & Berger, 1993). Unlike the adult, right and left ventricular outputs are not equal. The right ventricle ejects 60-65% of CVO, whilst the left ventricle ejects only 35-40% (Rudolph, 1985).

Actual CVO increases with increasing gestational age, increasing from about 250 ml/min at 80 d GA to about 2000 ml min^{-1} at 140 d GA in the fetal sheep (Rudolph & Heymann, 1970). This increase is due to the growth of the fetus which means that there is also an increase in cardiac volume (Thornburg & Morton, 1994). However, CVO per kg of fetal body weight does not change significantly with increasing age and weight (Rudolph & Heymann, 1970).

1.9.2 Blood pressure and heart rate

1.9.2.i Early gestation

An organism's blood pressure is lower during fetal life than at any other time during its existence, its circulation being characterised by the placenta which is a very low resistance shunt. The normal fetal MAP during late gestation (as measured in the fetal sheep) is about 45 mmHg (cf. about 100 mmHg in the adult) (Thornburg & Morton, 1994). The two main components that determine MAP are combined ventricular output (cardiac output), which is affected by heart rate and stroke volume, and total peripheral resistance.

In early gestation, prior to autonomic innervation of the heart and vessels and before the adrenal gland has been formed, blood is pumped around the system. This suggests that the heart and vessels must be under intrinsic control. It has been shown by Wagman *et al.* (1990), in the chick embryo,

that the Frank-Starling mechanism is particularly important for cardiac function during the early stages of development. It has also been suggested that the embryonic heart and vasculature may be responsive to circulating vasoactive substances, e.g. adrenaline, noradrenaline, serotonin (Nakazawa *et al.*, 1986; Nakazawa *et al.*, 1995). Embryonic cardiac output is determined by both heart rate (Nakazawa *et al.*, 1991) and stroke volume (Wagman *et al.*, 1990), thus alterations in either of these variables as well as vascular resistance will affect embryonic blood pressure. During the early stages of embryonic and fetal development there is a large increase in FHR which is accompanied by a rise in MAP (Fig. 1.13). In the developing rat embryo Nakazawa *et al.* (1988) measured an increase in MAP from 0.2 mmHg on day 11 to about 2.5 mmHg at day 15 of gestation, and an increase in FHR from about 100 beats min^{-1} at 11 days gestation to 200 beats min^{-1} at 15 days gestation. The rise in MAP is not as great as one may perhaps expect, given the huge increase in FHR and the fact that stroke volume also increases (Hu & Clark, 1989). This is because, occurring concomitantly, there is a large decrease in vascular resistance (Hu & Clark, 1989; Nakazawa *et al.*, 1986).

Presumably as long as blood volume (normalised for weight) continues to increase in the fetus, which is for the first 40% of gestation in the human and the sheep (Brace, 1993), stroke volume will continue to increase. Ultrastructure of the myocardium may also affect stroke volume. Early on, in embryonic life, the myofibrils of the myocardial cells show a marked degree of disarray (Wagman *et al.*, 1990). With increasing maturity the myofibrils become more organised, thus it is conceivable that as this is occurring the force of contractility that the cardiac muscle is able to exert also increases, so causing an increase in stroke volume. Presumably greater myofibrillar organisation and contractility may also impart more energy per ml of blood each heart beat, which would also cause blood pressure to increase. During early gestation the heart must operate at the bottom of the cardiac function curve (Fig. 1.12) in order for the Frank-Starling mechanism to function. However, as the heart grows, wall stress will increase (according to the law of Laplace:

$$S_w = 0.5(Pr/h)$$

where S_w is wall stress, P is transmural pressure, r is the radius, and h is wall thickness) and pressure will also increase, as described by pressure-volume

curves (Thornburg & Morton, 1993). These two factors result in an upwards displacement along the cardiac function curve.

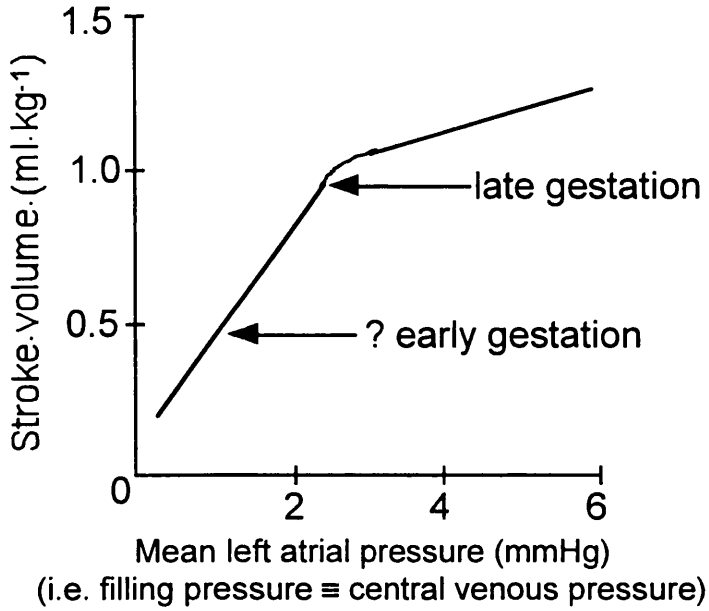

Figure 1.12. Cardiac function curve for fetal sheep. (Adapted from Thornburg & Morton, 1994).

1.9.2.ii Late gestation

In this section I shall mention the roles of chemo- and baroreflexes in the control of cardiovascular function, however I shall discuss them more fully later on (section 1.10).

Prior to autonomic innervation of the heart, adrenergic and muscarinic receptors are functional. Autonomic innervation then occurs, with parasympathetic preceding sympathetic innervation in all species (Teitel & Hoffman, 1996). In the more mature fetus, once cardiac and vascular innervation is established, control of the cardiovascular system occurs through both extrinsic and intrinsic mechanisms. Myocardial sympathetic nerves are present in the sheep by mid-gestation and in the rabbit by 0.7 of gestation (Thornburg & Morton, 1993). This illustrates the variation in development between different species, which is further emphasised by the different levels of maturity at which offspring are born. Generally, for most

physiological systems, precocious animals (e.g. guinea pig) tend to be more mature at birth than altricial animals (e.g. rat).

During late gestation in the fetal sheep the peripheral chemoreceptors have been found to play a role in the control of FHR (Hanson, 1993). Despite the fact that they are functioning by 90 days gestation (0.6 gestation) in the fetal sheep (Blanco *et al.*, 1984) they appear to have little effect on FHR until about 110 days gestation (0.75 gestation) (Iwamoto *et al.*, 1989). Plasma catecholamines and other humoral agents such as AVP (Rose *et al.*, 1981; Martin *et al.*, 1987; Raff *et al.*, 1991; Giussani *et al.*, 1994c), ACTH and cortisol (Rose *et al.*, 1981; Giussani *et al.*, 1994b), renin (Martin *et al.*, 1987; Rawashdeh *et al.*, 1988) and atrial natriuretic peptide (ANP) (Lawrence *et al.*, 1990). are also involved in FHR control. As was mentioned at the beginning of this section, the main determinants of MAP are cardiac output and total peripheral resistance, which are under both extrinsic and intrinsic control by this stage of gestation. Unlike the embryo, stroke volume has little effect on combined ventricular output in the late gestation fetus, and output is determined primarily by heart rate. This is because the late gestation fetal heart operates at the shoulder of the ventricular function curve (Fig. 1.12), thus any increase in filling pressure, i.e. preload, has little effect on stroke volume (Thornburg & Morton, 1993). The baroreflex plays an important role in the maintenance of MAP in the late gestation fetus, as it does in the adult. This was confirmed in the fetal sheep where, after arterial baroreceptor denervation there was an increase in arterial pressure variability (Yardley *et al.*, 1983). Exogenous and endogenous factors acting locally on the blood vessels also contribute to the control and maintenance of blood pressure. Recently it has been shown that nitric oxide (NO) contributes to the control of basal peripheral vascular resistance in the late-gestation fetal sheep (Green *et al.*, 1996), and endothelin-1 (ET-1) has also been shown to be vasoactive in the late-gestation ovine fetus (Chatfield *et al.*, 1991).

Late gestation is characterised by a decrease in FHR and an increase in MAP (Fig. 1.13). The drop in FHR is due to increasing vagal tone acting on the heart (Walker, 1993) and it is thought that the fall in baroreceptor sensitivity (see section 1.10.1.i) may also be a contributory factor (Hanson, 1993). The exact mechanisms responsible for the gestational increase in MAP are not known, however it is possible that it may in part be due to resetting of the baroreceptors. There is also an increase in peripheral vascular resistance

during late gestation, which will drive pressure up. It has been suggested that vascular resistance rises because vascularity does not increase at the same rate as the large increase in fetal growth which occurs at this time (Fig. 1.10) (Hanson, 1993). However, it is also possible that there is an increase in circulating vasoconstrictor agents and an increase in placental vascular resistance.

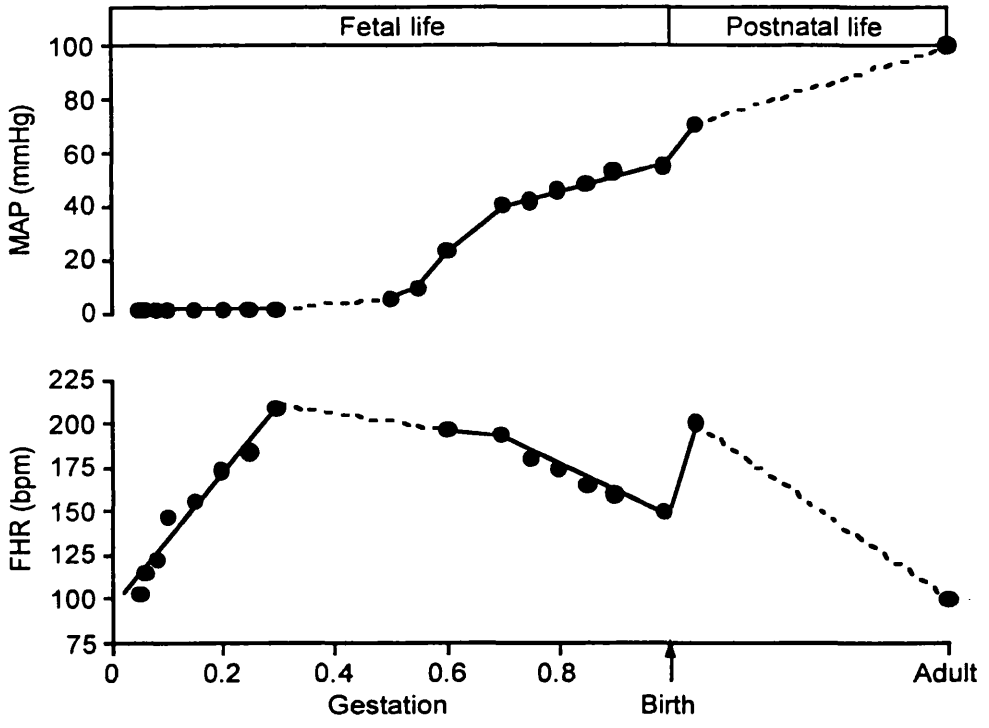

Figure 1.13. An estimate of the changes in MAP and FHR during embryonic, fetal and postnatal life. Based on data in the chick and ovine fetus, from: Hu & Clark (1989); Nakazawa *et al.* (1988); Kamitomo *et al.* (1994); Gagnon *et al.* (1994); Mostello *et al.* (1991); Kitanaka *et al.* (1989); Walker (1993).

1.9.3 Blood flow

1.9.3.i Umbilical-placental

Between about 60 and 100 d GA in the sheep, the umbilical-placental circulation receives about 50% of CVO (Rudolph & Heymann, 1970;

Iwamoto, 1989). After 100d GA, CVO to the placenta decreases to about 40 % (Rudolph & Heymann, 1970; Iwamoto, 1989; Jensen & Berger, 1993). It has been suggested that this may be due to a decrease in total fetal body circulatory resistance and perhaps an increase in placental vascular resistance (Rudolph & Heymann, 1970).

However, despite the proportional decrease in placental blood flow, actual umbilical-placental flow increases with increasing gestational age in the ovine fetus. This increase in actual flow is due mainly to a fall in placental vascular resistance up to 115 d GA, after which it is mainly accounted for by the rise in arterial pressure (Carter, 1993).

1.9.3.ii Brain

Only about 2-4% of CVO goes to the brain, and this value does not change significantly with increasing age in the sheep fetus between 60 d and 140 d GA (Rudolph & Heymann, 1970). The fraction is greater in the human than in the sheep, because the brain/body weight ratio of the human is larger (Jensen & Berger, 1993). Although the fraction of CVO remains fairly constant over this period of gestation, there is a progressive and considerable increase in flow from about 3 ml $100g^{-1}$ min^{-1} to about 130 ml $100g^{-1}$ min^{-1} (Rudolph & Heymann, 1970; Iwamoto, 1989).

There are regional differences within the brain both in terms of the amount of CVO received and flow per 100 g of tissue. The cerebrum receives the greatest proportion of CVO (2%), but flow is relatively low (about 80 ml/min/100g) compared to the medulla (about 145 ml min^{-1} $100g^{-1}$) which receives only 0.15% of CVO (Jensen & Berger).

1.9.3.iii Myocardial

The proportion of blood flow to the myocardium is 2-4% and does not change significantly with age, between 60 and 140 d GA in the sheep fetus (Rudolph & Heymann, 1970). Similarly, myocardial blood flow (170-290 ml/min/kg) does not change significantly, though there is some amount of variability.

At first sight it may seem odd that myocardial blood supply does not increase. However, although CVO increases with gestational age (see section 1.9.1) there is a gestational decrease in heart rate (see section 1.9.2), therefore the work load on the heart probably remains constant, so myocardial oxygen

consumption does not increase and there is therefore no need for increased myocardial blood supply.

1.9.3.iv Pulmonary

As has been described extensively earlier in this chapter (section 1.3.1.i), the placenta is the site of gaseous exchange in the fetus, as, being *in utero*, it is obviously not able to breathe using its lungs. Thus, blood supply to the lungs is to deliver nutrients for growth. The distribution of CVO to the lungs remains constant in the fetal sheep between 60 d and 100d GA at about 4%, then there is a significant rise to about 7% at 140 d GA. Similarly, pulmonary blood flow is about 38 ml/100g/min in mid-gestation and rises to about 130 ml/100g/min near to term (Rudolph & Heymann, 1970). The increase in pulmonary blood flow during the last part of gestation is due to decreased vascular resistance which may be the result of vasculogenesis (Harding, 1994), though increased metabolic activity and local endothelial factors probably also play a role.

1.9.3.v Liver and ductus venosus

Blood is delivered to the fetal liver via the umbilical vein, hepatic artery and portal vein. About 90% of the blood supplying the left lobe of the liver comes from the umbilical vein, and the other 10% is delivered by the hepatic artery. The right lobe receives 60% of its blood from the umbilical vein , about 35% from the portal vein and the remaining 5% from the hepatic artery. The proportion of umbilical venous blood flow that bypasses the liver and shunts through the *ductus venosus* to the inferior vena cava varies depending on species and state, however in the unstressed state it is about 50%. Less than 10% of the blood delivered to the right lobe of the liver via the portal vein is shunted through the *ductus venosus*. The *ductus venosus*, therefore, provides a bypass of the hepatic circulation and is important in providing sufficient venous return to the heart (Rudolph, 1985). It is interesting to note that several mammalian species do not have a *ductus venosus*, e.g. horse, pig, and guinea pig (Rudolph, 1996).

1.9.3.vi Renal

The proportion of CVO to the kidneys seems to remain fairly constant (about 2.5%) between 60 d and 140 d GA (Iwamoto *et al.*, 1989; Rudolph & Heymann, 1970), though there is the suggestion that it may decrease

(Rudolph & Heymann, 1970). Renal blood flow seems to fall between 60 d and 100d GA from about 122 ml $100g^{-1}$ min^{-1} to about 85 ml min^{-1} $100g^{-1}$, then there is a rise towards term to about 170 ml $100g^{-1}$ min^{-1} (Rudolph and Heymann, 1970). Iwamoto *et al.* (1989) showed a similar pattern of renal blood flow development. However, the proposed rise in renal flow during late gestation was not totally convincing in either study.

1.9.3.vii Adrenal

The adrenals receive about 1% of the CVO (Jensen & Berger, 1995), a value which does not change significantly between 80 d and 100 d GA in the fetal sheep (Iwamoto *et al.*, 1989). Likewise, adrenal blood flow (about 0.18 ml min^{-1} kg^{-1}) seems to remain fairly constant.

1.9.3.viii Gut, skin and skeletal muscle

Initially, in young (60-120 d GA) fetuses, the percentage of CVO to the gut is quite low, being only about 3%. But, it then increases to about 6%. This is concomitant with an increase in blood flow from about 30 ml $100g^{-1}$ min^{-1} to about 60 ml $100g^{-1}$ min^{-1} (Rudolph & Heymann, 1970).

Neither proportion of CVO nor blood flow to the carcass (body with the viscera, brain and eyes removed, i.e. skin, skeletal muscle and bones) change significantly with gestational age in the sheep, and are about 20% and 25 ml min^{-1} kg^{-1}, respectively Rudolph & Heymann, 1970). Similarly, Iwamoto *et al.* (1989) found that there was no significant change with gestational age in the proportion of CVO (about 30%) or blood flow (about 25 ml min^{-1} kg^{-1}) to the periphery. They did not define what they meant by the 'periphery'.

Femoral blood flow may be used as an indication of blood flow to the skeletal muscles. Dawes *et al.* (1968) demonstrated that between 90 d GA and 130 d GA flow remains fairly constant at about 5 ml $100g^{-1}$ min^{-1}. Then, up to 140 d GA, there is a decrease to about 2.8 ml $100g^{-1}$ min^{-1}, attributable to an increase in vascular resistance. It is worth noting that actual femoral blood flow increases with gestational age. Thus, if vascular resistance is increasing, the increased flow must be entirely due to the increase in blood pressure that occurs with increasing gestational age (see section 1.9.2).

1.10 FETAL CARDIOVASCULAR CONTROL

The fetal cardiovascular system is under both neural and endocrine control, with both systems acting in concert, exerting their actions on vascular resistance, and hence blood flow, and on myocardial performance. In this section I shall consider first the neuronal reflex control of the circulation and then the endocrine component of cardiovascular control.

1.10.1 Reflex

Circulatory function is continually being monitored by regional (peripheral) and central chemo- and baroreceptors. These receptors, when stimulated, adjust blood flow through the body by eliciting a number of reflex responses. Thus, both chemoreflex and baroreflex mechanisms play a role in maintaining adequate cardiovascular function, as mentioned above.

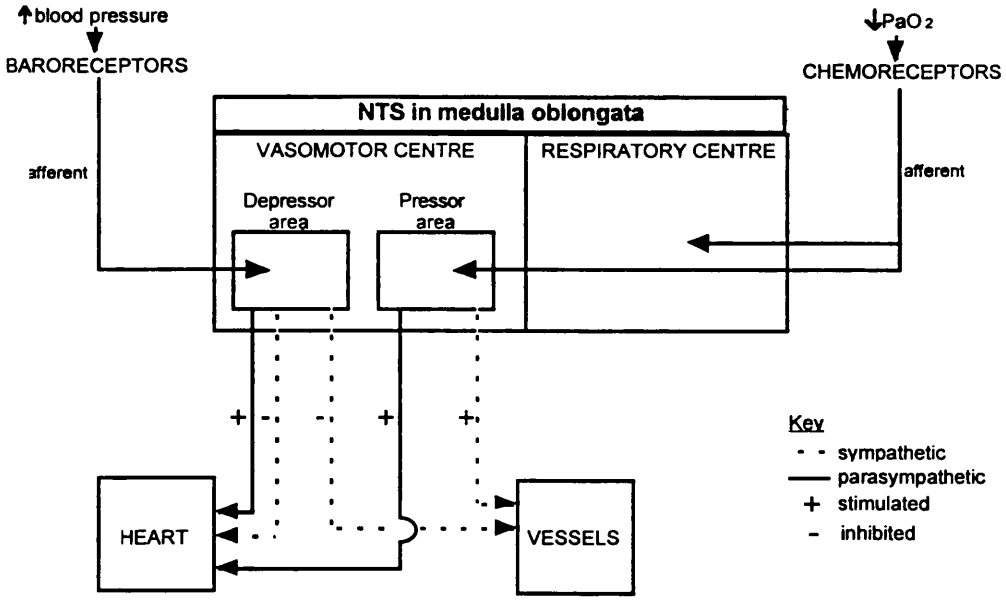

Figure 1.14. Illustration of the efferent baro- and chemoreflex pathways.

1.10.1.i Baroreflex

The principal sites in which the baroreceptors are located are the aortic arch and in the carotid sinuses. Arterial baroreceptors are nerve endings that are

stimulated by deformation or strain of the vessel walls in which they are located. It is incorrect to say that they respond to pressure *per se*, as they are not activated by pressure changes in the absence of vascular deformation (Brown, 1980) i.e. they can be considered to be distortion receptors. There are two types of baroreceptor sensory nerve endings: type 1 receptors which contain relatively few thin myelinated fibres which run together for a fairly long distance before branching into a diffuse plexus, and type 2 receptors which consist of a single thick myelinated fibre that also runs for quite a distance before arborising into a number of very fine branches that terminate in neurofibrillar end plates. Most baroreceptors are connected to A-type (myelinated) fibres, which have been found to have a conduction velocity of 10-55 m s^{-1}, though there have also been found to be a few which are connected to C-type (non-myelinated) fibres that conduct at a velocity of 2 m s^{-1} or less (Kirchheim, 1976). Mechanical deformation in the vessel wall causes mechanical deformation of the receptor generator region which is then transduced into an electrical signal (Brown, 1980; Kirchheim, 1976).

Afferent impulses from the baroreceptors project to the vasomotor centre located in the *nucleus tractus solitarius* (NTS), which is situated in the medullary region of the brainstem. These afferents reach the brainstem via the carotid sinus nerves and glossopharyngeal nerves from the carotid baroreceptors, and via the aortic depressor nerves in the vagus from the aortic baroreceptors. The transmitter substance in the NTS is unknown, but it may be L-glutamate (L-GLU). Second-order neurones then project from the NTS to cardiac preganglionic neurones, and to GABA-containing pacemaker neurones in a region of the medulla near the nucleus ambiguus (Spyer, 1994) The efferent limb of the baroreflex consists of vagal parasympathetic cholinergic fibres to the heart, and sympathetic adrenergic fibres to the heart and blood vessels. It is thought that the pacemaker neurones project directly to the sympathetic preganglionic neurones in the intermediolateral cell column of the spinal cord, possibly using L-glutamate as a transmitter. An increase in MAP causes enhanced parasympathetic activity resulting in a vagally mediated decrease in heart rate, and an inhibition of sympathetic activity which has a negative chronotropic and inotropic effect on the heart as well as causing vasodilatation of the vessels. These effects of the baroreflex result in a decrease in MAP.

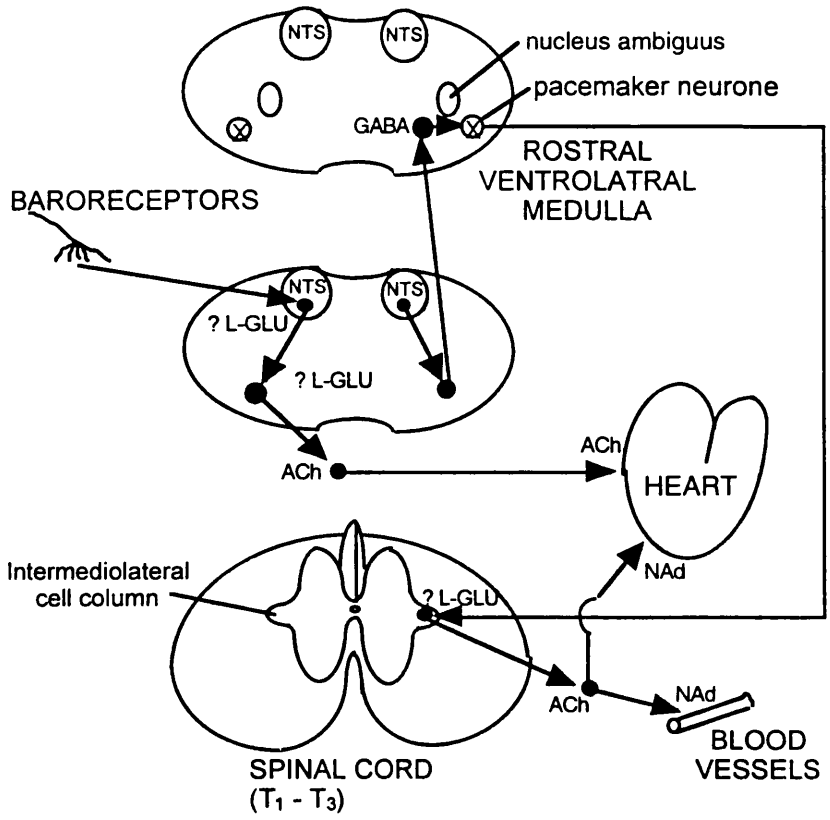

Figure 1.15. Illustration of the afferent and efferent limbs of the baroreflex. (Adapted from: Spyer 1994).

Baroreflex activity has been recorded from 88 days gestation (Blanco *et al.*, 1988) and thereafter (Maloney *et al.*, 1977; Blanco *et al.*, 1988) in the fetal lamb. It has been shown that as gestation increases, between 88 d GA and term in the fetal lamb, there is a reduction in sensitivity of the baroreceptors as blood pressure increases (Blanco *et al.*, 1988). This is seen as a shift to the right of the stimulus-response curve relating MAP to baroreceptor discharge and a decrease in its slope (Fig. 1.16). This resetting of the baroreceptors maintains their operation within the normal blood pressure range at any age.

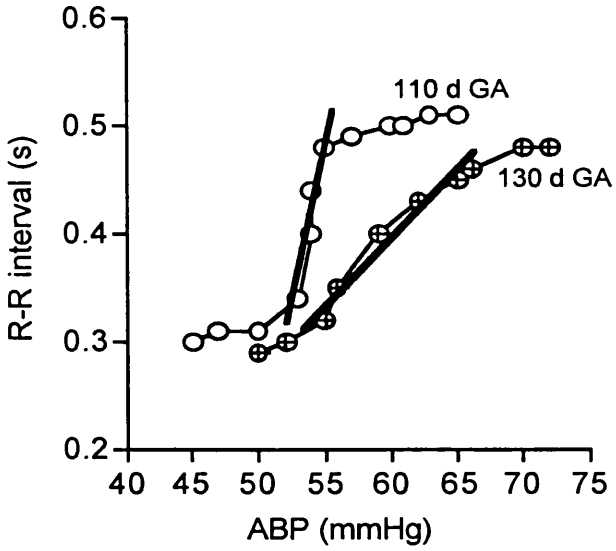

Figure 1.16. Illustration of the baroreflex curve in the late gestation fetal sheep, showing maturation of the baroreceptors.

1.10.1.ii Chemoreflex

The peripheral chemoreceptors are located in the in the carotid and aortic bodies. Arterial chemoreceptors detect changes in blood gas tensions, particularly oxygen, though the response may be modulated by pH and carbon dioxide. They are stimulated by a fall in PaO_2 or pH or a rise in $PaCO_2$. Chemoreceptor tissue is thought to consist of groups of type I cells enclosed by glial-like type II cells with associated nerve endings. It is generally accepted that the type I cells are the receptor cells which release excitatory transmitter substances affecting the associated afferent nerve terminals (Biscoe & Duchen, 1990).

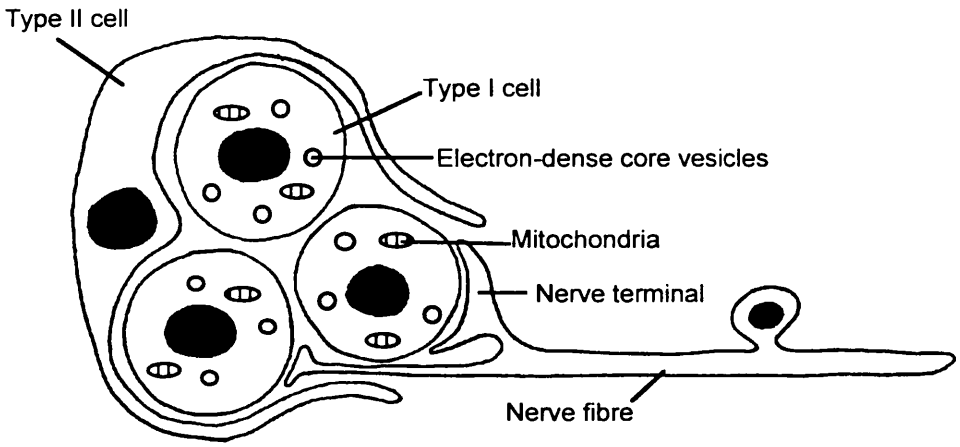

Figure 1.17. Schematic diagram of Type I and Type II chemoreceptor cells from within the carotid body. The electron-dense core vesicles contain catecholamines. (Adapted from Biscoe & Duchen, 1990).

Afferent fibres from the carotid chemoreceptors run via the carotid sinus nerve to the vasomotor centre in the NTS where they cause excitation of the vasoconstrictor centres in the medulla. The aortic afferents run to the NTS in the aortic branch of the vagus. (Chemoreceptor afferent fibres also run to the "respiratory centres" in the medulla). The efferent chemoreflex pathway consists of vagal cholinergic pathways to the heart and α-adrenergic fibres to the blood vessels. Stimulation of the chemoreceptors, e.g. by hypoxaemia, results in bradycardia due to increased vagal activity, and vasoconstriction due to increased sympathetic (α-adrenergic) activity. In the fetus, which is not breathing, the increase in peripheral resistance outweighs the reduction in heart rate, so there is an overall rise in blood pressure.

Blanco *et al.* (1984) recorded chemoreceptor afferent activity in anaesthetised, exteriorised sheep fetuses between 90 and 143 days gestation. They also demonstrated that discharge of the fetal chemoreceptors is much lower than in the adult for a given PaO_2, but that they are sensitive to changes in PaO_2 within the fetal range. Furthermore, it was demonstrated that the rise in PaO_2 that occurs in the first few minutes after birth more or less silences the chemoreceptors.

1.10.2 Endocrine

1.10.2.i Catecholamines

Basal resting catecholamine concentrations in the circulation have been found to be less than 70 pg ml^{-1} in the fetus (Jones & Robinson, 1975), and basal concentrations remain the same between 85 d and 142 d GA (Comline & Silver, 1965; Jones & Robinson, 1975). Jones & Robinson (1975) calculated that the clearance rate of catecholamines is 840 ml min^{-1} in the fetus (cf. 4200 ml min^{-1} for the adult sheep), which gives a secretion rate of 42 pg min^{-1}, assuming a resting level of 50 pg ml^{-1}. Within the adrenal, noradrenaline is the major catecholamine and adrenaline is found in about one-twentieth the concentration of noradrenaline, but no more than 30% of the basal resting catecholamine concentration is contributed by the adrenal medulla (Jones *et al.*, 1987). Jones *et al.* (1987) showed that the adrenal supply of catecholamines is too slow to carry out minute to minute fine-tuning of the cardiovascular system required to maintain blood pressure and heart rate, thus an intact sympathetic nervous system is essential. However, Jensen & Lang (1992) demonstrated that during normoxaemia the peripheral sympathetic nervous system is not essential for the maintenance of cardiac output and blood gases, which they suggested is due to a compensatory increase in adrenaline production by the adrenal medulla.

1.10.2.ii Arginine vasopressin (AVP)

AVP is a potent vasoconstrictor in the fetal circulation, and increases in circulating plasma levels cause an increase in mean arterial blood pressure. AVP also causes bradycardia and a redistribution of CVO in favour of the placenta, heart and brain, at the expense of the gastrointestinal and peripheral circulations (Iwamoto *et al.*, 1979).

AVP mRNA has been detected in the paraventricular nucleus of the hypothalamus from as early as 60 d GA in the fetal sheep, expression being similar in the magnocellular and parvocellular neurones. AVP mRNA levels within the paraventricular nucleus do not change as gestation progresses, however expression becomes greater in the magnocellular neurones by 80 d GA, and by term (147 d) they contain 5 times greater concentrations of AVP mRNA than the parvocellular neurones (Matthews & Challis, 1995). Immunoreactive AVP has been measured at 70 d GA in the hypothalamus of

fetal sheep, and it has been shown that the concentration increases progressively with age between 70 d and 130 d GA, the increase being particularly marked between 100d and 130 d GA (70 d: 1 ng mg protein^{-1}; 100 d: 2 ng mg protein^{-1}; 9 ng mg protein^{-1}) (Currie & Brooks, 1992).

There are 2 subtypes of AVP receptors, V_1 and V_2. The vasoconstrictor actions of AVP are mediated by vascular V_1-receptors, though, in the fetus they are not critical for the maintenance of blood pressure during normoxia. The characteristic bradycardia seen in response to AVP is not mediated by either V_1- or V_2-receptors (Ervin *et al.*, 1992).

1.10.2.iii Atrial natriuretic peptide (ANP)

ANP is synthesised and secreted by the heart. Its release is induced by atrial distension, though hormones, e.g. AII, AVP α-adrenoceptor agonists, acetylcholine, androgens, glucocorticoids and thyroid hormones may also cause its release(Smith *et al.*, 1989), as does hyperosmolality (Cheung *et al.*, 1987). It inhibits renin, aldosterone and AVP release, and induces diuresis, natriuresis and vasodilatation. These various effects of ANP mean that it affects cardiovascular homeostasis. Recently, 2 other members of the natriuretic peptide family have been identified, brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). The natriuretic peptides exert their effects through specific membrane-bound receptors that are of 2 subtypes, high-molecular-weight guanylate cyclase-bound (A and B receptors) and low-molecular-weight nonguanylate cyclase-bound (C) receptors. The former are thought to mediate most of the effects of the natriuretic peptides, and the C receptor is thought to be a clearance receptor. (Garcia, 1993).

Fetal plasma concentrations of ANP are more than 5-times greater than maternal plasma levels (fetal: 265 pg ml^{-1}, maternal (47 pg ml^{-1}), however urine concentrations are similar for both (about 14 pg ml^{-1}). (Cheung *et al.*, 1987). Furthermore, Cheung *et al.* (1987) suggested that ANP is either metabolised or reabsorbed by the fetal kidney. Decreased levels of ANP cause an initial decrease in arterial blood pressure, followed by a sustained increase, suggesting involvement of endogenous ANP in the maintenance of arterial pressure (Cheung, 1991).

1.10.2.iv ACTH and cortisol

ACTH is secreted by the pituitary corticotroph, whose function is regulated primarily by the hypothalamic secretion of corticotrophin releasing hormone (CRH) and AVP. ACTH then stimulates the adrenal cortex to secrete cortisol, which provides feedback inhibition of ACTH synthesis, both at the level of the pituitary and at the hypothalamus.

Cortisol produces increases in blood pressure by causing an increase in vascular resistance, but it is not a direct vasoconstrictor. The mechanisms by which cortisol affects vascular tone have not been conclusively established, however there are several mechanisms whereby it may exert its effects. Cortisol is involved in the synthesis of adrenaline, therefore it may affect vascular tone through its permissive effect on catecholamine synthesis. It has also been suggested that it may act in the brain to increase sympathetic efferent tone (Wood, 1995). Walker & Williams (1992) suggested that cortisol may affect the action and expression of various biochemical signals in the vascular wall, e.g. guanylate cyclase, ACE, PGE_1, AII, and their associated cell-surface receptors.

CRH mRNA is present in parvocellular fields of the paraventricular nucleus of the hypothalamus by 60 d GA in the fetal sheep. CRH mRNA increase progressively from 100 d GA to term (147 d GA) in all regions of the paraventricular nucleus (Matthews & Challis, 1995). Similarly, immunoreactive CRH within the hypothalamus increases between 70 d and 130 d GA, with the increase being particularly striking between 100 d and 130 d GA (70 d: 0.2 ng mg protein^{-1}, 100 d: 0.4 ng mg protein^{-1}, 130 d: 5 ng mg protein^{-1}) (Currie & Brooks, 1992). The concentration of AVP is always greater than that of CRH, although by 130 d GA the difference is diminished (Currie & Brooks, 1992). (AVP is discussed in section 1.10.2.ii).

There is a significant increase in ACTH and cortisol during late gestation (after 125-139 d GA) (Boddy *et al.*, 1975; Rose *et al.*, 1978; Tangalakis *et al.*, 1992; Matthews, 1995) despite the negative feedback effects of cortisol on ACTH. It has been suggested that this may be due to increasing pituitary expression of proopiomelanocortin (POMC) which is the precursor to ACTH, resetting of the HPA axis, and attenuation by cortico-steroid-binding globulin (CBG) (Matthews, 1995). Cortisol infusion in the fetal lamb between 100-120 d GA causes an increase in blood pressure, however at 130 d GA

increased blood cortisol concentrations did not cause resting blood pressure to increase (Tangalakis *et al.*, 1992). As a result, they suggested that, prior to 130 d GA cortisol is effective in regulating basal blood pressure levels, but not in the more mature fetus. The increase in basal arterial blood pressure during late gestation may, in part, be due to the increasing cortisol levels.

1.10.2.v Angiotensin II (AII)

AII is formed from AI in a reaction catalysed by the action of angiotensin-converting enzyme (ACE). The precursor to AI is angiotensinogen. The conversion of angiotensinogen to AI is catalysed by renin. Angiotensinogen is located in the plasma and organs and increases with development, during late gestation in the sheep and in the first 24 hours after birth in the rat. Renin is found in the kidney and adrenals of the fetus, and in the kidney it is developmentally regulated. In the human fetus, biologically active renin cells are found in the mesonephros at 5-6 weeks GA, where they are loosely arranged around the afferent glomerular vessels and larger vessels. With increasing gestational age there is a shift in the distribution of renin from the entire renal arterial tree to the arcuate and interlobular arteries and then to the juxtaglomerular apparatus, as seen in the mature animal. ACE, localised in the endothelium of epithelial cells, and in the fetus is found in early differentiating proximal tubules and glomerular capillaries, is measurable by 18 d GA in the fetal rat and its activity increases 2-fold by 24 hours after birth. (Guillery & Robillard, 1993).

AII, the primary biologically active hormone of the renin-angiotensin system (RAS), is a potent vasoconstrictor of the fetal body, though it does not cause vasoconstriction of the placental vascular bed because of the counteractive effects of the vasodilator PGI_2 (Wood, 1995). The increase in arterial blood pressure evoked by physiological increases in AII is accompanied initially by bradycardia, which is probably reflexly mediated, then there is a significant increase in heart rate which may be due to direct actions of AII on the heart. CVO is increased, and there is vasodilatation of the pulmonary and myocardial vascular beds (Iwamoto & Rudolph, 1981). AII also regulates renal water and electrolyte excretion by stimulating the adrenal cortex to secrete aldosterone (Guillery & Robillard, 1993; Wood, 1995), a mineralocorticoid that increases Na^+ reabsorption from and K^+ secretion into the tubule.

AII exerts its effects through specific AII receptors that are located in its target tissue. Two distinct subtypes of the AII receptor have been described, AII-1 and AII-2 receptors (Chiu *et al.*, 1989).

1.10.3 Local

1.10.3.i Nitric oxide (NO)

NO is synthesised from the amino-acid L-arginine, in a reaction catalysed by the enzyme NO synthase. It exerts its effects by stimulating the membrane-bound enzyme guanylate cyclase. Thus it is one of the mechanisms involved in the control of cellular functions. NO release in the cardiovascular system acts as an adaptive mechanism enabling the vascular endothelium to adapt to changes in its environment by acting on the vascular smooth muscle to regulate blood flow and blood pressure (Moncada *et al.*, 1991).

The release of NO is induced by a number of different stimuli, the principal ones of which appear to be pulsatile flow and shear stress (Moncada *et al.*, 1991; Busse *et al.*, 1993; Smiesco & Johnson, 1993; Melkumyants *et al.*, 1995). NO release is also stimulated by hypoxia, and it is dependent on the presence of extracellular Ca^{2+} (Busse *et al.*, 1993), as the NO synthase that releases physiologically active NO is Ca^{2+}/calmodulin dependent (Moncada *et al.*, 1991). Receptor-dependent agonists, e.g. acetylcholine (ACh), ATP, bradykinin, also enhance endothelial NO release (Busse *et al.*, 1993). Basal NO release is probably under the influence of one/or several of the above stimuli, and probably represents one of the simplest and most fundamental adaptive mechanisms in the cardiovascular system (Moncada *et al.*, 1991; Busse *et al.*, 1993).

1.10.3.ii Endothelin (ET)

Four different ET isoforms have so far been described, ET-1, ET-2, ET-3, and vasoactive intestinal contractor (only found in the mouse intestine). Generally ET is considered to be a potent vasoconstrictor, however it may also cause vasodilatation. These responses are mediated by different receptors. To date, 2 subtypes of the ET receptor have been found to exist, ET_A and ET_B. Vasoconstriction is mediated by ET_A receptors located on smooth muscle cells, and vasodilatation is caused by the release of NO or PGI_2 that are released from endothelial cells via ET_B receptors. Masaki, 1993).

1.10.3.iii Local renin-angiotensin system

Dzau (1984) demonstrated the ability of cultured vascular smooth muscle cells to produce AII. He thus proposed that an intrinsic RAS exists in the vessel wall and exerts autocrine or paracrine influences on local vascular function.

1.11 INVESTIGATION OF FETAL CARDIOVASCULAR RESPONSES

In order to investigate the mechanisms involved in the control of the cardiovascular system it is necessary to stimulate the animal in such a way as to evoke a cardiovascular response. In the fetus several methods have been employed. Blood pressure and heart rate responses have been investigated in response to both fetal (Iwamoto & Rudolph, 1981) and maternal (Mostello *et al.*, 1991) haemorrhage, and hypotension. The most common method employed to study fetal cardiovascular responses, however, is the induction of fetal hypoxaemia.

1.11.1 Hypoxia

In the adult animal, a decrease in the ventilation-perfusion ratio in the lungs results in a decrease in the partial pressure of O_2, i.e. hypoxia, and thus a decrease in the O_2 concentration in the blood, i.e. hypoxaemia. The fetus derives all its oxygen in the blood, thus it is incorrect to refer to hypoxia in the fetus, rather it is referred to as hypoxaemia.

There are several types of hypoxia. Hypoxic hypoxia refers simply to a decrease in the arterial partial pressure of O_2. Anaemic hypoxia occurs as a result of reduced oxygen carrying capacity of the blood, which could be due to reduced haemoglobin synthesis (anaemia), or because of the formation of methaemoglobin or CO poisoning (functional anaemia). Histoxic hypoxia occurs when oxygen utilisation is decreased as a result of poisoning e.g. by cyanide. Stagnant hypoxia occurs as a result of impaired oxygen transport because of inadequate blood flow and reduced cardiac output, e.g. heart failure.

1.11.1.i Acute hypoxaemia

There are a variety of methods whereby acute hypoxaemia is induced in the fetus.

Artery occlusion

Vessels that may be occluded so as to result in fetal hypoxaemia are the maternal aorta (Jensen & Hanson, 1995), maternal uterine artery (Ball *et al.*, 1994), umbilical cord (Giussani *et al.*, 1996a), or fetal abdominal aorta (Wilkening & Meschia, 1989).

Reduced maternal inspired fraction of O_2 (FiO_2)

There are 2 methods that have been employed to decrease maternal FiO_2.

One involves performing a maternal tracheostomy and chronically implanting a tracheal tube. Nitrogen is then administered directly into the ewe's trachea (Kamitomo *et al.*, 1992).

The other method does not involve any surgery, and is achieved by simply placing a plastic bag over the ewe's head and introducing a tube into the bag through which the desired composition of gases is administered. This is the method that was employed in this thesis to achieve fetal hypoxaemia.

1.11.1.ii Chronic hypoxaemia

Chronic fetal hypoxaemia has been produced in a number of different ways. However, some methods also result in hypercapnia and changes in fetal haematocrit, which complicate the interpretation of results.

Artery occlusion

This has been done by either ligating one of the uterine arteries (Jansson & Persson, 1990; Detmer *et al.*, 1991); or by reversible uterine artery occlusion (Bocking *et al.*, 1988; Bennet & Hanson, 1994; McLellan *et al.*, 1995). Occlusion of the fetal distal aorta has also been employed to produce chronic hypoxaemia (Anderson *et al.*, 1986).

Reduced maternal FiO_2

In order to produce chronic hypoxaemia maternal FiO_2 has been decreased by placing the ewe in an air-tight perspex chamber into which a gas supply is introduced that can be manipulated as desired (Kamitomo *et al.*, 1993;

Matsuda *et al.*, 1994). The chamber is large enough for the ewe to eat and drink, sit and stand, urinate and defecate.

Embolization

Embolization of either the maternal (Creasy *et al.*, 1972) or fetal (Trudinger *et al.*, 1987; Block *et al.*, 1989; Gagnon *et al.*, 1994) placental vascular bed results in reduced oxygen delivery to the fetus and hence fetal hypoxaemia. The hypoxaemia produced by this method becomes progressively more severe as time goes by, but the hypoxaemia produced is relatively mild (fetal PaO_2 of about 16 mmHg).

Caruncleclectomy

Caruncleclectomy performed prior to mating reduces the number of sites available for placentation during pregnancy. Therefore, placental size and transport capacity are reduced. This has been shown to result in hypoxaemia (Harding *et al.*, 1985).

Anaemia

Chronic fetal hypoxaemia has also been produced in the fetal sheep by isovolemic exchange transfusion at regular intervals (every day or every other day) throughout the study (Davis & Hohimer, 1991; Papparella *et al.*, 1994).

High maternal O_2 affinity

Hebbel *et al.* (1980) increased the O_2 affinity of the blood of pregnant female rats, so narrowing the P_{50} difference between mother and fetus. This resulted in reduced fetal growth presumably due to hypoxaemia.

Reduced maternal plasma volume

During pregnancy there is an increase in maternal plasma volume. When this volume is prevented from expanding, by daily withdrawal of blood whilst maintaining the maternal haematocrit, chronic fetal hypoxaemia results (Daniel *et al.*, 1989).

1.12 FETAL CARDIOVASCULAR RESPONSES TO HYPOXAEMIA

1.12.1 Acute hypoxaemia

1.12.1.i Reflex responses

The fetal cardiovascular reflex responses to hypoxaemia have been recently reviewed by Giussani *et al.* (1994). It has been found that prior to 110 d GA acute hypoxaemia causes a rise in heart rate, which is maintained, and a fall in blood pressure (Iwamoto *et al.*, 1989), but after 110 d GA the response is the opposite (Iwamoto *et al.*, 1989). There is an initial bradycardia, within 2-3 minutes after the onset of hypoxaemia, and a transitory rise in blood pressure, the magnitude of each being dependent on the extent to which the blood gases and pH change.

In the more mature fetus, the initial bradycardia is due to a carotid (not aortic) chemoreflexly mediated increase in vagal activity, and the peripheral vasoconstriction is partly due to a carotid chemoreflexly mediated increase in sympathetic activity operating on the α-adrenergic receptors (Giussani *et al.*, 1993). Part of the vasoconstriction is also non-reflex in nature and is the result of elevated levels of circulating hormones (see section 1.12.1.iii). The decrease in heart rate is thought to be chemoreflexly, rather than baroreflexly, mediated, because heart rate drops before the increase in pressure.

1.12.1.ii Changes in blood flow

CVO is maintained during acute hypoxaemia (Court *et al.*, 1984; Jensen & Berger, 1993), but it is redistributed in favour of those organs that are essential for keeping the fetus alive. There is a decrease in vascular resistance in the heart, brain and adrenals, such that blood flow to those organs is increased (Court *et al.*, 1984). At the same time, there is an intense vasoconstriction of the peripheral arterioles (starting within 5 minutes of the onset of hypoxaemia), kidneys, gut, spleen, liver, carcass and lungs (Court *et al.*, 1984), so blood flow to those organs decreases.

Court *et al.* (1984) observed a decrease in placental vascular resistance, whereas Jensen & Lang (1992) saw an increase. However, despite the increase in placental resistance observed by Jensen & Lang (1992), the percent of cardiac output to the placenta was increased and blood flow

remained the same. Thus, in response to acute hypoxaemia, there appears to be a maintenance of blood flow to the placenta.

1.12.1.iii Endocrine responses

There is an increase in the concentration of circulating plasma catecholamines during acute hypoxaemia due to the direct effects of hypoxaemia on the adrenal medulla (Cheung, 1989), and through chemoreflexly mediated sympathetic stimulation of the adrenals (Jensen & Hanson, 1995).

Hypoxaemia also causes an increase in the release of AVP (Iwamoto *et al.*, 1989; Raff *et al.*, 1991; Giussani *et al.*, 1994), and release is further augmented by acidaemia (Wood & Chen, 1989; Raff *et al.*, 1991), through mechanisms that are not mediated by cortisol (Akagi *et al.*, 1990), or by the peripheral baroreceptors (Wood *et al.*, 1989) or chemoreceptors (Raff *et al.*, 1991), contrary to the adult animal. It has been shown, however, that adenosine mediates the release of AVP in response to acute hypoxaemia, probably through the stimulation of central adenosine receptors in the brain (Koos *et al.*, 1994).

In the fetus, hypoxaemia is an extremely potent stimulus for the release of ANP (Cheung & Brace, 1988), with increases in ANP concentration during hypoxaemia being greater in the immature (110-119 d GA) than mature (130-135 d GA) fetal lamb (Cheung, 1992). It has been suggested that ANP release in response to hypoxaemia may be caused by direct effects on the heart and by the rise in plasma catecholamines (Cheung & Brace, 1988), and it has been demonstrated that the rise in ANP concentration during hypoxaemia is modulated by AVP and the autonomic nervous system (Cheung, 1992). Endothelin also regulates the release of ANP, perhaps by mediating its release from atrial cardiocytes, though its role during hypoxaemia is not understood (Cheung, 1994).

The rise in plasma concentrations of cortisol and ACTH during acute hypoxaemia is by now well established (Boddy *et al.*, 1975; Jackson *et al.*, 1989; Giussani *et al.*, 1994b). The elevation in ACTH concentration is greater in young (125-129 d GA) than older (134-147 d GA) fetal sheep, possibly due to the negative feedback effects of cortisol on ACTH (Akagi *et al.*, 1990). Cortisol concentrations rise during late gestation (see section 1.10.2.iv). A functional fetal hypothalamic-pituitary connection is essential for the ACTH stimulated rises in cortisol in response to hypoxaemia (Ozolins *et al.*, 1992).

ACTH release is stimulated by acidaemia (Wood, Chen & Bell, 1989; Wood & Chen, 1989), which suggests that the response is chemoreceptor mediated, though the relative contributions of central and peripheral chemoreceptors are unknown. Recently it has been shown that the rise in plasma cortisol concentration is delayed in response to carotid sinus nerve section, but ACTH concentration is still increased significantly, suggesting a possible role of the carotid chemo- and baroreceptors in the release of cortisol (Giussani *et al.*, 1994b).

AII levels increase during acute hypoxaemia concurrently with a rise in plasma renin concentrations (Broughton-Pipkin *et al.*, 1974), and contribute to the rise in blood pressure that is observed during hypoxaemia. We may speculate that impaired development of renal nephrons, such as has been observed in babies with IUGR (Hinchliffe *et al.*, 1992), may affect the fetal angiotensin response to hypoxaemia. The arterial chemoreceptors are involved in mediating the release of renin, which has been demonstrated by the fact that hypercapnia is a more potent stimulus to renin secretion than hypoxaemia (Wood, 1995).

Factors acting at the local level are also released in response to hypoxaemia. Both *in vitro* and *in vivo* studies show that NO is released in response to hypoxaemia (Busse *et al.*, 1993) and it is also released in the fetus (Green *et al.*, 1996). In response to fetal hypoxaemia, NO is important in regulating the increase in myocardial blood flow (Reller *et al.*, 1995), carotid blood flow (Green *et al.*, 1996) and cerebral blood flow (Iwamoto *et al.*, 1992).

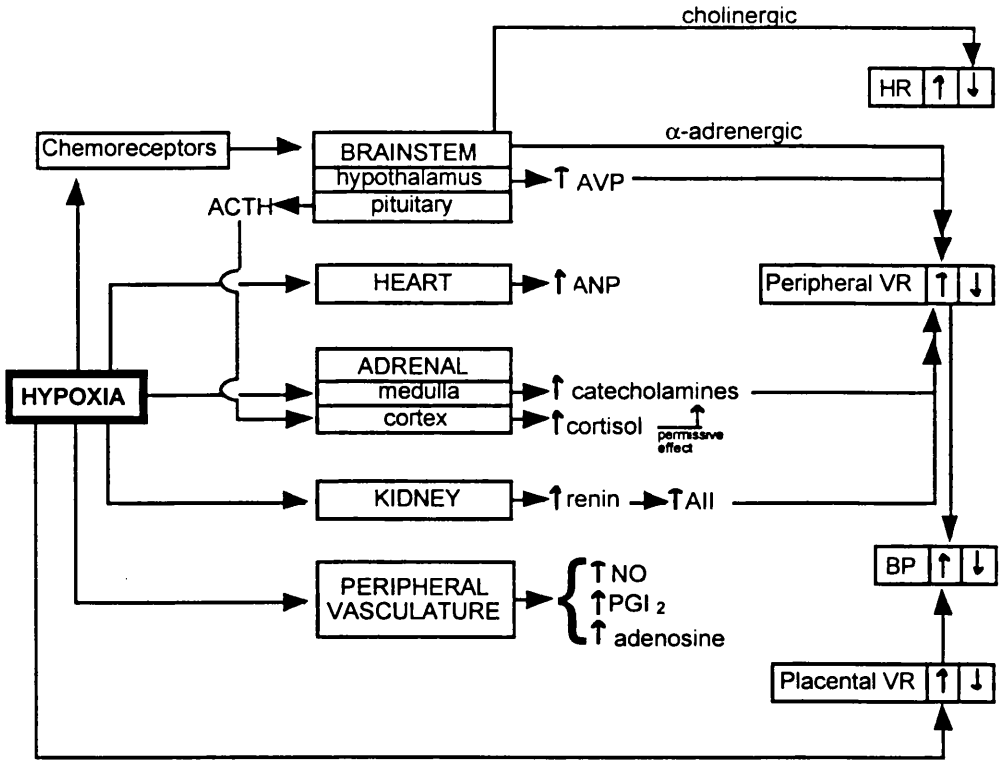

Figure 1.18. Diagram summarising the responses to acute hypoxaemia of the fetus.

1.12.2 Chronic hypoxaemia

The initial responses to chronic hypoxaemia are as one would expect in response to acute hypoxaemia, however, subsequently there is a return of heart rate and blood pressure to baseline levels (Bocking *et al.*, 1988). The reflex responses to chronic hypoxaemia have not been extensively studied and are not well understood, however there is information on the pattern of blood flow and endocrine responses. Redistribution of blood flow to the brain, heart and adrenals is maintained for at least 48 hours (Bocking *et al.*, 1988) and there is a sustained increase in ACTH and cortisol for up to 8 hours (Richardson *et al.*, 1996). Progressive hypoxaemia for up to 10 days is associated with elevated plasma noradrenaline concentrations (Gagnon *et al.*, 1994), and when prolonged for up to 21 days there is also an increase in ACTH and cortisol concentrations in the plasma (Murotsuki *et al.*, 1996b).

1.13 CARDIOVASCULAR TRANSITIONS AT BIRTH

There are a large number of changes that take place at birth enabling the newborn to adapt to extrauterine life. To remain within the confines of this thesis I shall describe only the cardiovascular adjustments that take place.

1.13.1 Circulation

After birth, when the umbilical cord is severed and thus umbilical blood flow ceases, there is a very large fall in blood flow through the *ductus venosus* and gradually within a matter of hours (human) or a few days (sheep) the *ductus venosus* becomes functionally closed. However, the *ductus venosus* is not physically closed until about 20 days after birth in the human infant. There is a rapid cessation of blood flow through the *foramen ovale* secondary to the decreased pulmonary resistance and large increase in pulmonary blood flow after the first breaths have been taken. Despite this more or less immediate functional closure of the *foramen ovale*, left-to-right shunting can persist for several days. In fact, anatomical closure of the *foramen ovale* usually takes up to a year to occur. The *ductus arteriosus* becomes functionally shut shortly after the first breaths of air and is anatomically shut within a few hours in both sheep and human newborns. Thus, ventilation and oxygenation, which result in a decrease in pulmonary vascular resistance, and umbilical cord occlusion, which removes the placenta (a low resistance vascular shunt) and results in an increase in systemic vascular resistance, result in the transition from the fetal to the immediate neonatal circulatory pattern (Teitel, 1988; Walker, 1993). Figure 1.7 shows the circulatory changes that take place after birth.

1.13.2 Blood pressure and heart rate

The structural changes in the circulation described above are accompanied by alterations in blood pressure, heart rate and the cardiovascular reflexes.

Removal of the placenta causes a dramatic increase in systemic vascular resistance. There is also a steep increase in FHR (Fig. 1.8). These two factors are major contributors to the rise in blood pressure that also occurs after birth. As the animal matures heart rate decreases until it reaches adult levels and there is an increase in blood pressure to adult values. (Walker, 1993).

1.13.3 Cardiovascular reflexes

Similar to the pattern of development seen in the baroreflex during late gestation, at birth (when there is a large increase in MAP) and during the first few weeks of postnatal life (when MAP is gradually increasing), the sensitivity of the baroreceptors decreases and they reset, seen as a shift to the right of the baroreflex curve and a decrease in its slope (Tomomatsu & Nishi, 1982; Blanco *et al.*, 1988; Segar *et al.*, 1992). At birth, the rise in PaO_2 and the decrease in $PaCO_2$ silences the chemoreceptors, which reset to their adult sensitivity over the first few postnatal days (Blanco *et al.*, 1984). This is an important adaptation for the neonate to make to its new hyper-oxygenated environment, compared to the *in utero* environment.

1.14 GROWTH AND DEVELOPMENT *IN UTERO*. DOES IT IMPACT ON ADULT HEALTH?

Above I have described normal placental growth and development, and the growth and cardiovascular development of the normal healthy fetus. Unfortunately, however, not all fetuses are exposed to an optimum uterine environment, which means that their growth and development is affected, so they may be born growth retarded or with other abnormalities. Cerebral palsy, a tragedy of the newborn period, is the result of perinatal events in 10% of cases, and is highly associated with low birthweight (Cunningham *et al.*, 1993). Sudden Infant Death Syndrome (SIDS), which is one of the leading causes of infant mortality between the ages of one and twelve months in the U.S.A., killing 1-2 of every 1000 live births, is thought to be the consequence of abnormal fetal development (Nathanielsz, 1992).

Over the last decade or so, there has been growing interest in the possible consequences of fetal development on pathological conditions, not only of newborn infants and children but also of adults. Diseases of particular interest have been hypertension, coronary heart disease and stroke, non-insulin dependent diabetes, and chronic bronchitis. Traditionally, these diseases that have been presumed to be either genetically inherited and/or caused as a result of adult lifestyle factors.

I would now like to present the evidence in humans, for and against the notion that events *in utero* may predispose to disease in later life.

1.14.1 Evidence in support

1.14.1.i Perinatal nutrition and various diseases in later life

Low birth weight (an indication of reduced fetal growth) is a risk factor for perinatal morbidity and mortality (Danielian *et al.*, 1992), and in 1967 Gruenwald (1967) made the suggestion that socio-economic factors have a prominent influence on fetal growth. He made a study of the birth weights of Japanese children born in the years 1945-46 (early post-war period), 1957-58 and 1963-64. He observed a large increase in birthweights in the 12 years between 1945-46 and 1957-58 and another increase, albeit smaller, in the 6 years between 1957-58 and 1963-64. This improvement in birth weights may have been related to better nutrition associated with improving socio-economic status in the years after the war. Gruenwald (1967) addressed the question of whether environmental factors affect fetal growth, so he did not investigate whether improved fetal growth was accompanied by improved health. However, the subsequent studies of Professor Barker and his group in Southampton suggest that this may indeed have been the case, as they have made observations linking various cardiovascular diseases (which I will discuss below) and non-insulin dependent diabetes with impaired growth during fetal life and infancy. They have suggested that reduced growth and development *in utero* may be the result of maternal nutrition before and during pregnancy (Barker, 1994). Other workers (McCance *et al.*, 1994; Lithell *et al.*, 1996) have also associated low birth weight with non-insulin dependent diabetes. Neurodevelopment (Lucas *et al.*, 1989; Amiel-Tison & Pettigrew, 1991), schizophrenia (Munk-Jorgansen & Mortensen, 1993) and retarded social behaviour (Osofsky, 1975) have been associated with a poor *in utero* environment and poor nutrition early in life. There have also been reports suggesting that prenatal factors are important influences for the risk of breast cancer (Ekbom *et al.*, 1992) and prostate cancer (Ekbom *et al.*, 1996). Thus, clearly there is support for the idea that the prenatal environment influences neonatal and adult outcome. I shall now discuss the evidence in more depth, but, to remain within the confines of this thesis, I shall concentrate only on the factors relating to cardiovascular events.

1.14.1.ii Perinatal nutrition and cardiovascular disease

Ten years ago, epidemiological findings linked coronary heart disease and death from stroke with fetal growth and development (Barker & Osmond,

1986 & 1987; Barker *et al.*, 1989a; Barker, 1990, Barker, 1991). It was found that infant mortality in 1921-25 was highest in poor rural areas and some northern industrial towns and, furthermore, that death from ischaemic heart disease in 1968-78 followed a similar geographic distribution. Nutrition during early life was implicated as a causative factor, because the people living in those areas were generally of low socio-economic status. Maternal mortality (from causes attributable to pregnancy or childbirth, other than puerperal fever) was also highest in those areas, as a result of the poor physique and health of mothers, which was again attributed to poor nutrition. Thus, poor nutrition during pregnancy was suggested to be a risk factor for hypertension in offspring. Further evidence in support of these findings emerged as findings in 5654 men born during 1911-30 in Hertfordshire showed that death rates from ischaemic heart disease were highest in those men with the lowest birth weights (Barker *et al.*, 1989b; Barker, 1991), and 16000 men and women born in Hertfordshire showed a similar relationship of cardiovascular disease with birth weight (Osmond *et al.*, 1993). Another study showed similar findings in children too (Barker *et al.*, 1989c). There was an inverse relation between systolic blood pressure and birth weight, in a group of 10 year old children and 36 year old adults.

1.14.1.iii Initiation and amplification

The relationship between blood pressure and birth weight described above (Barker *et al.*, 1989c) was found to be stronger in adults than children. This suggests that factors acting both before and after birth are involved in the aetiology of cardiovascular disease. This was investigated in a study of children aged 0-10 years who were followed-up at 4 days, 6 months, 1 year and every year thereafter, and adults aged 36 years, 46-54 years, and 59-71 years (Barker *et al.*, 1993b). In all age groups people with lower birth weights had higher systolic blood pressure. But, blood pressure was highest in those people who were small at birth and became heaviest later on, as was also found to be the case in Swedish men (Leon *et al.*, 1996). A further finding of the study was that the relation between systolic blood pressure and birth weight became stronger with increasing age, as evidenced by improved correlation coefficients. Thus, it appears that high blood pressure is initiated during intrauterine life, that it is amplified during life after infancy, and that factors after infancy are also contributory.

1.14.1.iv An effect mediated by the placenta

Further information came from a group of 449 men and women born in Preston during 1935-43 and still living in the area (Barker *et al.*, 1990; Barker, 1990). The same association as had previously been observed between low birth weight and high blood pressure was found. But, it was also observed that the highest blood pressure occurred in those people who were not only small at birth but also had a large placenta. In a study of 8684 women who gave birth in Oxford during 1987-89, Godfrey *et al.* (1991) showed that high placental/birth weight ratio was associated with low maternal haemoglobin levels. Maternal anaemia was linked to low socio-economic status, so probably reflected poor nutrition. Thus, Godfrey *et al.* (1991) suggested that maternal anaemia during pregnancy, as a result of poor nutrition, may have implications for the development of hypertension in offspring. This was supported by a study carried out in Kingston, Jamaica, where it was found that there was an inverse relationship between maternal haemoglobin during pregnancy and children's mean systolic blood pressure measured at 10-12 years of age (Godfrey *et al.*, 1994). Children's blood pressure was also inversely related to maternal triceps skinfold thickness and weight loss during pregnancy, so further implicating maternal nutrition as a potential cause for reduced growth *in utero*. There have been other studies that have associated decreased placental weight and low birth weight with maternal nutritional status both prior to (Martyn *et al.*, 1996) and during pregnancy (Stein & Susser, 1975a & b; Rosso *et al.*, 1992; Godfrey *et al.*, 1996; Campbell *et al.*, 1996). Thus, it appears that placental size, large or small, is predictive of adverse outcome. Certainly placental hypertrophy as a result of severe maternal anaemia is associated with low birth weight (Beischer *et al.*, 1970). High maternal haemoglobin levels during pregnancy are also related with poor pregnancy outcome (Garn *et al.*, 1981; Rasmussen & Øian, 1993), which suggests that anaemia *per se* is not the cause of unfavourable neonatal outcome, but that the placenta is an important factor. This is further borne out by the finding that maternal haemoglobin and ferritin concentrations at 14 weeks gestation are inversely related to placental volume measured at 18 weeks gestation (Howe *et al.*, 1995). Furthermore, haemoglobin concentration is inversely related to the concentration of human chorionic gonadotrophin (hCG) and human placental lactogen (hPL) measured at 10 weeks gestation (Wheeler *et al.*, 1994), both of which are important during embryonic and fetal development (see section 1.3.2.ii).

Reduced blood flow in the umbilical vein may be the result of increased placental resistance. It has been found that umbilical vein blood flow at caesarean section correlates positively with birth weight (Konje *et al.*, 1996), which suggests that reduced growth *in utero* may be the consequence of increased placental resistance. Placentae from IUGR pregnancies associated with chronic fetal hypoxaemia were found to have abnormal structural features (Macara *et al.*, 1996). Also, reduced placental volume, surface area, and number of cotyledons have been observed in placentae from women who were undernourished during pregnancy and gave birth to growth retarded babies (Murthy *et al.*, 1976).

1.14.1.v Body proportions

Another study (Barker *et al.*, 1992a) of men and women born in Preston between 1935 and 1943 has shown that not only is altered placental growth a risk factor for hypertension, but so are altered body proportions at birth. It was found that thinness at birth (low ponderal index) and increased head circumference/length ratio at birth were associated with elevated systolic blood pressure in adulthood. This pattern of growth, resulting in asymmetrical growth and wasting is suggestive of factors acting during late gestation (see section 1.7.1). However, later studies have related small head circumference and thinness (Barker *et al.*, 1993) or low weight (Stein *et al.*, 1996) at birth, i.e. symmetrical growth, to death from cardiovascular disease. Symmetrical growth retardation is associated with events early in gestation (see section 1.7.1).

1.14.1.vi Effects other than on blood pressure

There are yet more studies linking poor nutrition early in life with low birth weight and poor development (Prentice *et al.*, 1983; Lucas, 1990; Cogswell & Yip, 1995) and with high blood pressure (Forrester *et al.*, 1996; Taittonen *et al.*, 1996). However, added support for the intrauterine origins of cardiovascular disease comes from findings that nutritional deprivation during pregnancy results in altered vascular structure that may be associated with perturbed cardiovascular development *in utero*. For instance, arterial compliance in the conduit arteries of the trunk and legs, measured by pulse wave velocity, has been found to be lower in men and women who were small at birth and who developed hypertension (Martyn *et al.*, 1994). This is interesting because of the fact that changes in compliance may be the result of

altered hemodynamics during fetal life, as demonstrated by findings that children who had only one umbilical artery *in utero* have anomalous iliac compliance (Berry *et al.*, 1976). Altered fetal hemodynamics may also be implicated in the left ventricular hypertrophy observed in people who had high systolic blood pressure, low birth weight, and reduced growth rate during infancy (Vijayakumar *et al.*, 1994; Zureik *et al.*, 1996). Blood pressure development may also be affected by perturbations of the renin-angiotensin system. Of interest in this regard is the finding that plasma angiotensin II levels were elevated in IUGR babies (Kingdom *et al.*, 1993), and that asymmetrical growth retardation has been associated with decreased nephron number (Hinchliffe *et al.*, 1992). It may be that asymmetrical IUGR babies have abnormal kidneys and perturbed functioning of the renin-angiotensin system that could perhaps result in abnormal blood pressure development. Lastly, an association has also been made between low birth weight and high plasma concentrations of fibrinogen which is involved in the clotting of blood and is produced by the liver (Barker *et al.*, 1992c), and small abdominal circumference perhaps reflecting decreased liver growth, and increased death from coronary heart disease (Barker *et al.*, 1995).

1.14.1.vii Summary of evidence in support

There is a definite association of maternal nutrition during pregnancy with restricted intrauterine growth and hypertension in later life. There would appear to be critical periods during pregnancy that determine outcome, but their timing is as yet uncertain in relation to blood pressure development. Also, uncertain is the relative contribution of factors acting in early infancy and adolescence.

1.14.2 Evidence against

1.14.2.i Nutrition in utero is not associated with later blood pressure

Lucas & Morley (1994) and Morley *et al.* (1994) challenged the view that blood pressure in later life is related to fetal nutrition and growth, on the grounds that, in preterm babies, they could find no association of high blood pressure with low birth weight, or with extremes of both protein and energy intake during early life. The babies in their studies were born prematurely at 34 weeks gestation, thus they suggested that if the events associated with

reduced fetal growth programme for hypertension, then early postnatal nutrition should be expected to influence later blood pressure. Similarly, Seidman *et al.* (1991) found no strong association of birth weight with blood pressure at 17 years of age in men and women born in Jerusalem.

1.14.2.ii Postnatal factors and blood pressure

The Jerusalem study (Seidman *et al.*, 1991) found that high blood pressure was more closely related to a high current body weight than to birth weight. It is possible, however, that their findings were confounded by the age (17 years) at which they chose to study their subjects, because of the growth, hormonal and blood pressure changes that take place at around that time. Nonetheless, although Taittonen *et al.* (1996) found that birth weight was associated with blood pressure in Swedish children and adolescents between 3 and 18 years old, they also found an association between blood pressure and other factors such as current age and weight, the duration of breast feeding, birth rank, and maternal hypertension during pregnancy. Thus, they suggested that these other pre- and post-natal factors may be important predictors for future blood pressure, as well as birth weight. Similarly, Whincup *et al.* (1991 & 1994) found that whilst birth weight was associated with adult blood pressure, events during childhood and adult lifestyle factors were of greater importance. Further studies (Shaper & Elford, 1991; Wannamethee *et al.*, 1996) are in support of this view. The British Regional Heart Study showed that long-term cardiovascular outcome is determined by the geographical location (associated with regional variations in socio-economic status and nutrition) in which individuals live later in life and not that in which they are born (Shaper & Elford, 1991).Wannamethee *et al.* (1996) showed an association between middle-aged men whose father's social class was manual and ischaemic heart disease. They suggested that this may be a reflection of socioeconomic status during childhood.

1.4.2.iii Summary of evidence against

Clearly there are studies that do not support the hypothesis that intrauterine events determine the risk for cardiovascular disease in later life. Despite this, however, it seems that there is some agreement that perinatal events may be important for future blood pressure development.

1.15 AIMS OF THE WORK REPORTED IN THIS THESIS

The search for the causes of coronary heart disease and the attempt to prevent disease in the next generation has necessitated investigation into the observations of Barker and his colleagues (Barker, 1994). Their initial observations (Barker *et al.*, 1990) suggest that an adverse intrauterine environment, as the result of poor maternal nutrition, has an impact on fetal growth and development and is involved in the aetiology of cardiovascular disease in adult life.

Nutrition is an aspect of welfare that has attracted much attention for many generations and it is as much an issue today as it has ever been. It is generally accepted that improved living standards in the Developed World over the past hundred years or so have had a significant positive effect on nutrition, particularly of those people in the lower socio-economic classes; but, malnutrition due to poverty is still a major problem in the Third World. Undernutrition in women living in industrialised countries is not, however, uncommon. The fashionable concept of 'thin is beautiful' means that there are those people who choose to eat insufficiently to achieve this goal. The ideal shape and weight, as portrayed by womens' magazines, is, for many people, below their optimum condition. Anorexia nervosa is a growing problem, particularly in young women of child-bearing age, with a doubling in incidence over a 10 year period between 1970 and 1980 to 0.64 per 100,000 of the population (Thompson, 1993). Anorexic women who become pregnant give birth to growth retarded babies, often prematurely (Brown *et al.*, 1981; Treasure & Russell, 1988). Over-eating and obesity are also a common problem in today's affluent societies. In the U.S.A. 13.5% of the adult population are overweight, 37% of these morbidly so. Maternal obesity is also associated with poor perinatal outcome. There is a proportion of the population who, whilst they are not undernourished, are malnourished. This results from an unbalanced diet, high in fat and sugar content.

Ischaemia, coronary heart disease and stroke are the most common causes of morbidity and mortality in the West. The incidence of coronary heart disease in Britain in 1994 was 845 people per 100,000 of the population (Dept. of Health, 1996). Clearly, therefore, it would be a huge benefit to society if the incidence of these pathologies was decreased. Up to now governments and health workers have tried to address the problem by urging people to change their lifestyles by eating a diet lower in salt and fat, reducing alcohol intake,

not smoking, taking regular exercise, etc. However, if improving the health and nutrition of women of reproductive age, and therefore the nutrition of the fetus, produces greater long term benefits, then the implications are immeasurable. Quality of life for a large proportion of the general populous would be improved, and the huge amount of money currently spent by both individuals and governments on drugs, surgery, and both hospital and home care would be reduced. Clearly then, there is huge potential social and economic advantage in investigating the 'Barker phenomenon'.

Thus, the overall aim of this thesis was to elucidate the mechanisms involved in the development of fetal and neonatal blood pressure as a consequence of growth. My hypothesis was that maternal nutrition during pregnancy, operating via unknown mechanisms mediated by the placenta, results in altered fetal cardiovascular development and hypertension postnatally. In pursuing this question the aims of the study were threefold:

1) To investigate whether or not postnatal hypertension could be induced as a result of undernutrition *in utero*, in an animal model.

The general uncertainty surrounding the association of intrauterine events with hypertension made this a necessary starting point.

As described in Chapter 3, this aim was successfully achieved using the anaemic rat as a model. However, it was still only possible to speculate on the mechanisms involved. On the strength of my results, the association of hypoxaemia with IUGR in the human (Soothill *et al.*, 1986), and the well documented fact that, in the acute situation, hypoxaemia causes an elevation in blood pressure (Boddy *et al.*, 1974; Cohn *et al.*, 1974; Hanson, 1993), hypoxaemia seemed a likely cause.

2) To investigate the possible cause(s) linking maternal nutrition during pregnancy and blood pressure of the offspring.

Hypoxaemia being the likely cause, I studied the effects of repeated acute hypoxaemia on fetal cardiovascular development and fetal size. The results showed that repeated acute hypoxaemia of a moderate degree did not result in

significantly altered cardiovascular development (Chapter 4). However, it became clear that fetal blood pressure development may follow different trajectories, perhaps as a result of effects on the placenta caused by maternal undernutrition (Chapter 5).

3) To investigate the possible mechanisms involved linking maternal periconceptual undernutrition and fetal cardiovascular development .

In adressing this aim, I first established that there is a link between periconceptual maternal undernutrition and cardiovascular development in the fetus (Chapter 6). These findings were accompanied by alterations in placental gross structure, so I then went on to investigate more closely the effects of such an insult on placental gross morphology (Chapter 7).

Chapter 2

METHODS

2.1 GENERAL INTRODUCTION

Some of the work described in this thesis was carried out in a small animal model, using the rat, and the remainder was carried out in a large animal model, where the sheep was the species of choice.

There were several reasons why the rat was my initial choice of species. As stated in the introduction, my initial aim was to try to reproduce in an animal model the phenomenon whereby maternal nutrition resulted in hypertension in the offspring. The first criterion was to choose a species with a short gestational period and a rapid rate of development. Gestation in the rat is only 21 days, and rats reach sexual maturity quickly, by about 40 days of age. Another advantage of the rat is that average litter size is 11 pups, so achieving a reasonable number of observations can be achieved relatively quickly. The second criterion was that dietary intake could easily be manipulated and monitored. This is easily achieved in rats, as they can be housed individually. The final criterion for the study was to choose a species where technology was available to study blood pressure non-invasively in neonates and adults. In rats this is possible using a rat tail blood pressure monitor (see section 2.2.3.i). One of the drawbacks of the rat, from the point of view of my studies, is that the dam eats the placentae at birth, so it is not possible to study both placentae and pups. However, on balance, the rat seemed the obvious species to choose.

My findings in the rat (see later, Chapter 3) made it clear that it was necessary to study development *in utero*, particularly of blood pressure. Whilst hemodynamic measurements have successfully been measured in the rat fetus (Nakazawa *et al.*, 1988; Nakazawa *et al.*, 1985), the technique has not yet been developed whereby longitudinal recordings can be made. The reason that fetal measurements are difficult to obtain in rat fetuses is because of their small size. So, the species of choice needed to be one in which instrumentation is possible. The sheep, therefore, seemed the obvious species in which to carry out further investigations. As I mentioned in Chapter 1, much of the work pertaining to fetal physiology has been done in the sheep, therefore there is a large literature on the fetal sheep, particularly in late gestation. Secondly, since it first started to be used in the 1960's, the chronic sheep preparation has come to be a fairly standard procedure. The sheep also

lends itself to research because it is relatively docile and is therefore amenable to handling, it is not too large, and it tolerates anaesthesia well.

2.2 RATS

2.2.1 General

Sprague Dawley albino rats were used in all the following studies. Dams were housed singly in plastic cages with stainless-steel mesh lids, which contained sawdust as a bedding material. They were kept in the animal house under conditions of constant temperature (20°C) and humidity (55%), and a fixed 12 hours light/12 hours dark cycle. Food and water were available *ad libitum*. Each dam had a number written on her tail in permanent ink for identification. All dams were about 16 weeks old and had had one successful pregnancy previously.

For mating, the female and male rats were put in a cage that had a stainless-steel mesh floor beneath which was a tray. Conception was judged to be the day on which a vaginal plug was observed in the tray. A vaginal plug is a mixture of coagulated sperm and cells that is formed after copulation. It hardens whilst in the vaginal cavity then falls out, though it may remain inside for up to twelve hours. After plugging the female was removed and placed back in her cage.

When the pups were born they remained with their mothers until weaning at 21 days of age. Pups were identified by ear-punching.

2.2.2 Diet

Control (C) rats were fed a diet of normal rat chow *ad libitum*. Rats in the anaemic (AN) group, also fed *ad libitum*, were on a low-iron diet which contained less than 6 p.p.m. iron (Special Diet Services).

2.2.3 Apparatus

2.2.3.i Indirect rat tail blood pressure monitor

Maternal and neonatal systolic blood pressures were measured using a rat tail blood pressure monitor (Harvard Apparatus Ltd., Edenbridge, Kent, U.K.).

The set up consists of a holding tube in which the rat is placed and restrained so that it can be connected to the monitor (Fig. 2.1).

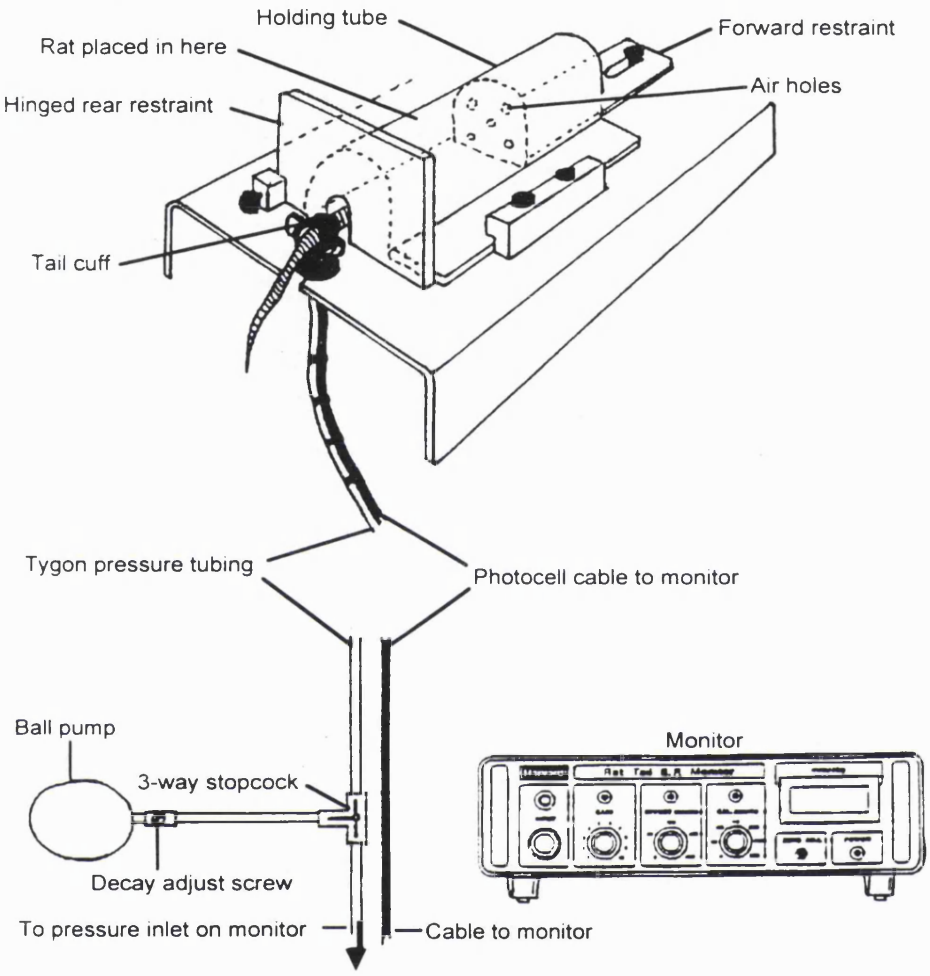

Figure 2.1. Diagram showing the set-up for measuring blood pressure in the rat using an indirect rat tail blood pressure monitor.

The blood pressure measuring device itself is an electronic version of the traditional sphygmomanometer cuff that is used to determine human blood pressure indirectly. The rat tail blood pressure monitor consists of an inflatable latex occlusion cuff that contains a light source and a photo detector. There are different sizes of cuff and the one with the most appropriate diameter for the size of rat is selected. The cuff, placed around the base of the tail of the rat, is inflated using a hand inflation bulb and this causes the blood flow to become occluded. The photo cell in the cuff detects the flow. A

pressure transducer in the monitor amplifier, to which the cuff assembly is connected, converts pressure to an analogue voltage. The blood flow changes are also converted into a voltage. Thus, both pressure and flow can be recorded onto a chart recorder which is connected to the amplifier. The cuff is inflated to a pressure that occludes blood flow and is then allowed to deflate slowly until blood flow waveforms (which are pulsatile) are observed on the pulse channel of the recorder. Both cuff pressure and flow are recorded simultaneously, and the pressure at which flow first appears is considered to be the systolic blood pressure (Fig. 2.2). It is evident from looking at Figure 2.2a) that there is no absolutely definitive point on the trace where blood flow first appears which can be said to define SBP, and the point chosen is dependent upon the experience and consistency of the investigator. This can therefore contribute to error in evaluation of the pressure. It is important that the rat is acclimatised to the apparatus prior to pressure measurement so that its blood pressure is not elevated due to stress. It is also a necessity to keep the rat still, as any body movement results in movement artefact on the trace (Fig. 2.2b) and thus makes it impossible to determine systolic blood pressure.

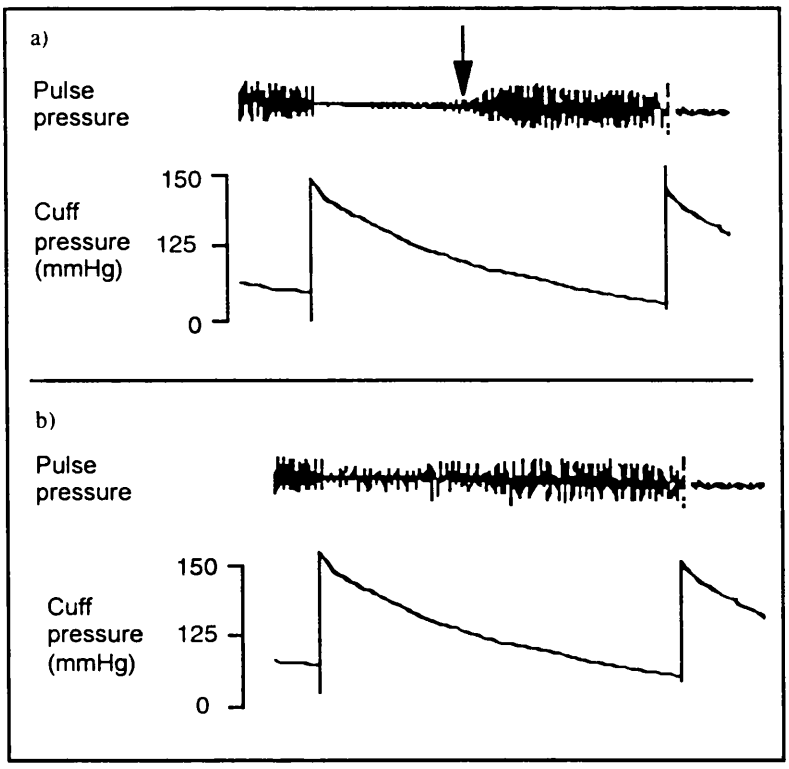

Figure 2.2. Examples of blood pressure recordings made using the rat tail blood pressure monitor. a) This represents a good pulse pressure recording (upper part of trace) from which SBP could be detected. The arrow indicates the the point at which SBP was measured. b) This trace, however, cannot be used as there is movement artefact making it impossible to detect the point at which the flow becomes pulsatile and thus to determine the SBP.

2.2.3.ii Haemoglobin measurement

Haemoglobin levels of the rats were determined using the cyanmethaemoglobin method (Sigma Diagnostic Kit) which was developed by Drabkin and Austin (1932). This is a quantitative colourimetric test involving the conversion of haemoglobin to its cyano-derivative. The principle of the method is that haemoglobin reacts with Drabkin's reagent (potassium cyanide, potassium ferricyanide and sodium bicarbonate) and is converted into methaemoglobin by the action of the ferricyanide. The methaemoglobin then reacts with cyanide to form cyanmethaemoglobin and

the absorbance of this derivative at 530-550 nm is proportional to the total haemoglobin content of the blood.

The concentration of total haemoglobin in blood was calculated from a standard curve (Fig. 2.3), which was derived each time a new stock of Drabkin's reagent was made up. The standard curve was generated by preparing working standards containing known concentrations of haemoglobin (Sigma, from bovine blood), measuring each of their absorbencies at 540 nm using a spectrophotometer (Kontron Uvikon 810), then plotting the absorbance values obtained against haemoglobin concentration.

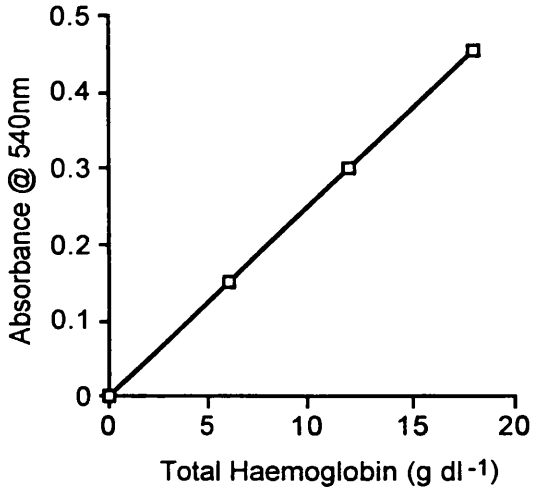

Figure 2.3. Example of a haemoglobin standard curve used to calculate the concentration of total haemoglobin in rat blood.

2.2.4 Experimental protocol

2.2.4.i Fetal and placental measurements

At 20 days of gestation, just prior to the expected day of birth (21 days), the pregnant dams were placed in an air-tight perspex box, with an inlet at one end attached to a gas supply and an outlet at the other end. They were then killed using 100% CO_2. (Home Office Schedule 1).

Fetal and maternal body weights and haemoglobin levels, and placental weight were then recorded. Blood samples for fetal haemoglobin measurements were collected into capillary tubes after decapitation of the fetuses, the blood from up to four fetuses being pooled so as to obtain an adequate volume for the assay. Maternal blood samples were obtained by puncturing the tail vein and collecting the blood in a heparinised capillary tube.

In preparation for weighing, the placentae were separated from the fetuses, membranes were removed, and they were blotted dry.

2.2.4.ii Maternal and neonatal measurements

Both maternal and neonatal blood pressures were recorded using the tail cuff method. At least three readings were obtained for each animal. Systolic blood pressure was calculated from the mean of three recordings obtained. When more than three recordings were made, three were selected at random to obtain the mean.

Blood samples for haemoglobin measurement were obtained from the tail vein. This was done by pricking the tail vein and collecting the blood in a heparinised capillary tube.

2.2.4.iii Organ weights

Neonatal rats were killed in the CO_2 killing chamber. The heart, lungs, liver and kidneys were then removed from each animal, blotted dry and weighed.

2.2.5 Data analysis

2.2.5.i Blood pressure

Blood pressure values for each animal were taken as the mean of three recordings. The individual means were then pooled for each group, C and AN, to obtain a group mean.

2.2.5.ii Weights

The individual body and organ weights for each animal were pooled for each group and a group mean for the different weights calculated .

2.1.6 Statistics

In all cases there was considered to be a statistical difference when $P < 0.05$.

The individual mean SBP recordings for AN and C groups showed a Normal distribution (Fig. 2.4).

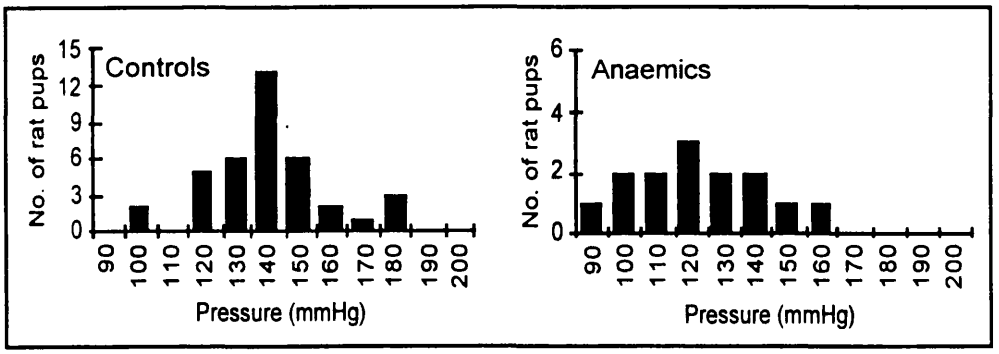

Figure 2.4. The Normal distribution of mean SBP in C ($P < 0.05$, χ^2 test) and AN ($P < 0.05$, χ^2 test) groups.

Statistical differences in SBP between AN and C animals in each group could therefore be analysed using a parametric method. The statistical method used was Student's unpaired *t*-test.

Statistical differences in the various weights measured betweeen the two groups were also analysed using Student's unpaired *t*-test.

2.3 SHEEP

2.3.1 General

The sheep used in Chapters 4, 5 and 6 were a Mule cross, the Scottish half breed (Border Leicester x Cheviot), and those used in Chapter 7 were Clun Forest. The advantage of the pure-bred Cluns over the Mules is that there is less genetic variation.

Mating was time dated so that the conception date, and thus length of gestation, of all ewes was known. This was achieved by the use of sponges.

Sponges are progestogen-impregnated pessaries that are inserted intravaginally for 12-14 days. The ewes are put to the tup about 48 hours after removal of the sponges. Those ewes that were required to conceive out of season i.e. July, August, September, were administered a follicle stimulating hormone, pregnant mare's serum gonadotrophin (PMSG), at the time of sponge removal. The dose of PMSG injected was 300-750 iu PMSG, depending on the month of tupping

An ultrasound scan was performed on each ewe at about 60 days of gestation to identify any that were barren, to confirm gestational age, and to ascertain the number of fetuses.

Ewes were brought into the animal house at least two days prior to surgery so that they could acclimatise. They were housed first in a holding pen (maximum capacity of four adult sheep) then individually in metabolic crates in rooms of a constant temperature and humidity with a fixed light/dark cycle. They were fed hay and water *ad libitum* and were given a daily ration (approx. 600g) of sheep nuts.

2.3.2 Materials

2.3.2.i Catheters

Both fetal and maternal catheters were made of transparent vinyl tubing (Portex) with an internal diameter (ID) of 1.0 mm and an outside diameter (OD) of 2.0 mm. An 18 gauge blunt needle (Sherwood Medical) was inserted into the distal end of the tubing and the proximal end was bevelled. All catheters were 1.5m long and were colour coded at each end for identification (Fig. 2.5). A lockable three-way stopcock was attached to the distal end of each catheter.

2.3.2.ii Electrodes

EMG electrodes were made of a 90 cm length of Portex transparent vinyl tubing (ID 1.5 mm, OD 2.1 mm) through which two 1.1m lengths of teflon-coated multi-stranded stainless steel wire (Cooner Wire Co., U.S.A.) were threaded, so that there was about 2 cm of wire at the distal end and 18 cm at the proximal end. Each end of the tubing was sealed by injecting silicone sealant (RS Ltd.) into it for distance of about 2 cm. At the proximal end a double knot was tied in each wire 5-6 cm from the end of the tubing, and a

few millimetres of teflon coating was removed immediately below each knot (Fig.2.5). A gold pin was soldered onto each of the distal wires. All electrodes were colour coded at each end.

Cortical (ECoG) electrodes were similar to EMG. electrodes, but had three wires rather than two, the third wire being an earth. At the proximal end, the earth wire was knotted and bared in the same way as the wires on the EMG electrodes. The signal wires, however, had a rubber disc slid onto each of them, they were double knotted in the same way as the earth wire and all of the remaining length of wire below the knots was bared and rolled up into a ball.

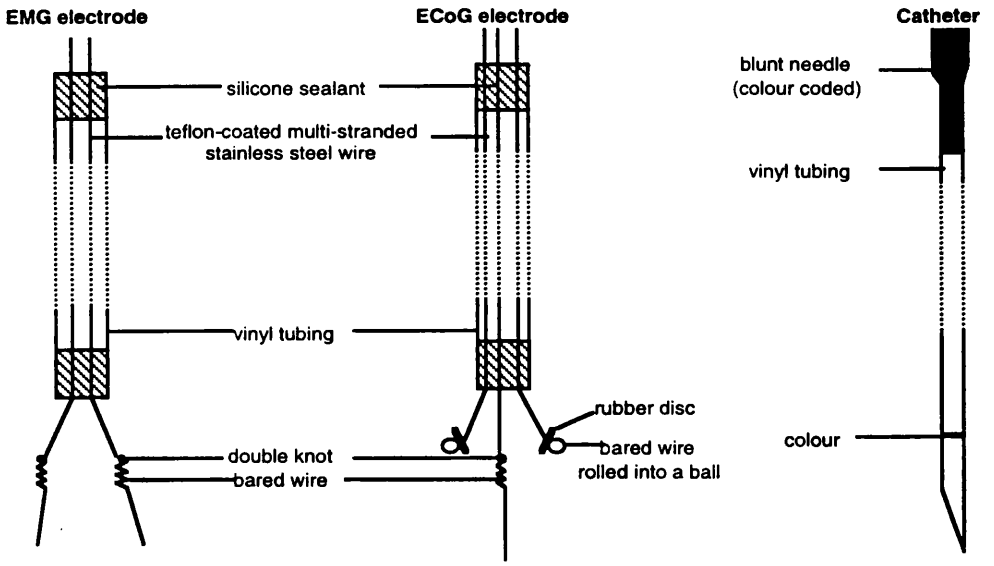

Figure 2.5. Diagram of EMG and ECoG electrodes and a catheter.

2.3.2.iii Flow probes

Chronically implanted perivascular flow probes (Transonic Systems Inc., Ithaca, NY, USA) were used to measure volume flow. Size 2R and 3R probes were used, with either "U" or "L" reflectors (Fig. 2.7). I found that sometimes the vessel would slip out of the probe with the "L" reflector, therefore it was preferable to use a "U" reflector. There was a silicone flange

attached to the probe body so that the probe could be sutured to the surrounding tissue for anchorage so as to ensure the minimum of movement.

Calibration of the probes is carried out at the factory using a gravity-fed constant flow set-up. The fluid used is water at room temperature, which is circulated through tubing attached to a thin-walled latex tube within a water bath and around which is positioned the perivascular flow probe for calibration. The flow profile is always laminar. The calculated flow through the system is plotted against the flow measured by the probe to obtain a calibration curve (Fig. 2.6).

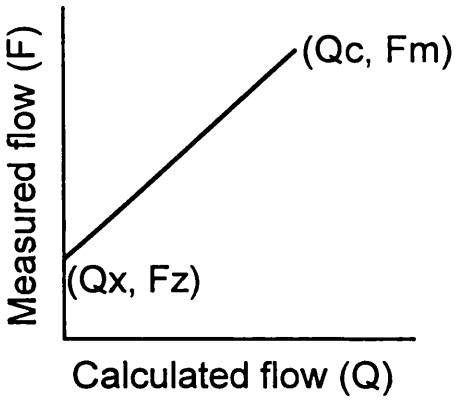

Figure 2.6. Calibration curve for Transonic flow probes.

The point at which the calibration line crosses the y-axis gives the zero offset of the probe. The slope of the resulting line, which should approximate 1, is then calculated:

$$\text{Slope} = \frac{Fm - Fz}{Qc}$$

Prior to surgery, each probe was assembled and placed in water where it was ensured that the zero offset was within the unadjusted flow error specified by the factory. If it was not, that probe was not implanted.

Theory of operation

Transonic flow probes consist of a probe body in which are housed two ultrasonic transducers and to which is attached a fixed acoustic reflector. The vessel lies between the probe body and the reflector, which is midway between the two transducers (Fig. 2.7).

A plane wave of ultrasound, emitted from one of the transducers, intersects the vessel, bounces off the reflector, again intersects the vessel, and is received by the other transducer where it is converted into an electrical signal. The flowmeter derives an accurate measure of the 'transit time' it took for the ultrasonic wave to travel from one transducer to the other. The motion of the blood flowing through the vessel affects the transit time of the ultrasonic signal, much as water current affects the speed of a swimmer. This transmit-receive sequence is repeated alternately in upstream and downstream directions. The integrated difference in transit time between the upstream and downstream signals gives the volume flow (rather than the velocity).

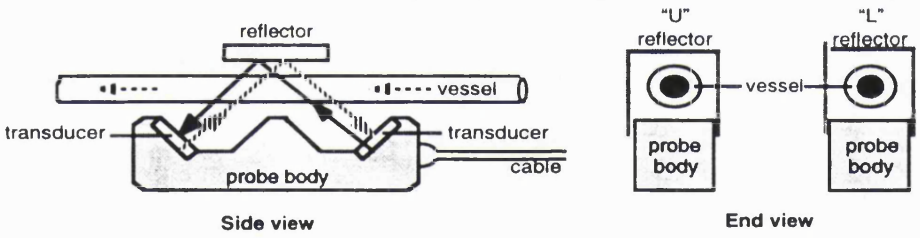

Figure 2.7. Schematic views of the perivascular Transonic flow probe sensor, showing operation and "U" and "L" reflector types.

2.3.3 Animal preparation and anaesthesia

Ewes were starved 12 hours prior to surgery. Anaesthesia was induced by an injection of thiopentone sodium (Intraval sodium, Rhône Mérieux Ltd., Harlow, Essex, UK; 1.2 g in 12 ml) into the jugular vein of the ewe, her neck having been shaved first. The ewe was then placed on her back on the operating table and was intubated with a cuffed tracheal tube (9.0 mm ID, Portex) through which 3-4% halothane (Fluothane, ICI, UK) in oxygen (2l/min) was administered. Each leg of the ewe was then secured. Once the

ewe was completely anaesthetised, which was established by loss of the corneal reflex, absence of a response to a noxious stimulus such as pinching the ear lobe, and lack of postural muscle tone in the limbs, halothane delivery was reduced to 2-3% for maintenance of anaesthesia. Artificial ventilation was not used unless a ewe did not breathe spontaneously.

Having established stable surgical anaesthesia, the abdomen and one hind leg were shaved and the skin was scrubbed with Povidine antiseptic solution (BK Veterinary Products, Bury St. Edmunds, UK) and water and sprayed with a 2% solution of chlorhexidine (Hibitane, ICI Pharmaceuticals, Macclesfield, Cheshire, England) in IMS. After this, the ewe was draped with a sterile laparotomy drape, so that all unclean areas were covered, leaving only a window for the midline incision to be made. All the surgeons' work surfaces were also covered with sterile drapes.

2.3.4 Fetal instrumentation

Strict aseptic conditions were maintained during surgery, all surgeons' gowns, instruments, catheters and electrodes having been autoclaved, and flow probes cold-sterilised in a solution of Novasapa (Willows Francis Veterinary, Crawley, W.Sussex. England).

A midline skin incision of about 10 cm was made in the maternal abdomen using a scalpel with the aid of electrocautery, avoiding the mammary veins. The abdominal cavity was opened along the line of the *linea alba*, using a pair of blunt, curved scissors. The uterus was then located, the number of fetuses confirmed and its/their position ascertained.

Next, the electrodes, catheters and flow probe were inserted into the maternal abdominal cavity. This was done by pushing a trochar and cannula through the ewe's peritoneum and abdominal wall, making sure that there was no gut in the way, so that they exited on the maternal flank. The trochar was removed, leaving the cannula, and the electrodes etc. were passed through the cannula. The cannula was then removed.

The fetus could now be exteriorised. The fetal tail was located and, whilst holding on to it, a 4-5 cm incision was made in the uterus, running parallel to the uterine vessels and avoiding any placentomes to minimise bleeding. Babcock forceps were used to hold the edges of the wound and the amniotic

membranes, helping to reduce tearing of the wound and amniotic fluid loss, and the fetus was exteriorised.

The fetal hindquarters were exteriorised first and the flow probe was implanted. An incision was made in the fetal skin over the anticipated position of the femoral artery. The muscles were then dissected apart by blunt dissection to expose the femoral artery. The artery was carefully separated from the femoral vein and nerve and the probe was implanted around it. The probe was anchored in place by stitching the four corners of its silicone flange to the surrounding tissue (US 3.0 braided silk suture, size 20 curved round-bodied needle). The wound was then closed and the cable of the probe stitched securely to the fetus in two places (US 2.0 braided silk suture, size 16 curved triangular needle) to ensure that the probe was not dislodged.

The fetus was then put back into the uterus and turned around so that the fetal head could be exteriorised. Great care was taken to ensure that the cable of the flow probe did not get twisted around the umbilical cord. Once the fetus had been successfully rotated, the head was exteriorised.

The diaphragm electrode was the first to be implanted, so the front legs were also exteriorised and the fetus was pulled out until the lower margin of its rib cage was exposed, care being taken all the time not to occlude the cord. The diaphragm was exposed on the right hand side by an incision made in the skin and blunt dissection of the muscle between the third and the fourth most caudal ribs. The ribs were retracted using Allis tissue forceps. The two signal wires of the electrode were sewn into the diaphragm as far apart as possible, a double knot was made and the remaining wire cut, quite close to the knots so as to minimise abrasion. The ribs were then stitched together and the wound sutured closed (US 2.0 braided silk suture, size 16 curved triangular needle). The electrode lead was secured to the body in two separate places. The amniotic catheter was also secured to the fetus at the second anchor point.

The fetus was manoeuvred back into the uterus until only its forelimbs and upper body were exteriorised. The ECG electrode was then attached, one of each of the signal wires being sewn onto either side of the chest at the level of the heart. The cable was then secured in two places.

This having been done, the fetus was eased further back into the uterus so that only its head and neck remained exposed. An incision was made to one side of the trachea and the carotid vein and jugular artery were located by

blunt dissection. Each vessel was ligated distally (US 2.0 braided silk suture) and a second ligature tied in a loose loop about 1 cm centrally. A small hole was cut in the vessel using Vannas scissors and the catheter was inserted. The catheter were advanced along the inside of the vessel for a distance of about 7 cm. After testing the catheter to make sure that blood could be withdrawn and that saline could be flushed in, the central ligature was tied down, securing the catheter in the vessel.

The trachea was located by blunt dissection, a curved haemostat was placed underneath it to hold it up, and a ligature (US 1 braided silk suture) was tied loosely around it. A small hole was made in the trachea, between the cartilaginous discs, using a needle (size 6 half curved triangular) and the catheter was inserted and advanced for a distance of about 3 cm. The ligature was then tied around the catheter to secure it in the trachea. The wound was then closed and the catheters secured together at two points on the neck of the fetus.

The fetal head was then placed prone and an incision of about 3 cm made in the skin on the top of the skull to enable ECoG electrode placement. The skin was peeled back from the skull and the bone was cleared of fascia using a swab. Bone wax (Ethicon) was used to stem any bleeding of the bone. Holes were then drilled bilaterally through the skull about 2 cm lateral to the mid-line suture and about 0.5 cm posterior to bregma. The signal wires of the electrode (rolled up into a ball) were inserted into the holes to lie on top of the dura and the rubber discs were pushed down over the holes and glued into place with cyanoacrylate adhesive (RS Components, Corby, Northhants., UK), securing the electrodes within the holes. The head wound was then glued closed, ensuring that as little glue as possible was exposed on the top of the head, as it sets hard and is abrasive to the uterus. The earth wire was sewn into the scalp, as were the two wires of a separate earth electrode. The two electrodes were then secured firmly at two points on the fetal neck.

The fetal instrumentation was now complete and the fetus was put back in the uterus in the same position as it was found. The uterus was sutured closed (US 2.0 braided silk suture, size 16 curved round bodied needle) around the various catheters and leads, taking care to pick up all the amniotic membranes. The wound was then oversewn, to ensure that there would be no leakage of amniotic fluid or rupture of the wound. The catheters and leads were pulled to ensure adequate length on the exterior, but at the same time

ensuring that there was enough length remaining inside to allow for inadvertent pulling and fetal movement. The maternal peritoneum was then securely closed (umbilical cotton tape, medium curved gallie needle), and the skin wound closed (US 1 braided silk suture, size 4 straight triangular needle).

The vascular catheters were heparinised by flushing heparinised saline (1000 iu heparin in 500 ml saline) into them, and antibiotics were administered via the amniotic catheter: 2 ml gentamicin (80 mg $2ml^{-1}$, Cidomycin, Roussel Ltd., Uxbridge, England) and 4 ml penicillin G (600 mg made up in 4 ml saline, Crystapen, Glaxo, Greenford, England). The catheters, electrodes and flow probe were collected into a plastic bag. The halothane was turned down to 0.5-1%.

2.3.5 Maternal instrumentation

Maternal instrumentation consisted of anterior metatarsal vein and artery catheterisation.

The ewe's leg was pulled across her body and secured on the opposite side of the table so as to expose the lateral aspect of the shaved and cleaned leg. The area was sprayed once again with Hibitane and draped so that only a small area around the knee was exposed. A 2-3 cm incision was made and the pedal vein and artery located by blunt dissection. A trochar and cannula (5 mm OD, 40 cm long) were then inserted into the wound and tunnelled sub-cutaneously to exit on the upper part of the leg. The trochar was removed, the arterial and venous catheters were threaded through the cannula, and the cannula was removed. The vein and artery were catheterised in the same way as was described for the fetus, and the wound was closed (US 1 braided silk suture, size 4 straight triangular needle). The catheters were heparinised.

A couple of stitches were put in the leg and flank exit wounds, and the maternal catheters were secured to the maternal skin in the area of the flank exit wound. All three of the maternal wounds were sprayed with Terramycin aerosol spray (Pfizer Ltd., Sandwich, England). An i.m. injection of Streptopen was given to the ewe.

2.3.6 Post-operative care

The halothane was turned off and the ewe was disconnected from the anaesthetic machine, though the tracheal tube was not removed yet. Whilst

still anaesthetised, the ewe was returned to a clean crate. A length of umbilical tape was sewn into the skin on her back and used to tie up the bag containing the catheters etc. Once her swallowing reflex had returned the tracheal tube was removed and the ewe was returned to the animal room. The ewe was closely observed until she regained consciousness, at which time she was given sheep nuts, water and hay.

The ewe and fetus were given a five day post-operative recovery period before experiments began. Over this five day period fetal blood gases were measured daily and antibiotics were administered (days 1 & 2: Crystapen - 300 mg iv. to ewe, 150 mg i.v. to fetus, 150 mg intra-amniotically; Gentamicin: 40 mg i.v. to ewe, 40 mg intra-amniotically. Days 3 - 5: Crystapen: 300 mg i.v. to ewe, 150 mg i.v. to fetus, 150 mg intra-amniotically).

Vascular and tracheal catheters were flushed daily during the five day recovery period and throughout the time of experimentation with heparinised saline (5000 iu heparin in 500 ml saline).

2.3.7 Apparatus

2.3.7.i Recording equipment

The recording equipment was situated in a separate, adjoining room to the ewes. The ewes stood with their tails facing the researcher in the other room and could be observed through a window in the separating wall. There was also a hole in the wall through which cables could be passed from the sheep to the recording equipment.

A specially constructed perspex box for holding the pressure transducers was attached to the side of the ewe's crate. This box was attached at the estimated height of the fetal heart, when the ewe was standing.

Arterial, venous, tracheal and amniotic pressures were measured using pressure transducers and amplifiers (Gould Ltd.; Hainault, Essex, England). Diaphragm activity, ECoG and ECG signals were measured using Gould universal amplifiers. Fetal heart rate (FHR) was calculated from the ECG using a tachometer (Gould Biotach). Mean blood flow was measured using a flow meter (T201, Transonic Systems Inc., Ithaca, NY, USA). All signals were taken down to an eight channel chart recorder (Linearcorder F WR3701, Graphtec UK Ltd.) and, via an eight channel data acquisition system

(MacLab/8; ADInstruments, Hastings, E. Sussex, England), to a PC (Apple Macintosh LCIII). Signals were stored on 88 Mb SyQuest cartridges, as well as paper chart records, for future analysis. Analysis of the raw signals was carried out using the MacLab software, and the resulting data was put into a spreadsheet (Microsoft Excel).

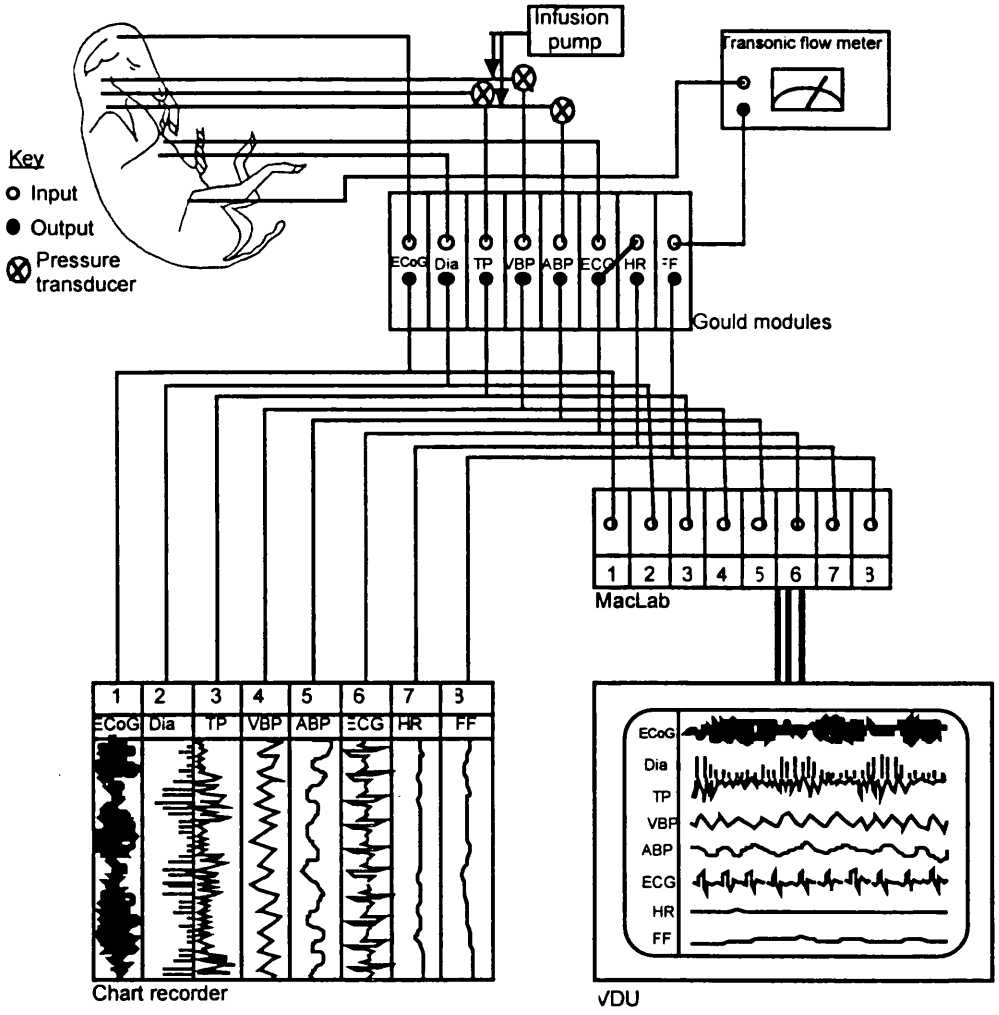

Figure 2.8. Diagrammatic representation of the sheep recording equipment.

2.3.7.ii *Measurement of blood gases and related variables, glucose and lactate*

Blood gases, pH, ion concentrations(Na^+, K^+, Ca^{2+}) and haematocrit were measured on a blood gas analyser (BGE; Instrumentation Laboratory Ltd., Warrington, Cheshire, England). Fetal values were corrected to 39.5°C and maternal values to 39°C. A haemoximeter (CO-Oximeter 482; Instrumentation Laboratory Ltd.) was used to measure haemoglobin, and glucose and lactate were measured using a glucose-lactate analyser (YSI, 2300 STAT PLUS; YSI, Farnborough, Hants., England).

2.3.8 Experimental procedures

2.3.8.i *Acute isocapnic hypoxaemia*

Acute isocapnic hypoxaemia of the fetus was induced by lowering the maternal inspired oxygen. Maternal inspired gases were manipulated by tying a clear plastic bag, into was introduced a pipe from a gas supply, over the ewe's head. The induced hypoxaemia lasted for a period of 1 hour, however the whole protocol lasted for 3 hours.

During the first hour (control) the ewe breathed air only (44 l/min), during the hour of hypoxia a mixture of air (14 l/min), nitrogen (24 l/min) and carbon dioxide (1.2 l/min), then in the final hour (recovery) she breathed air again as in the control period. The ewe's inspired gases during the hypoxic period were designed to decrease the fetal PaO_2 by about 50% to ca. 13 mmHg, keeping $PaCO_2$ constant. During the course of the 3 hour protocol 5 fetal arterial blood samples (0.5 ml) for blood gas, glucose and lactate analysis were taken at 30 min, 70 min, 100 min, 135 min and 165 min (Fig. 2.9).

	CONTROL	HYPOXIA
pH	7.326	7.277
$PaCO_2$ (mmHg)	42.3	43.3
PaO_2 (mmHg)	25	13
Haematocrit (%)	22	24
HCO^{3-} (mmol l^{-1})	23.1	19.8
Base excess (mmol l^{-1})	0.2	-2.5
CaO_2 (Vol%O_2)	8.5	4.3
Haemoglobin (g dl^{-1})	7.5	5.6
SaO_2 (%)	79.1	39.7
Lactate (mmol l^{-1})	1.01	5.05
Glucose (mmol l^{-1})	0.594	0.973
Na^+	140	142
K^+	2.8	2.6
Ca^{2+}	1.16	0.83

Table 2.1. An example of the blood gas and glucose and lactate changes that occurred in response to an imposed hypoxic challenge.

Three arterial samples (1.5 ml) were taken at 30 min, 100 min and 165 min for hormone analysis. These samples were collected into EDTA tubes, spun at 3000 revs/min in a cool centrifuge (4°C) for 10-15 min and were then stored in a -20 °C freezer.

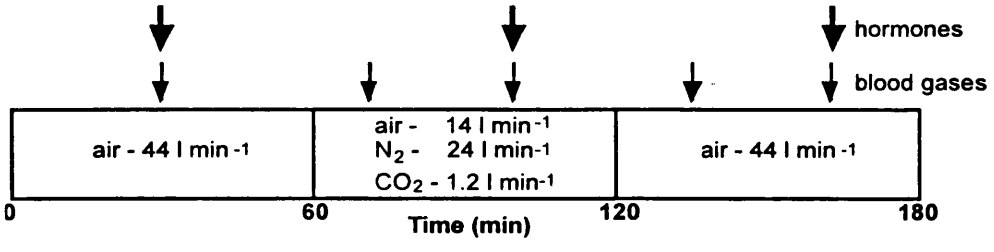

Figure 2.9. Schematic representation of the 3-hour acute hypoxia protocol.

2.3.8.ii *Fetal monitoring over a 3-week period*

Fetuses that were monitored for three weeks were studied for a period of two hours every other day. During the two hour recordings the ewe was normoxic and did not have a bag on her head. A fetal and a maternal arterial blood sample (0.5 ml) were taken for blood gas, glucose and lactate analysis during the first 15 min of the recording.

2.3.8.iii *Assessment of the baroreflex*

The baroreflex was assessed by giving the fetus a single i.v. (jugular) bolus dose (75 - 100 μg) of phenylephrine to elevate blood pressure transiently (Fig. 2.10).

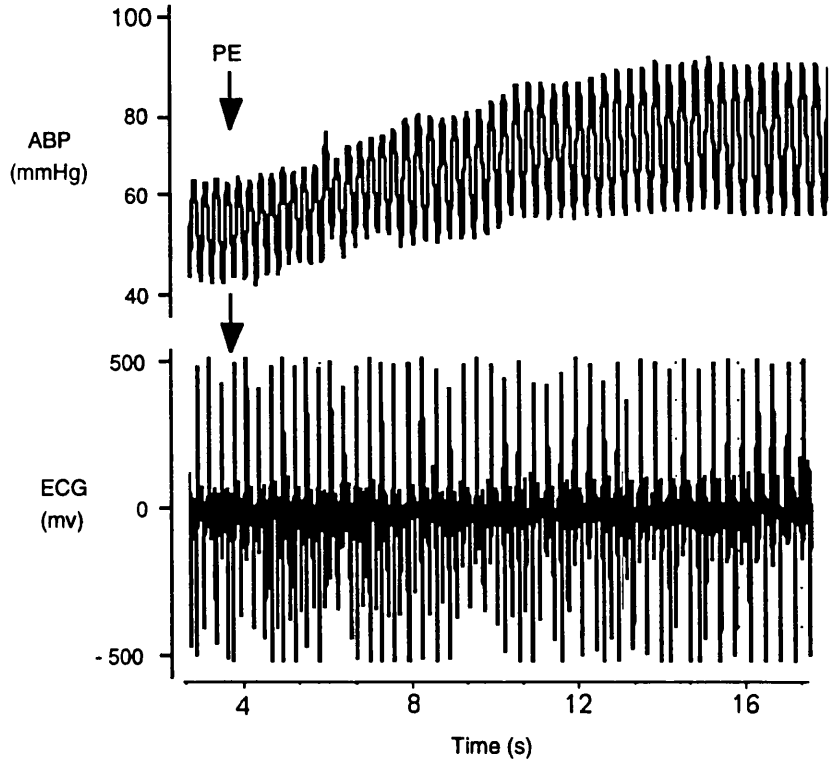

Figure 2.10. Part of a trace showing arterial blood pressure and ECG during the first 12 s after an injection of 75μg phenylephrine into the jugular vein. There is a pronounced increase in blood pressure and an increase in R-R interval.

2.3.9 Post mortem

When the experiments had been completed the ewes were sacrificed by an i.v. overdose of pentobarbitone (40 ml [8g] Euthatal, Rhône Mérieux, Harlow, Essex, UK). There were two post-mortem procedures, one basic and another extensive.

2.3.9.i Basic post-mortem

When the ewe was confirmed to be dead, her abdomen was opened along the midline, the uterus opened and the fetus(es) removed. The fetus(es) were weighed and crown-rump length (CRL) was recorded. The flow probe was then removed from the instrumented fetus. The heart, lungs, liver and kidneys were dissected out and weighed.

2.3.9.ii Extensive post-mortem

The ewe was weighed then the abdominal cavity was opened, as above, and the uterus was removed by cutting as low down the neck of the uterus as possible. The uterus and contents were weighed and the singleton fetus was removed from the uterus via the cervix, by cutting the umbilical cord at the level of the fetal abdomen. Care was taken not to spill any amniotic fluid out of the uterus. The fluid was then carefully poured out of the uterus into a measuring jug so that its volume and weight could be recorded.

The fetus was weighed and CRL, abdominal circumference (AC) and femur length (FL) recorded. The heart, lungs, liver, kidneys, adrenals, perirenal fat, spleen, pancreas, thymus, thyroid and brain were dissected out and weighed. Paired organs were weighed separately.

The uterus was laid out and a sketch of its position and orientation made. The uterus was then cut open along the midline and through the middle of each horn, the incision lines being noted on the sketch. Once the uterus was opened out, a second sketch was made recording the shape of the opened uterus and the position of the umbilical cord. The length of the cord was recorded. Each placentome was then individually dissected from the uterus and its position was recorded on the diagram of the opened uterus. Each placentome was trimmed of membranes, blotted dry, weighed and the thickness (using calipers) and diameter (using a ruler) of each was measured (Fig. 2.11).

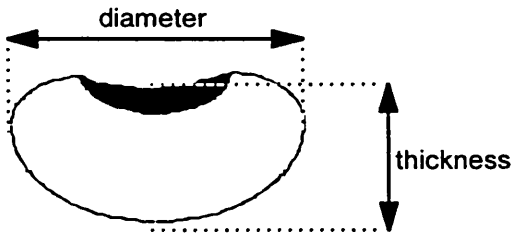

Figure 2.11. Illustration showing how placentome diameter and thickness were measured.

Each placentome was graded A, B, C or D on the basis of gross morphology, based on the classification system of Vatnick *et al* (1991) (Fig. 2.12).

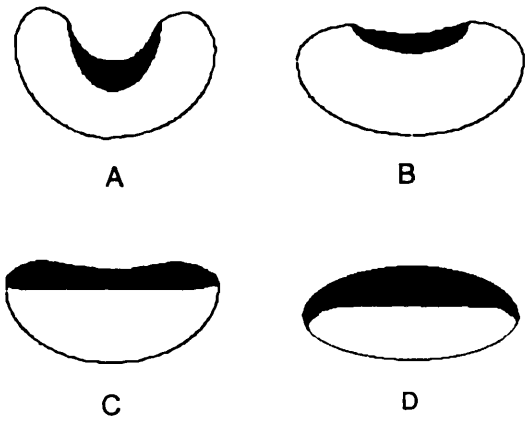

Figure 2.12. Schematic illustration of the morphological characteristics used to classify placentomes based on the system of Vatnick *et al.* ((1991). The black area represents fetal and the white maternal tissue. Thus, going from A to D the fetal tissue occupies relatively more of the placentome. Note that in this diagram the diameters and depths are shown to be relatively similar. In practice, they vary in each of the cotyledon types.

2.3.10 Data analysis

2.3.10.i Gestational age, weights and body measurements

The individual values for each animal on a particular day were put into groups and the mean value for each group on that day was calculated.

2.3.10.ii Arterial blood pressure, heart rate and blood flow

Mean minute values (μ^F_t, where μ is the mean for a fetus (F) at time t) for each of the parameters arterial blood pressure, systolic and diastolic blood pressures, heart rate and blood flow were obtained for each individual fetus, by collecting the data in 1 min. bins for the whole experiment and averaging the values in each bin. So an average value was obtained for each consecutive minute: 1 min, 2 min, 3 min, etc.

Basal values

For protocols that involved acute isocapnic hypoxaemia, individual basal mean values on any one day (gestational age) were then obtained by calculating the mean of the average minute values obtained during normoxia at 0, 15, 30 and 45 min:

Individual basal mean for fetus A on day 1, $\mathbf{A}_1 = (\mu^A_0 + \mu^A_{15} + \mu^A_{30} + \mu^A_{45}) / 4$

In those protocols that involved monitoring during normoxia for two hours, basal mean was taken as the mean of the average minute values recorded at 0, 15,30, 45, 60, 75, 90, 105 and 120 min

The group basal mean (Ψ^B) for a particular treatment group on that day was calculated by adding together the basal means of all the animals in that group on that day:

Group basal mean on day 1, $\Psi^B_1 = (\mathbf{A}_1 + \mathbf{B}_1 + \mathbf{C}_1 + \mathbf{D}_1 + \mathbf{E}_1) / 5$

where animals A, B, C, D and E are the animals in a particular treatment group.

Values during hypoxia

Individual mean values during hypoxia on any one day (gestational age) were taken as being the average minute value obtained at the time of interest. Thus,

the individual mean value at 15 min of hypoxia was the average minute value at that time:

Individual mean value at 15 min of hypoxia in fetus A, $A_1 = \mu^{A}_{75}$

(15 min is 75 min into the experiment - see Fig. 2.9)

The group mean value during hypoxia (Ψ^{H}) for a particular treatment group on that day was calculated by adding together the individual means during hypoxia of all the animals in that group on that day:

Group mean during hypoxia on day 1,

$$\Psi^{H}_1 = (A_1 + B_1 + C_1 + D_1 + E_1) / 5$$

where animals A, B, C, D and E are all the animals in a particular treatment group.

2.3.10.iii Vascular resistance

Vascular resistance (R) was calculated using the following equation:

$$R = (P_a - P_v) / Q$$

where P_a is arterial pressure, P_v is venous pressure, and Q is blood flow. The above equation is derived from Ohm's law and assumes steady flow. Blood flow is, of course, pulsatile, but this is the best derivation of flow that can be calculated with the available information.

2.3.10.iii Blood gases, glucose and lactate

Individual values for each fetus were the absolute values that were measured at the time of blood sampling. Group means for a specified time on a particular day (gestational age) were obtained by calculating the mean of the individual values for all the animals in that particular group at the specified time, in the same way as is described above for blood pressure, etc.

2.3.10.iv Baroreflex

Individual fetal values of R-R interval and MAP were collected in 2 s bins and the values in each bin averaged. So, individual average values for each fetus were obtained at 0, 2, 4, 6 s, etc. The baroreflex for individual animals was measured by plotting the mean R-R interval measured at 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60 s (i.e. while the pressure was rising) against MAP

measured at the same times. The resulting curve was taken to represent the baroreflex, and the slope of the steep part of the curve defines the sensitivity of the baroreflex (Fig. 2.13). The slope of the curve for individual animals was obtained in this way.

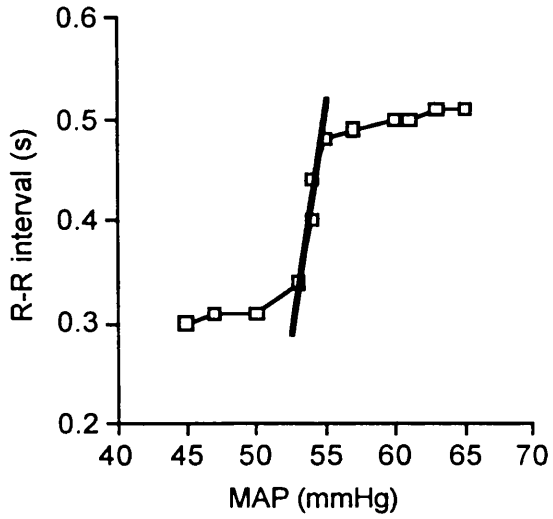

Figure 2.13. Example of the resulting baroreflex curve, when R-R interval was plotted against MAP. A line has been fitted to the steep part of the curve and it is the slope of this line which represents the sensitivity of the baroreflex.

The group mean at any one time was calculated as the mean of the individual average values for all the animals in that particular group at the desired time. Group means were calculated at 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50 and 60 s. The group means were then plotted, R-R interval against MAP, to obtain a baroreflex curve for the whole group (e.g. Fig. 4.4).

2.3.10.v Chemoreflex

The heart rate, femoral vascular resistance, femoral flow and blood pressure responses to hypoxia were analysed to give a measure of the chemoreflex response (see section 1.11).

In order to assess the chemoreflex, basal mean values at 0, 15, 30 and 45 min were averaged for each animal so as to obtain a single mean basal value for each animal. This mean basal value was then subtracted from the individual mean at 5 min hypoxia (65 min into the experiment, see Fig. 2.9) for femoral flow, femoral vascular resistance and heart rate, and the individual mean at 15 min hypoxia (75 min into the experiment) for MAP. The resultant value for each animal represented the individual response to hypoxia. The group mean of this delta value was worked out for each group.

2.3.11 Statistics

In all cases $P < 0.05$ was considered significant.

2.3.11.i Student's t-test

The *t*-test can only be used when dealing with data that has come from a population with a normal distribution. It tests the null hypothesis that data are a sample from a population with a specific 'hypothesised' mean. Either a one-tail or a two-tail *t*-test may be carried out. The difference between one-tail and two-tail is explained in terms of the alternative hypothesis. If the alternative hypothesis is that the population mean is not equal to, i.e. is more than or less than, the hypothesised mean then the test is two-tailed. If, on the other hand, the alternative hypothesis is that the population mean is greater than the hypothesised mean, or that the population mean is less than the hypothesised mean, the test is one-tailed. In addition, the test will be either paired or unpaired depending on whether the values being tested are intra- or inter-group, respectively.

I used the *t*-test to analyse differences between group mean values, e.g. heart weight, basal blood pressure difference between two groups on a certain day, the difference between CRL at the start and the end of the study in a particular group.

2.3.11.ii Analysis of variance (ANOVA)

ANOVA also assumes that the population has a normal distribution. It is a method of analysing the variability between sets of data.

In order to determine whether or not there was a difference between groups in developmental trends I used 1-way ANOVA. I did not use 2-way ANOVA because it is a paired test, and I did not always have the same number of

observations in each group for a variety of reasons e.g. death of an animal just before the end of the experimental period, blocked catheter, malfunctioning flow probe.

I used 1-way ANOVA to analyse trends in MAP, SBP, DBP, FHR, blood flow, vascular resistance, blood gases, haemoglobin, haematocrit, ion concentrations, glucose and lactate.

2.3.11.iii Summary measures

Summary measures is a useful method for analysing serial measurements. It considers the response of each individual within a group, and uses these individual responses to form a single number that summarises some aspect of the response of that individual i.e. a summary measure. The summary measure may be derived in many different ways, and the method chosen will depend upon the aspect of the individual's response which is of interest. For example, in this study I was interested in the trend in blood pressure development in fetuses over a period of time i.e. was there an increase, a decrease, or no change in pressure? Therefore, I chose to look at the individual responses in terms of the slope of a straight line fitted to the values obtained for individual fetuses. Thus, in this case, my summary measure was slope. This is a useful way of looking at the values because it is descriptive of the data in a highly relevant way. What better way to look at a linear trend over time than to describe it as a slope? The individual slopes for the animals in each treatment group were then pooled and compared using Student's unpaired *t*-test.

I also applied this method of analysis when looking at the development of heart rate, femoral flow and femoral vascular resistance, and when examining the slope of the baroreflex curve. I did not analyse any of the other variables using this method of analysis because it was difficult to define a suitable summary measure and I could not justify fitting a straight line to the data as I was not convinced that there was a linear change with age, e.g. for the change in haemoglobin.

2.3.11.iv Linear regression

Linear regression draws a line of best fit through a set of points. It is useful for describing the relationship between two variables. By determining the standard deviation of each point from the line of best fit, it is possible to

calculate the correlation coefficient for the line i.e. the linear association between the two variables being studied. The correlation coefficient lies within the range -1 to +1, with a midpoint of zero indicating no linear correlation. It is not sufficient to assume that because the correlation coefficient is close to 1 (+ or -) there is a good association, particularly if the sample size is small. It is therefore necessary to ascertain the probability (*P*) of Pearson's correlation coefficient from a set of tables and to determine the *t* statistic. The former determines whether the correlation is significant or not, and the latter determines whether the association can indeed be described as linear.

2.3.11.v Chi squared (χ^2) test

The χ^2 test is a non-parametric method of analysis, i.e. it makes no assumption about the distribution of a population and can thus be used to test whether a particular data set comes from the same population distribution as another.

I used χ^2 to test whether the SBP values I obtained in my rats were from a normal population. I also used it to test whether the population distribution of different placentome types was different between treatment groups (Chapter 7).

Chapter 3

FETAL AND NEONATAL GROWTH AND BLOOD PRESSURE DEVELOPMENT : THE EFFECTS OF ANAEMIA

3.1 INTRODUCTION

Over the last decade there has been growing interest in the idea that events which occur *in utero* may influence blood pressure during adult life (Barker & Osmond; 1987, Barker *et al.*, 1989) and studies have linked hypertension in adult life with a higher placental to birth weight ratio i.e. to small babies with large placentae (Barker *et al.*, 1990). It is not known whether placental growth directly affects the development of blood pressure, or whether other factors such as nutrition and oxygen supply are important determinants. Lucas and Morley (1994) showed that early nutrition in low birthweight preterm infants did not affect blood pressure measured at 7.5-8 years of age, which suggests that the relation between the fetus, placenta and the intra-uterine environment i.e. prenatal factors are important in determining outcome.

In the human, severe pregnancy anaemia is associated with placental hypertrophy (Beischer *et al.*, 1970) and maternal anaemia and iron deficiency during pregnancy result in a high placental to birthweight ratio (Godfrey *et al.*, 1991), which may subsequently predispose towards high blood pressure. The increased placental growth is thought to be a compensatory response and has been attributed to compensation for hypoxia resultant from the anaemia. The effects of hypoxia on fetal growth have been studied in many species including the sheep (Robinson *et al.*, 1983; Jacobs *et al.*, 1988a; Kitanaka *et al.*, 1989; Gagnon *et al.*, 1995), guinea pig (Jansson & Persson, 1991) and rat (Bruce & Cabral, 1975). The impact on growth is not uniform between studies, however, in that impaired fetal growth is not always an outcome. Similarly, the effects of chronic hypoxaemia on blood pressure are varied between studies. It is therefore clear that the physiological determination of fetal growth and development is complex and is not clearly understood. Perhaps even less understood are the implications of fetal growth and development for adult life

In this study I set out to reproduce the observations of Barker and his colleagues, namely of altered placental to birth weight ratio and hypertension, in the anaemic rat. Thus, the aim of the study was to examine the effects of the induced maternal iron-deficiency anaemia on placental and fetal weight, postnatal body and organ weight of the pups, and on the development of their blood pressure post-natally.

3.2 EXPERIMENTAL DESIGN

Female Sprague-Dawley rats were fed either a low-iron or a normal diet (as described in section 2.1.2) 3-4 weeks prior to mating. The haemoglobin levels in those rats on the low-iron diet fell from 17±2 g dl^{-1} to 11 ± 2 g dl^{-1}. After mating the rats were split into three groups, with anaemic (AN) and control (C) dams in each, and studied as follows:

Group 1- maternal and fetal body weights and haemoglobin levels, fetal organ weights, and placental weights were recorded (as described in section 2.2.4) at 20 days of gestation (E20). (AN dams, n=4; C dams, n=4; AN fetuses, n=47; C fetuses, n=59).

Group 2 - dams were allowed to litter and on postnatal day 20 (P20) systolic blood pressure was recorded in the conscious mother and pups, as described in section 2.2.3.i. Mothers and pups were then killed and their body weights and haemoglobin levels recorded, as well as fetal organ weights. (AN dams, n=4; C dams, n=6; AN pups, n=18; C pups, n=39).

Group 3 - the dams in this group were also allowed to litter, and maternal and neonatal systolic blood pressures, haemoglobin levels and body weights were recorded at P20. Both AN and C pups were weaned at P21 onto normal rat chow. They were then studied again at P40, when systolic blood pressure, haemoglobin levels, body and organ weights were measured. (AN dams, n=8; C dams, n=7; AN pups, n=80; C pups, n=64). After the pups were weaned at 21 days old, the mothers ceased to be a part of the experiment.

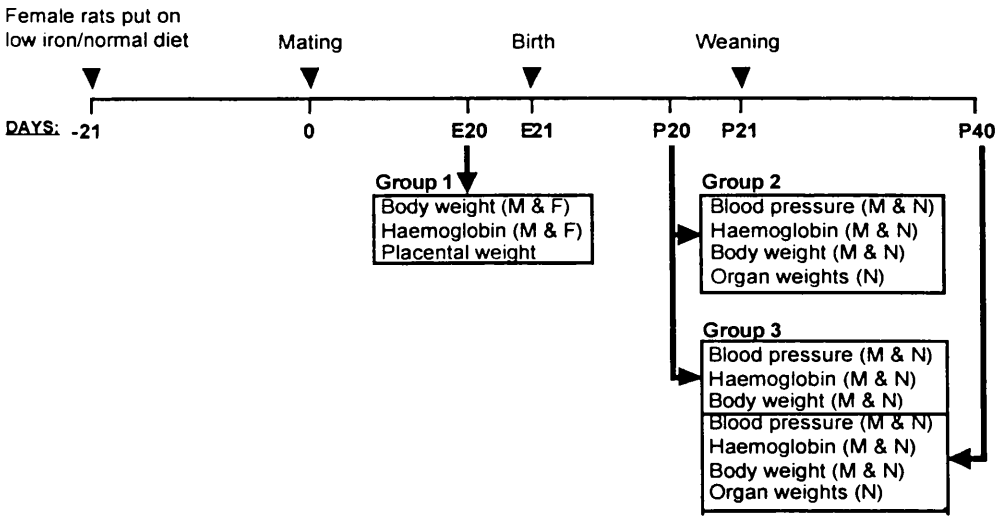

Figure 3.1. Experimental design, showing the protocol for fetal (F), neonatal (N) and maternal (M) rats in each of the three groups.

3.3 RESULTS

3.3.1 Haemoglobin

Maternal haemoglobin levels in AN dams (12.5 ± 0.5 g dl^{-1}) were significantly lower than those of C dams (19.5 ± 0.8 g dl^{-1}) at mating and throughout the 3 week suckling period.

At all ages studied (E20. P20 and P40), AN fetuses and pups had significantly lower haemoglobin levels than C. The value obtained for the mean haemoglobin level of C fetuses and pups lie within the range quoted for rats at each age (term: 7.4-9.1 g dl^{-1}; neonate: 6.3-9.0 g dl^{-1}; adult: 12-17.5 g dl^{-1}; Waynforth, 1980), and that for the anaemics falls outside of this range. AN rats can therefore be considered to be adequately anaemic.

Between P20 and P40 both AN and C pups showed a rise in haemoglobin levels. The rise was, however, steeper in the AN group, so that by P40 haemoglobin levels in AN animals approached those of C (Fig. 3.2).

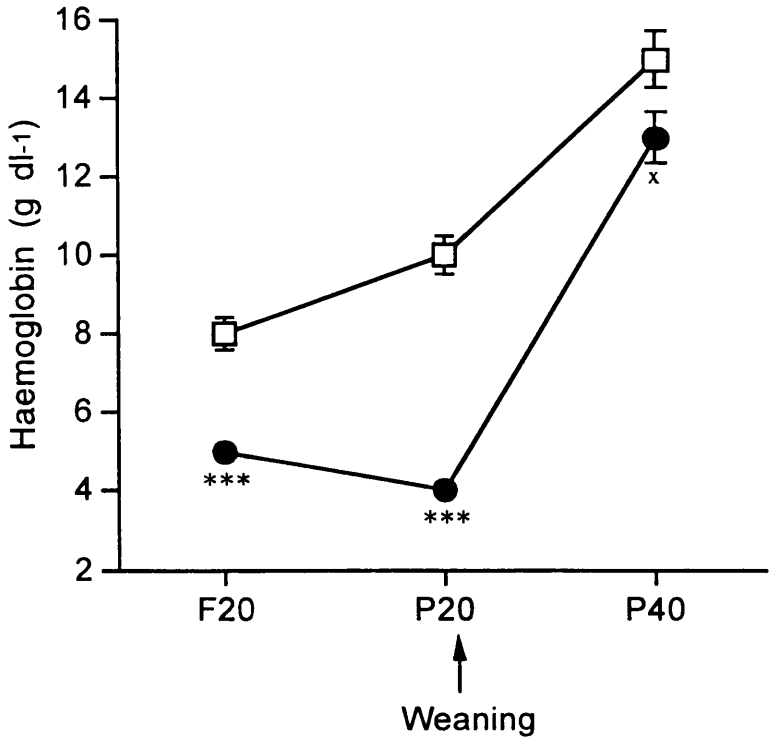

Figure 3.2. Haemoglobin values for C (□) and AN (●) fetuses and neonates. Values are mean ± S.E.M. ^{x} $P < 0.05$, *** $P < 0.001$, AN vs. C.

3.3.2 Placental weight

The placentae of AN rats were significantly lighter than those of C. Placental to body weight ratio was also significantly lower in AN compared to C (Fig. 3.3). The lower placental to body weight ratio represents a greater decrease of placental weight than in fetal body weight.

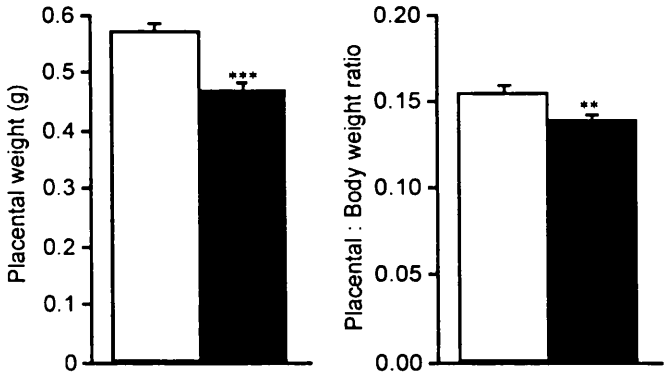

Figure 3.3. Placental weight and placental : body weight ratio in C (□) and AN (■) rats. Values are mean ± S.E.M. ** $P < 0.005$, *** $P < 0.001$, AN vs. C.

3.3.3 Body weights

The difference in mean body weights between AN and C rats at all ages was highly significant with that of C being much greater than that of AN at E20, P20 and P40 (Fig. 3.4).

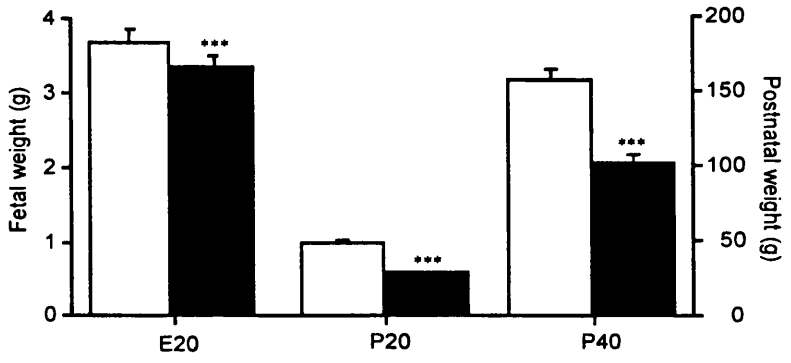

Fig. 3.4. Fetal and neonatal body weights in AN (■) and C (□) rats. Values are mean ± S.E.M. *** $P < 0.001$, AN vs. C.

However, it is interesting that the percentage increase in body weight between P20 and P40 was significantly greater in AN than in C animals (Fig. 3.5). In other words AN pups grew at a greater rate than C after weaning.

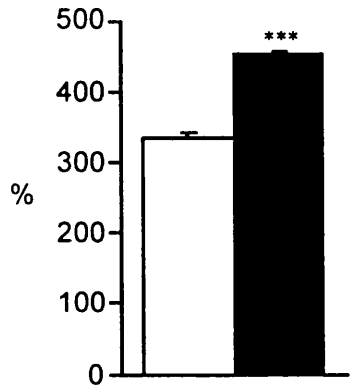

Fig. 3.5. Percent increase in body weight from P20 to P40 in C (□) and AN (■) pups. Values are mean ± S.E.M. *** $P < 0.001$, AN vs. C.

3.3.4 Organ weights

At P20, AN pups had significantly heavier hearts than C pups, but their lungs and livers were significantly lighter. AN kidneys were lighter than those of C, but not significantly so. At P40, AN rats had significantly lighter hearts than C rats, their livers were still significantly lighter and their kidneys were significantly lighter. AN lungs were no longer significantly lighter than those of C. Table 3.1).

		P20	P40
Heart weight (g)	**C**	0.30 ± 0.016	0.72 ± 0.025
	AN	0.54 ± 0.018 ***	0.61 ± 0.028 *
Lung weight (g)	**C**	0.65 ± 0.02	1.16 ± 0.045
	AN	0.48 ± 0.020 ***	1.08 ± 0.046
Liver weight (g)	**C**	1.96 ± 0.114	8.38 ± 0.268
	AN	1.41 ± 0.039 ***	5.52 ± 0.335 ***
Kidney weight (g)	**C**	0.34 ± 0.54	0.73 ± 0.026
	AN	0.28 ± 0.008	0.53 ± 0.032 ***

Table 3.1. Organ weights in C and AN pups at P20 and P40. Values are mean ± S.E.M. * $P < 0.01$, *** $P < 0.001$, AN vs. C.

Owing to the significant differences observed in body weight between the two groups, organ weight expressed as a percentage of body weight is a more meaningful comparative measure than the absolute weights *per se*.

When expressed thus, liver, kidney and heart weights were significantly greater in AN pups at P20 than C, but by P40 only kidney and heart weights were greater in AN, and lung also. Liver weights were not different between the two groups by this age.

Liver weight increased significantly between P20 and P40 in both AN and C animals, and kidney, lung and heart weights decreased significantly in both groups (Fig. 3.6). The decrease in weight, as a percentage of body weight, observed in the kidneys and hearts was, however, much steeper in AN pups than that seen in C. This indicates a lower rate of growth of these two organs in AN at this time.

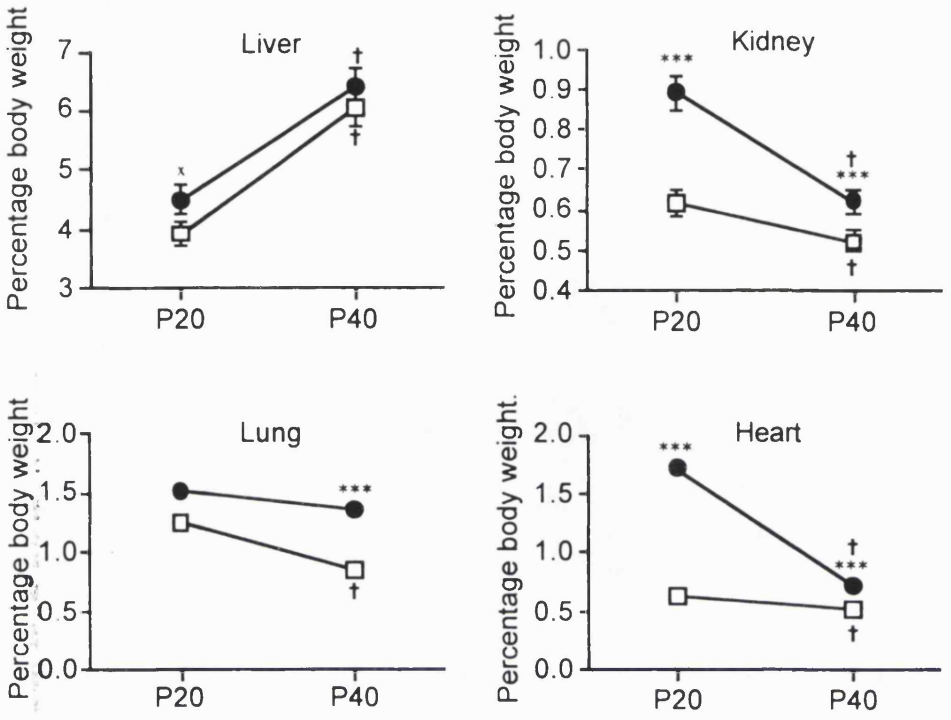

Figure 3.6. Liver, lung, kidney and heart weights expressed as a percentage of body weight in AN (●) and C (□) animals. Values are mean ± S.E.M. ^{x} $P < 0.05$, *** $P < 0.001$ AN vs. C; † $P < 0.001$, P20 vs. P40.

3.3.5 Systolic blood pressure

Evaluation of blood pressure in the rat pups proved to be an extremely arduous task, being both time-consuming and problematic. As described in the Methods chapter (in section 2.2.3.i), evaluation of SBP using the tail cuff required the tail of the rat to remain absolutely still so that a readable pressure trace could be obtained. It is also a requirement of this method that blood flow be occluded, thus it is important that the tail cuff selected is the correct size for the rat. Persuading the pups to keep still was usually not easy, and the smallest cuff available was not always as perfect a fit as one would have liked for some of the young 20-day old pups. It is perhaps due to these rather exacting requirements that I found a degree of intra-animal variation in the measurement of SBP (Fig. 3.7).

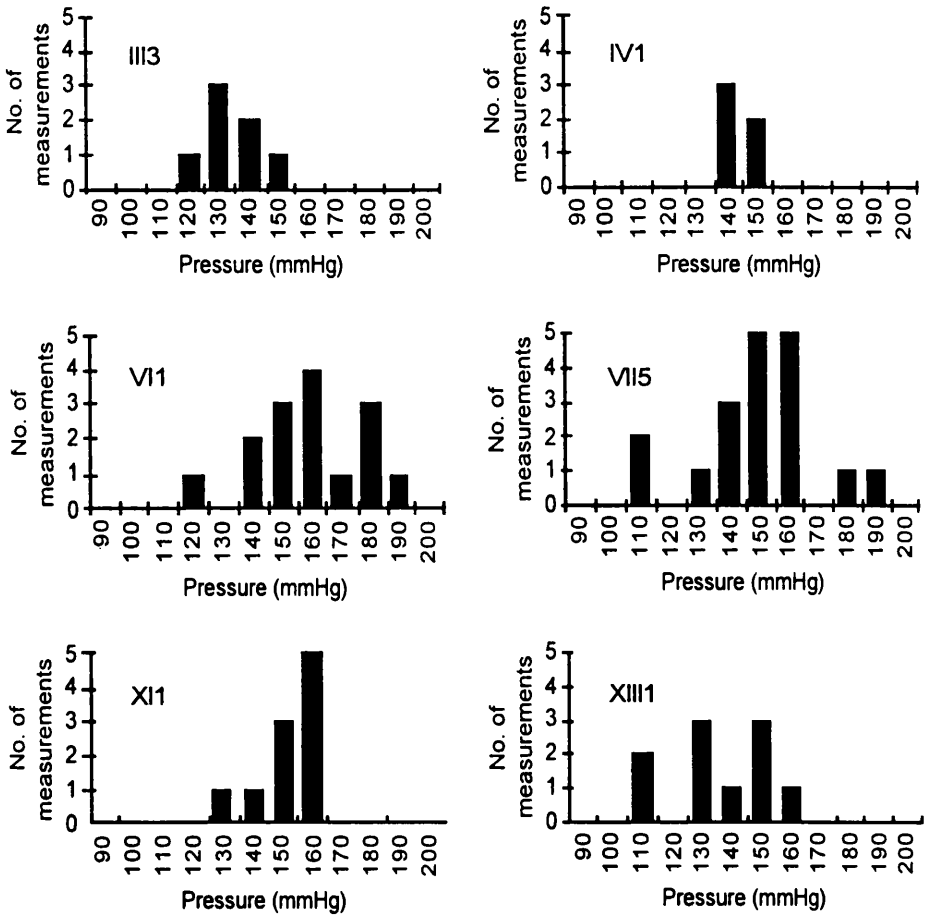

Figure 3.7. Illustration of intra-animal variation in SBP recordings. This figure is an example of the different SBP values obtained for individual pups.

This variation in individual SBP values was the cause of some concern, so it seemed appropriate to examine variation within litters.

As can be seen in figure 3.8 there was also variation in SBP recordings between littermates. It was, however, reassuring to find that despite its variation, generally, the distribution of SBP recordings obtained showed a bell-shaped Normal distribution.

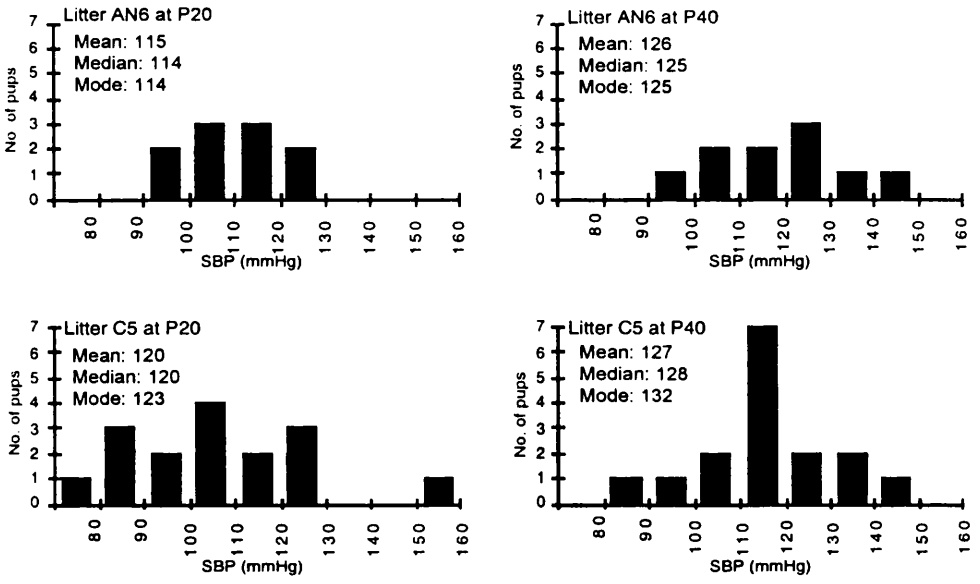

Figure 3.8. Illustration of intra-litter variation in SBP recordings. This figure shows the different SBP values obtained for two litters of rats, AN6 and C5, at 20 and 40 days of age.

The distribution of mean SBP data for C and AN rats was again found to show a Gaussian distributed at both 20 and 40 days of age (as determined by χ^2 test, see section 2.1.6) (Fig. 3.9). This confirmed that the animals came from a normally distributed population that it was valid to take the mean SBP for each group when making comparisons between them.

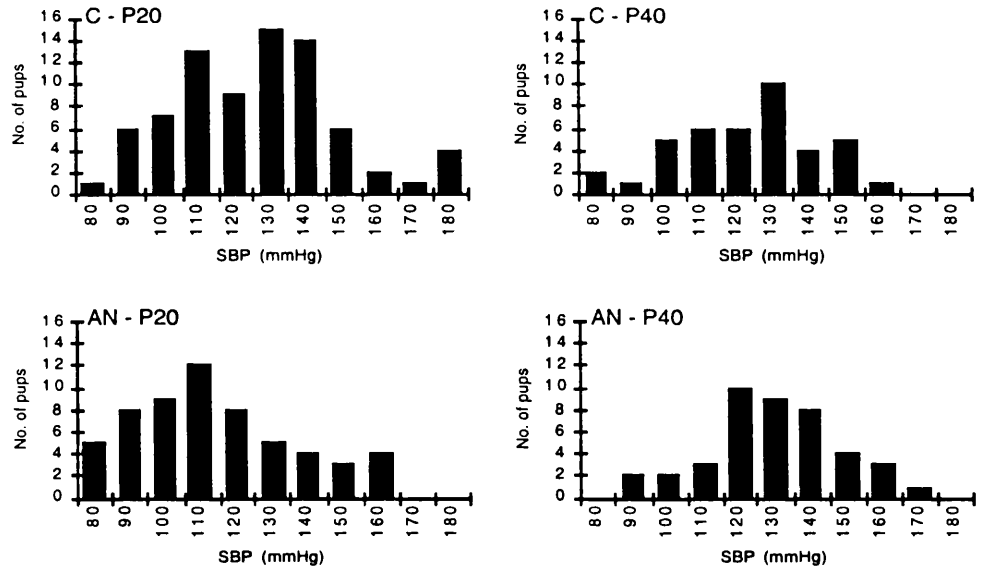

Figure 3.9. Illustration of intra-group variation in SBP recordings. This figure shows the distribution in C and AN at both 20 and 40 days of age. All are normally distributed (χ^2, $P < 0.05$).

So, looking now at mean SBP of the animals at P20 and P40 we can see that AN pups had a lower SBP than C at P20, however, between P20 and P40 there was a dramatic rise in pressure in AN pups and only a slight increase in C, so by P40 AN pups had a significantly higher SBP than controls (Fig. 3.10).

Maternal SBP was not different between AN (112 ± 5) and C (118 ± 3) dams at either E20, P20 or P40.

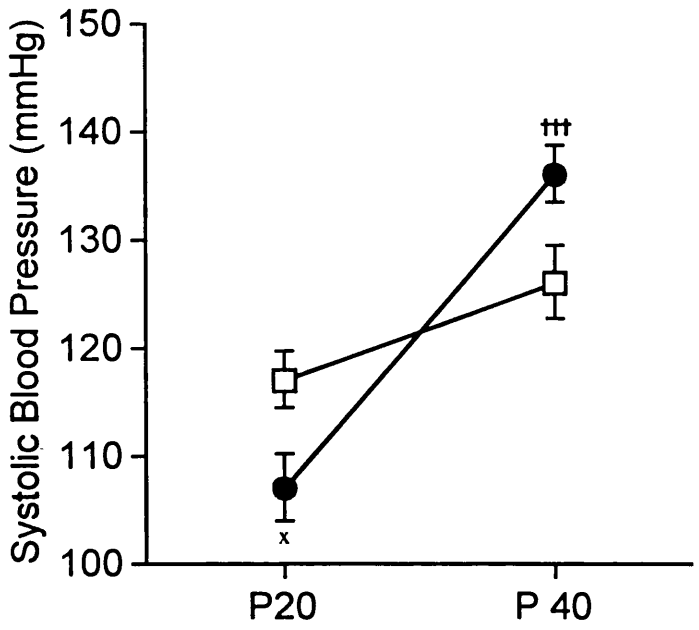

Figure 3.10. The change in systolic blood pressure between P20 and P40 in AN (●) and C (□) pups from group 3. Values are mean ± S.E.M. $^{x}P < 0.05$, AN vs. C; $^{\dagger\dagger\dagger}P < 0.001$, P20 vs. P40.

3.4 DISCUSSION

The principal finding of this study was that there is a pronounced postnatal rise in SBP associated with maternal anaemia during pregnancy, and that this is preceded by a low SBP before weaning in anaemic pups. The elevated SBP in the anaemic rats was not associated with a greater placental to body weight ratio, rather there was an association between the rate of growth postnatally (Fig. 3.5) and hypertension (Fig. 3.10).

I shall first discuss the possible mechanisms resulting in the low blood pressure of AN pups before weaning, before going on to discuss the potential mechanisms involved in their subsequent development of hypertension. I will then consider the changes seen in organ size and growth and their relation to the changes observed in blood pressure. Finally, I shall examine the role of the placenta with respect to growth and cardiovascular function.

3.4.1 Hypotension

3.4.1.i Cardiac and vascular remodelling

As described above, AN had heavier hearts (Fig. 3.6) and lower systolic blood pressure (Fig. 3.10) than C at P20. These findings suggest that the severe anaemia that AN were subjected to *in utero* and in the immediate post-natal period resulted in changes in their cardiovascular development. Whilst I did not investigate the structure of either cardiac or vascular tissue from the rats in this study, it is likely that this architecture was altered due to a process of both cardiac and vascular remodelling.

Davis and Hohimer (1991) made late gestation sheep fetuses anaemic by serial isovolaemic haemorrhage. They found that anaemic fetuses had larger hearts, greater combined ventricular output and lower mean arterial blood pressure than control fetuses. Similarly, Mostello *et al.* (1991) found that the fetuses in their study, where anaemia was induced in the ewe by repetitive isovolaemic haemorrhage three to five times a week from 110 to 138 days gestation, had lower MAP than controls. These findings are comparable to those which I observed in my rat pups. I did not measure CO in this study, but on the basis of the above evidence, it was probably increased in my AN fetuses and pups.

Since mean arterial blood pressure is determined by CO and total peripheral resistance, it seems reasonable to suppose that any increase of CO in AN must have been accompanied by a considerable fall in peripheral resistance. It is well known that patients with chronic severe anaemia have a high CO, low blood pressure and low systemic vascular resistance (Anand & Chandrashekhar, 1993). Similar observations have been made in the sheep (Delpapa *et al.*, 1992; Mostello *et al.*, 1991; Edelstone *et al.*, 1987). The cardiac hypertrophy in AN was presumably the result of increased CO, which itself may have been increased in response to a decrease in afterload i.e. low systemic vascular resistance. Decreased afterload depends upon both rheological (viscosity) and geometrical (vascular) factors. Fowler and Holmes (1975) showed in adult dogs made anaemic by exchange transfusion that there is a significant increase in CO as a result of decreased blood viscosity. Such a volume overload on the heart would then have resulted in cardiac remodelling, such as that which has been demonstrated in the anaemic rat (Olivetti *et al.*, 1989) where it was shown that ventricular hypertrophy is

accompanied by a significant degree of myocardial capillary proliferation. It therefore seems that the cardiac hypertrophy observed in AN may have been the result of increased CO which was in response to decreased afterload.

Severe anaemia also results in decreased blood viscosity, as was mentioned above in dogs (Fowler and Holmes, 1975) and as has been observed previously in rats (Olivetti *et al.*, 1993). It is therefore most likely that my AN rats had decreased blood viscosity. Combining Poiseuille's and Ohm's laws we see that a decrease in viscosity will result in an increase in flow, which will in turn result in a decrease in resistance:

$$R = \frac{8 \cdot l \cdot \eta}{\pi \cdot r4}$$

where R is resistance, l is the length of the vessel, η is blood viscosity, and r is the radius of the vessel. It has been demonstrated in lambs made chronically hypoxaemic by pulmonary stenosis, that there is no alteration in vascular hindrance (vascular resistance divided by whole blood viscosity) in response to chronic hypoxaemia, and that decreased blood flow to non-vital organs was the consequence of increased whole blood viscosity (Dalinghaus *et al.*, 1994). However, resistance to flow cannot be considered only in terms of blood viscosity. We must also take into account the contribution of vascular geometry.

The cardiovascular system is comprised of not only the heart but the blood vessels also, and together they form a continuous system of branching hollow tubes, the parameters of which are defined by the number of vessels forming the branches and the mass of the vessel wall. Thus, as intimated at the beginning of this section, alterations in cardiovascular function may involve changes not only in the heart but of the blood vessels also. Accordingly, it is likely that there was a degree of vascular as well as cardiac remodelling in AN. Remodelling of the vascular system involving vasculogenesis at the periphery would contribute to a decrease in peripheral vascular resistance because of the approximate parallel arrangement of the capillaries. This is described by Kirchhoff's law of tubes in parallel:

$$\frac{1}{R_{tot}} = \frac{1}{R_1} + \frac{1}{R_2} + \ldots \quad \rightarrow \quad R_{tot} = \frac{R_1 + R_2 + \ldots}{n}$$

where R_{tot} is the total resistance; R_1, R_2, etc. is the resistance of individual tubes (capillaries); and n is the total number of tubes. Thus, an increase in the number of vessels results in a decrease in total resistance.

Vascular remodelling occurs ordinarily in the normal fetus and neonate (Langille, 1993). He found that there is a rapid accumulation of elastin and collagen during the perinatal period in sheep, which may pre-adapt vessels to the large increases in pressure that occur after birth. He also observed a strong relationship between blood flow and developmental changes in vessel diameter during the postnatal period. If there is disturbance during this critical period when these numerous changes in vascular structure, which are important for adaptation to adult life, are taking place, the development of blood pressure would also be affected. In view of the proposed increase of CO in my AN rats and their low haemoglobin levels, it seems probable that their vascular development would have been affected, perhaps resulting in the observed hypotension.

As well as perturbations in normal vascular development, further vascular remodelling may have taken place secondary to the anaemia. Hypoxia for three weeks in adult rats caused brain angiogenesis, which was initially due to microvascular hypertrophy and later on to hyperplasia (Harik *et al.*, 1995). Dawson & Hudlicka (1993) showed in rats that in response to a long-term increase in blood flow, instigated by vasodilatation caused by administration of the nonselective α_1 adrenoceptor antagonist prazosin, there is increased capillary growth. They suggested that this angiogenesis was the result of increased velocity of flow and thus shear stress, which may have led to the activation of various growth factors. Hudlicka (1991) implicated fibroblast growth factor (FGF) release, in response to increased endothelial shear stress, as having a stimulatory effect on capillary growth. More recently Malek *et al.* (1993), using endothelial cells cultured *in vitro*, showed that increased shear stress caused differential modulation of the mRNA levels of the two potent growth factors platelet-derived growth factor B (PDGF-B) and basic FGF. Increased shear stress of differing amounts resulted in varying increases in mRNA expression of the two growth factors, suggesting that expression of each peptide growth factor gene is differentially regulated by fluid shear stress in vascular endothelial cells. Vascular remodelling could also comprise changes in the number or size of vascular smooth muscle cells. In their review of cardiovascular remodelling, Struijker-Boudier *et al.* (1995)

discuss the concept of "phenotypic modulation", where tissue structure is influenced by a modified cellular responsiveness to growth promoters and inhibitors. Interestingly, they also discuss the fact that flow-associated shear stress is an important factor in the modulation of vascular smooth muscle cell phenotype. This may be an important concept with regard to vascular smooth muscle hypertrophy and hyperplasia, and could be one of the mechanisms involved in the lower blood pressure of AN. Increased peripheral vascular density due to angiogenesis would cause there to be a decrease in the resistance to flow, as described above.

3.4.1.ii Local and endocrine factors

A further contributory factor to the decrease in vascular resistance may have been caused by nitric oxide (NO) (formerly known as endothelial-derived relaxing factor - EDRF), a potent vasodilator. Haemoglobin binds NO (Martin *et al.*, 1986; Jia *et al.*, 1996) and it has been postulated that in chronic severe anaemia, the low circulating haemoglobin concentration may result in reduced binding and thus inhibition of NO so leading to increased basal NO activity and therefore vasodilatation (Anand & Chandrashekhar, 1993). So, in my study, the anaemia itself may have resulted in peripheral vasodilatation by this mechanism in AN rats. Also, both increased shear stress at the endothelium (Melkumyants *et al.*, 1995; Smiesco & Johnson, 1993; Hudlicka, 1991) and hypoxia (Busse *et al.*, 1993; Hudlicka, 1991) have been shown to stimulate the release of endothelial vasodilator factors such as NO and prostacyclin.

Atrial natriuretic peptide (ANP) may also be implicated in contributing to the decreased systolic blood pressure observed in AN. ANP, as well as its natriuretic and diuretic properties, causes a decrease in blood pressure. It has been shown (Silberbach *et al.*, 1995) in the chronically anaemic sheep fetus that circulating levels of ANP are elevated, though they failed to demonstrate a significant decrease in mean arterial pressure in their study. It is possible that there was no significant fetal MAP response to ANP, such as is observed in adults, because of the presence of the low-resistance placental bed which may dampen changes in pressure. Also, the immature vasculature of the fetus may respond differently to ANP than that of the post-natal animal. Isolated rat hearts exposed to hypoxia show a dramatic increase in ANP release, which is partly mediated by α- and β- adrenergic stimulation, and they also show a decrease in systolic pressure (Lew & Baertschi, 1988). The concentration of

ANP in AN may have been elevated during fetal life and after birth and it may have contributed to the lower blood pressure that was measured at P20. Unfortunately I did not measure plasma levels of ANP or monitor urine output.

3.4.2 Hypertension

3.4.2.i Growth and blood pressure development

There were further changes in the cardiovascular development of the rats in my study, as can be seen in figure 3.10. I have already discussed the fact that SBP was lower in AN at P20, but by P40 it had risen to a higher level than that observed in C. It is also interesting to note that, although AN pups were lighter than C at both P20 and P40 (Fig. 3.4), the increase in weight in AN was greater than that of C between P20 and P40 (Fig. 3.5), which suggests a greater rate of growth of AN pups at this time. These findings complement those of Godfrey *et al.* (1991) where they found that maternal anaemia during pregnancy was associated with decreased birth weight. In a later study Godfrey *et al.* (1994) found that blood pressure in 10-12 year old Jamaican children was greatest in those children whose mothers were anaemic during pregnancy. They also found that systolic pressure rose by 0.5 mmHg for every 1 kg increase in the child's current weight. Findings in the guinea-pig (Persson & Jansson, 1992) have shown an association of IUGR with increased blood pressure at 3-4 months of age. Interestingly, although Persson and Jansson (1992) did not mention it, their IUGR guinea-pigs showed a 91% increase in weight between birth and 3-4 months of age whilst controls only increased in weight by 86%, i.e. IUGR pups grew faster than their normal-sized littermates. In young rats it has been shown that the rise in blood pressure which occurs normally with age is due predominantly to increases which occur concomitantly with growth spurts (Schork *et al.*, 1994). It seems, therefore, that the rate of growth, and not simply body size, is an important factor influencing the rapid rise in SBP that I saw in AN rats. This idea is further supported by the findings of Whincup *et al.* (1989), whose study of children aged 5-7 years showed that blood pressures were highest (especially in boys) in those who were of low birth weight and had gained most weight subsequently. As yet, however, the mechanisms linking growth and blood pressure are not known.

Obesity may provide a model for trying to understand the link between growth and blood pressure development. In man it is well recognised that there is a relationship between weight gain and the development of essential hypertension. It has been suggested (Hall, 1994) that extreme weight gain (obesity) is a very important cause of human essential hypertension, and that changes in kidney function are a key factor uniting the two. Impaired renal function is strongly associated with hypertension, and it has been shown in the dog that obesity results in alterations in kidney function as well as an increase in blood pressure and alterations in various hemodynamic variables (Hall, 1994). Maybe the rapid increase in body weight seen in my AN rats between P20 and P40 resulted in similar perturbations in renal function to those that occur in obesity.

3.4.2.ii Cardiovascular reflex responses

The developmental differences in blood pressure that are seen in this study, in response to maternal anaemia during pregnancy may in part be due to changes in cardiovascular reflex responses. Both chemo- and baroreflexes are known to be functional *in utero* from mid to late gestation. In the sheep the chemoreceptors are functionally active from 80 days (Hanson, 1988) and the baroreceptors from 85 days (Shinebourne *et al.*, 1972) gestation. However, prior to this age it is not known whether they operate and little is known of their development.

The chemoreflex responses to acute hypoxaemia are well established and include a transient bradycardia, rise in MAP, and redistribution of CO. The mechanisms involved in this response have been extensively studied (Iwamoto *et al.*, 1989; Itskovitz *et al.*, 1991; Giussani *et al.*, 1994). However, maintenance of hypoxia beyond an acute insult results in reversal of the above responses towards normoxaemic levels. The mechanisms responsible for this adaptation are unknown (Hanson, 1993). In neonatal life it is known that there is chemoreceptor resetting, i.e. a decrease in sensitivity, during the first few days after birth, as the receptors adjust their sensitivity towards the adult range of PaO_2 and $PaCO_2$ (Blanco *et al.*, 1982; Blanco *et al.*, 1984; Hanson, 1988). It has also been suggested in fetal lambs exposed to chronic mild hypoxaemia caused by uterine artery occlusion, that efferent chemoreflex activity appears to be increased (Bennet & Hanson, 1994). This leads one to speculate that perhaps sustained hypoxaemia, such as may have occurred as result of the severe anaemia in AN, resulted in an amplification of

the chemoreflex during fetal life resulting in perturbations of chemoreceptor resetting in neonatal life and affecting the control of blood pressure.

In normal fetal development there is a rise in MAP during late gestation (Kitanaka *et al.*, 1989; Daniel *et al.*, 1989; Mostello *et al.*, 1991; Kamitomo *et al.*, 1994), which is accompanied by a reduction in the gain of the baroreflex (Blanco *et al.*, 1988). However, as with the chemoreflex, the effects of chronic hypoxaemia on these adaptive mechanisms are unknown. It is possible that, as a result of vascular remodelling and a decrease in peripheral resistance, not to mention the hypoxaemia that can occur in severe anaemia, the normal development of the baroreflex response is altered. Changes in vessel wall properties could result in alterations in vessel compliance which would affect baroreceptor sensitivity. A change in baroreceptor sensitivity would result in altered blood pressure levels. For instance, it is a well known fact that in people with high blood pressure, the baroreceptors are reset to higher pressures.

Baroreflex function may also be affected by changes in the brain. The spontaneously hypertensive rat (SHR) is a good model for studying the development of hypertension, as neonatal SHRs are normotensive until about 5 weeks of age. Boone and McMillen (1994) investigated proenkephalin gene expression in the brains of SHR and Wistar-Kyoto (normotensive) rats at 4 and 14 weeks of age. In the central nervous system, the enkephalin peptides, which act as synaptic transmitters, are localised in areas involved in autonomic, cardiovascular and pulmonary regulation. They found that during the development of hypertension proenkephalin gene expression in the brain was altered with some regions showing an increase and others a decrease. Proenkephalin mRNA levels were lower in the nucleus tractus solitarius, caudal and rostral ventrolateral medulla, and higher in the locus coeruleus, anterior and lateral hypothalamus of the SHR. They suggested that these regional variations in proenkephalin mRNA levels may have resulted in a decrease in arterial baroreceptor activity and an increase in sympathoadrenal activity, thus resulting in the development of hypertension. It is also interesting to note that at 4 weeks of age Wistar-Kyoto pups were significantly heavier than SHRs, but up to the age of 14 weeks they showed an increase in body weight of only 62% compared to the 73% increase seen in SHRs. We may speculate that mRNA proenkephalin levels in my AN rats were also altered in a similar fashion to those of SHR.

3.4.2.iii Local and endocrine factors

In response to hypotension (Rose *et al.*, 1981; Wood, 1989; Anand *et al.*, 1993; Friess *et al.*, 1995) and hypoxaemia (Robinson *et al.*, 1983; Giussani *et al.*, 1994b & c; Murotsuki *et al.*, 1996) there is an increased release of adrenocorticotrophic hormone (ACTH), cortisol, vasopressin and renin. Elevated circulating levels of these hormones for a prolonged period, such as may have been the case in AN, could result in alterations in growth and development. Cortisol levels are elevated in sheep fetuses that are growth retarded (Murotsuki *et al.*, 1996; Robinson *et al.*, 1983), suggesting that cortisol may somehow affect fetal growth. Murotsuki *et al.* (1996) have suggested that prolonged hypoxaemia may blunt maturation of the HPA axis which may contribute to growth restriction *in utero*. Alterations in HPA function may also result in changes in blood pressure development.

3.4.2.iv Hemodynamic changes

Much of the discussion concerning hypotension centred around the idea of altered hemodynamics. Likewise, in the development of hypertension, hemodynamic changes may be a contributory factor. The increase in blood viscosity which would have occurred as the anaemic condition improved, would result in vasoconstriction (Dalinghaus *et al*, 1994). Also reversal of tissue hypoxia as a result of increasing haemoglobin levels would result in a decrease in NO release from the endothelium (Busse *et al.*, 1993; Hudlicka, 1991) and thus an increase in vascular tone. The elevated haemoglobin level itself would also mean that there was a further decrease in NO (due to the binding of NO to haemoglobin) (Martin *et al.*, 1986; Jia *et al.*, 1996).

Alternatively, the increased flow, due to the earlier proposed decrease in vascular resistance during hypotension, could have caused changes in the form of the large arteries such as has been observed in babies born with only a single umbilical artery (Meyer & Lind, 1974; Berry *et al.*, 1976). The sustained increase in flow may have altered the compliance of the vessels due to increased strain on the vessel walls.

3.4.3 Organ size, growth and function and blood pressure development

When investigating the link between growth and blood pressure development it may be important to consider the growth of individual organs as well as

body size *per se*. In response to chronic anaemia in the newborn lamb there is a redistribution of blood flow in favour of the heart, brain and adrenals, whilst flows to the GI tract, liver, carcass, adrenals, spleen and kidneys do not change (Bernstein *et al.*, 1988). A similar blood flow pattern is seen in anaemic pregnant ewes (Edelstone *et al.*, 1987). Somewhat contrary to this Silberbach *et al.* (1995) found there to be an increase in renal blood flow in chronically anaemic fetal lambs. In this study, I found that AN pups had significantly larger hearts, kidneys and, to a lesser degree, livers, as a percentage of body weight, than C at P20 and P40 (Fig. 3.6). This suggests that there may have been a redistribution of blood flow in favour of these organs, particularly the heart and kidneys, so preserving their growth. Lung weights were not different between the two groups at P20, but were bigger in AN at P40 (Fig. 3.6).

3.4.3.i Heart

Increased load on the heart and cardiac remodelling would have contributed to cardiac hypertrophy (as discussed above).

Between P20 and P40 growth rate of the heart of AN was lower than in C, although it was still heavier in AN at P40 (Fig. 3.6). This decreased rate of growth may have been due to the improvement of the anaemic condition over this 20 day period. Concomitant with the increasing haemoglobin levels, oxygen supply to the tissues would have increased so CO and thus the energy demand and work load of the heart would have decreased.

3.4.3.ii Kidney

The larger kidneys of AN may reflect an increase in erythropoietin production. Erythropoiesis is triggered by a decrease in PaO_2 and it has been demonstrated in the fetal lamb that there is a significant rise in erythropoietin levels after a moderately severe haemorrhage (Shields *et al.*, 1993). Erythropoietin levels in the human fetus are elevated in babies that are small for gestational age (Snijders *et al.*, 1993) and in severely anaemic fetuses of red blood cell-isoimmunised pregnancies (Thilaganathan *et al.*, 1992). There is an ontogenetic change in the site of erythropoietin production during fetal life, starting in the yolk sac then switching to the liver, with a small fraction being formed in the kidney (Huch & Huch, 1994). Perhaps, due to the severity of the anaemia, the kidney became a more important site of

erythropoietin production in my AN rats than it would normally be in fetal life. Hinchliffe *et al.* (1992) found that IUGR babies have a decreased number of nephrons and lighter kidneys than appropriately grown controls. The cause of IUGR and death in these babies was not, however, disclosed by the authors so it is impossible to determine whether they are comparable with IUGR resulting from severe anaemia. Osmotic diuresis in rats has been suggested to cause an increase in protein content and weight of the kidney in rats (Ogino *et al.*, 1994), however there is an increase in aldosterone concentration in anaemic babies (Ville *et al.*, 1994). If aldosterone concentrations also increase in anaemic rats it is unlikely that my AN rats were diuretic. Nonetheless, it seems certain that there was some alteration in kidney function of AN, which probably also affected blood pressure, but unfortunately it is only possible to speculate as to what these changes may have been.

Similar to the heart, between P20 and P40 growth rate of the kidneys of AN was lower than in C, although they were still heavier in AN at P40 (Fig. 3.6). The increasing haemoglobin concentration over this 20 day period would have meant that the hypoxic stimulus for erythropoietin production by the kidney would have decreased. (The kidney is the site of erythropoietin synthesis in the adult animal). This may have had some impact on the growth rate of the kidneys. It is interesting, in view of the dramatic increase in blood pressure seen in AN between P20 and P40 (Fig. 3.10) when haemoglobin levels were also increasing (Fig. 3.2), that patients suffering from renal anaemia and severe anaemia who are treated with recombinant human erythropoietin develop hypertension (Bode-Böger *et al.*, 1992; Eggena *et al.*, 1991). It may be that high levels of erythropoietin in AN were in part accountable for the hypertension that developed, as it has been suggested that erythropoietin affects the balance of the release of various vasodilating (prostacyclin) and vasoconstricting (prostaglandin $F_{2\alpha}$, thromboxane B_2 and endothelin-1) substances (Bode-Böger *et al.*, 1992).

3.4.3.iii Liver

The moderate increase of liver weight in AN (Fig. 3.6) may also be due to increased erythropoiesis and hematopoiesis in response to the anaemia, particularly during fetal life, since by the end of the first trimester the liver and spleen are the main sites of hematopoiesis and the liver of erythropoiesis in the fetus (Huch & Huch, 1994).

3.4.3.iv Lungs

The heavier lungs and slightly greater rate of lung growth seen in AN (Fig. 3.6) may have been related to the rate of body growth in these rats. As has already been discussed, AN grew at a faster rate than C. This may be of relevance so far as lung size and growth go, as it has been observed in the human that rapidly growing infants expend energy at a higher rate and also have a higher resting heart rate and respiratory rate than their normally growing counterparts (Schulze *et al.*, 1993). An elevated respiratory rate may promote lung growth and may have been the cause of the larger lungs seen in AN at P40. I did not measure respiratory rate in this study.

3.4.4 The relationship between placental weight and birth weight

The placental barrier plays a major part in determining the transfer of various substances from the maternal circulation to that of the fetus and *vice versa.* This being so, it is obvious that growth and development of the placenta have important consequences for fetal growth and development. In this study I measured placental weight and fetal body weight to get an indication of growth in each. I observed a lower fetal body weight (Fig. 3.4), and a lower placental weight and placental:body weight ratio (Fig. 3.3) in AN compared to C. This may not seem unexpected at first as it is generally observed in normal healthy pregnancies that there is a positive correlation between fetal and placental size (Calkins, 1937). However, contrary to my findings, maternal anaemia during pregnancy is strongly associated with placental hypertrophy and a greater placental:birth weight ratio than in normal pregnancies (Beischer *et al.*, 1970). So, we must ask why in this case AN had a lower placental:body weight ratio than C. However, first I would like to discuss briefly how useful or valid a measure placental weight is, in terms of whether it is a reasonable indicator of placental dysfunction.

Certain pathological conditions during pregnancy are associated with either placental overgrowth (maternal and fetal anaemia, congenital syphilis, maternal diabetes) or undergrowth (underweight mothers, low pregnancy weight gain, maternal hypertension), and associations have been made between placental undergrowth and reduced neonatal growth (Naeye, 1987). More recently Barker *et al.* (1990) have shown that babies who are small at birth and have a large placenta, i.e. a high placental:birth weight ratio, are

predisposed to developing hypertension in later life. Despite these connections there seems to be a degree of uncertainty as to the significance of placental weight as a measure of neonatal outcome. In the clinical setting most anxiety surrounding the recording of placental weight seems to centre around the accuracy of measurement, though our lack of understanding of the relationship between placental and fetal growth also adds to the uncertainty (Newton, 1993). Does the placenta control the size of the baby or does the baby control the size of the placenta? Our present understanding of the interaction that takes place between the two would suggest that there is no clear cut answer, but rather there is a complex interrelationship between the two. Endocrine factors, cytokines and various growth factors interact in precisely timed ways to regulate pregnancy, and placental and fetal growth and development (Robinson *et al.*, 1995). However, in situations where there are alterations in the maternal nutritional or hormonal environment, the balance between fetus and placenta is perturbed, which affects the growth of both (Robinson *et al.*, 1994). In fact, over 35 years ago it was shown that abnormally small or large placentae are associated with adverse perinatal outcome (Little, 1960). On balance then, placental weight is a quantity that is worth considering in relation to fetal and neonatal growth and development.

Clearly in this study the growth of AN fetuses was impaired (Fig. 3.4). This may have been a direct result of anaemic hypoxia since it has been shown in the fetal lamb that chronic hypoxaemia reduces growth (Gagnon *et al.*, 1995; Kamitomo *et al.*, 1994; Mostello *et al.*, 1991; Block *et al.*, 1989; Jacobs *et al.*, 1988; Owens *et al.*, 1987). Similarly, in the fetal rat, growth was impaired in response to increased maternal oxygen affinity (Hebbel *et al*, 1980). However, contrary to my findings, Hebbel *et al.* (1980) found an increase in placental:body weight ratio, as did Godfrey *et al.* (1991) in anaemic women. Whilst this increase in the ratio is partly due to reduced fetal growth, there would also appear to be a contribution from enhanced placental growth, which may be in response to hypoxia. The finding that the placenta is larger in women who live at high altitude (Krüger & Arias-Stella, 1970) and the observation of Alexander (1964) that surgical reduction in the number of caruncles resulted in a compensation during pregnancy by an increase in individual cotyledon weight, supports the idea of there being enhanced placental growth in response to hypoxia. However, Jackson *et al.*, (1987) found that placental weight was unchanged in Amerindian women living at altitude in Bolivia, although placental to birth weight ratio was increased

because of the reduced birth weight of babies born at altitude. Although they were not significantly different in weight, Jackson *et al.* (1987) found that the histological composition of highland placentae was significantly altered and oxygen transfer was enhanced by movement of the fetal capillaries to the periphery of the villi. Similarly, Reshetnikova *et al.* (1993) found that the villous membrane was thinner in the terminal villi of human placentae from altitude, and that there was an increased density of capillaries which seemed to be vasodilated. Following long-term exposure of guinea pigs to hypoxia, placentae are found to have an increased number of capillaries of reduced diameter which display increased branching and coiling, and a reduced trophoblast thickness which improves placental diffusing capacity and oxygen transfer (Scheffen *et al.*, 1990). These studies demonstrate that the placenta can adapt so as to increase diffusion capacity when exposed to hypoxia. No histology was carried out on the placentae from the rats in my study, but it seems likely that the placentae from AN animals would have shown structural differences from C, as well as being lighter. Several studies in both sheep (Alexander, 1964; Everitt, 1964; Harding *et al.*, 1985; Owens *et al.*, 1987) and humans (Naeye, 1987) show an association between reduced placental growth and reduced fetal growth. It is therefore likely that in this study also the small placentae of AN were linked with the reduction in fetal growth. The question of cause and effect, however, is one that my study is not able to address.

3.5 CONCLUSION

The results of the study described in this chapter show that the pronounced postnatal rise in SBP associated with maternal anaemia during pregnancy has its origins *in utero* and, and as such, this work adds support to the epidemiological findings of Barker *et al.* (1989). But, I did not find the elevated SBP of AN rats to be associated with a greater placental:body weight ratio; instead there was a decrease in placental weight. I did, however, find that there is an association between the rate of growth and blood pressure. Growth during fetal life appeared to be slower, judging by the fact that AN fetuses were lighter than C, and SBP was lower; and postnatally, hypertension developed in AN pups which showed an greater rate of growth than C.

The results of this study highlight several areas of work that need to be pursued. The possible mechanisms for the observed changes in blood pressure development as a result of maternal anaemia during pregnancy have been discussed, but cardiac output and peripheral vascular resistance need to be measured in the anaemic rat in order to confirm the mechanisms proposed above. Measurement of fetal blood pressure in this study would have provided us with valuable information, so it is clear that a study of development during fetal life, after birth and into adulthood needs to be done. The cogency of placental size as a marker of blood pressure development remains unclear, so an in depth study of the placenta e.g. histology, analysis of various enzymes, hormones and growth factors, would give us a clearer idea as to the importance of the placenta in blood pressure development. Lastly, the mechanisms linking growth, both pre-natally and post-natally, and blood pressure development are far from clear and further work is required if we are to understand the interaction between the two.

There is an important point that arises from the finding that hypertension was preceded by a low SBP before weaning in anaemic pups. This is that it is necessary to follow the development of individuals right through to maturity in this type of longitudinal study, where one wishes to study the effect of development during fetal and neonatal life on adult health. Retrospectively this is perhaps obvious, owing to the enormous physiological changes that take place in early life. We must be cautious if seeking to extrapolate from the infant to the adult.

3.6 SUMMARY

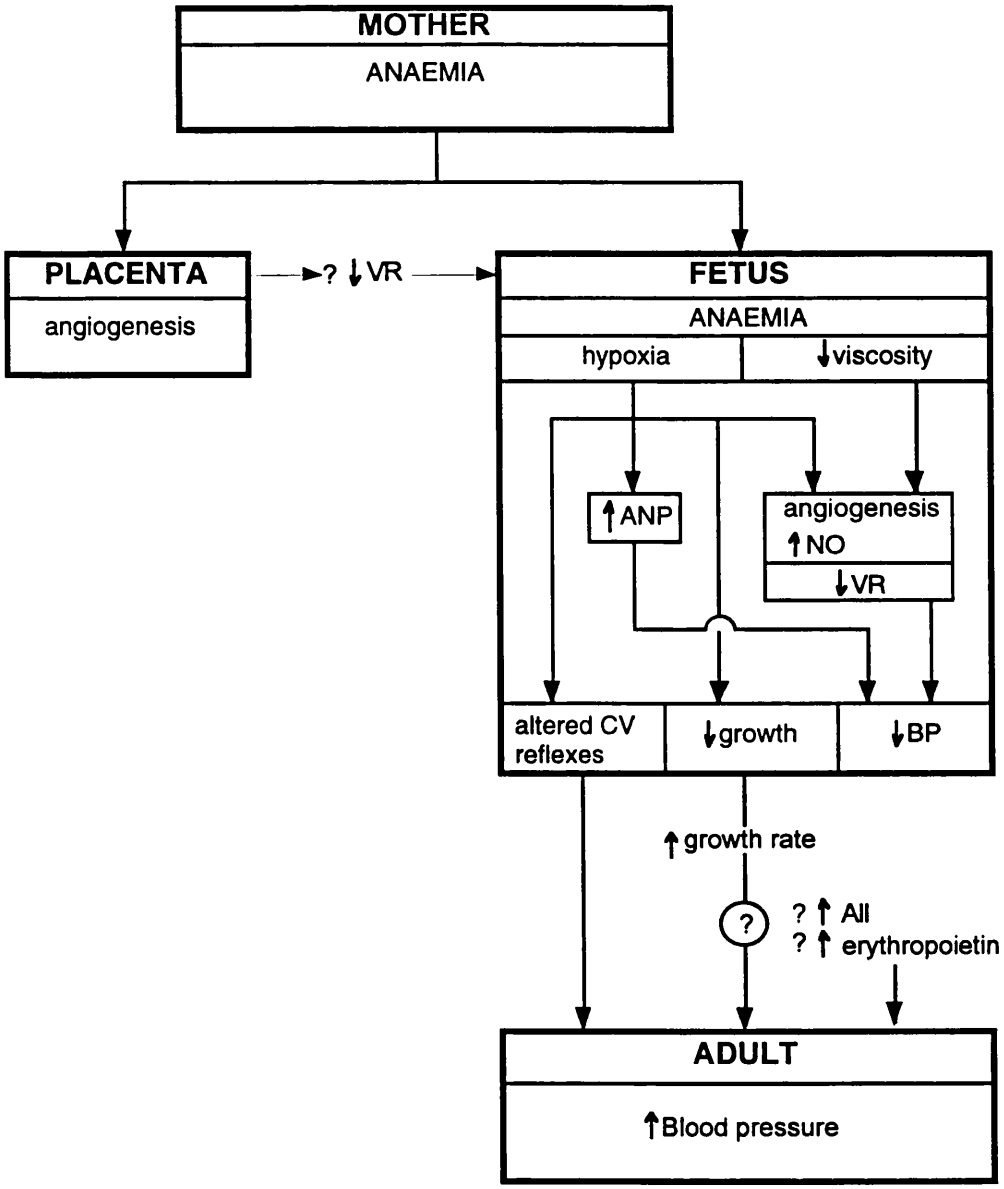

Figure 3.11. Diagram summarising the possible mechanisms involved in the development of hypertension as a result of maternal anaemia during pregnancy.

Chapter 4

FETAL CARDIOVASCULAR DEVELOPMENT: THE EFFECT OF REPEATED ACUTE HYPOXAEMIA

4.1 INTRODUCTION

The results described in Chapter 3 suggest that hypoxaemia during life *in utero* may play a major role in the development of hypertension in later life.

The fetal cardiovascular hemodynamic (Thornburg & Morton, 1993) and reflex (Giussani *et al.*, 1994) responses to a single acute hypoxaemic challenge in late gestation are now well established (discussed at length in Chapter 1, section 1.7.2.ii). In response to chronic hypoxaemia (in the absence of acidaemia) for up to 24 hours it has been demonstrated that there is a return of heart rate and blood pressure to normoxaemic levels, despite PaO_2 levels remaining low (Bocking *et al.*, 1992; Fujimora *et al.*, 1994) (discussed more fully later on in this chapter, section 4.4.1). The mechanisms responsible for this adaptation are as yet unknown. Investigations into the development of heart rate and blood pressure in response to longer periods of hypoxaemia (weeks) have so far yielded little further insight into the mechanisms involved, as the results obtained vary from investigator to investigator (discussed more fully later in this chapter, section 4.4.1). Whilst there is a growing literature on the effects of chronic hypoxaemia on the fetus, and there are a number of studies investigating the acute effects of repeated hypoxaemic/asphyxic insults (lasting seconds or minutes in duration) (Jensen *et al.*, 1985 & 1987; Giussani *et al.*, 1996a & c), there is, to my knowledge, no published information regarding the effects of repeated acute hypoxaemia on the development of the fetus, an insult that could perhaps be regarded as lying somewhere between acute and chronic hypoxaemia.

A physiological event that may be viewed as repeated acute hypoxia is the occurrence of contractures. There is little uterine activity during pregnancy, except for the last 6-18 hours before parturition. However, it is now well established that there are spontaneously occurring uterine contractions, known as 'contractures', that occur throughout pregnancy. These contractures are of low amplitude (causing only 3-10 mmHg changes in amniotic pressure) and low frequency (0.5-3/hour) with a duration of 3-15 minutes. Thus, they have a much longer duration and lower amplitude than the high-frequency, high-amplitude and relatively short duration 'contractions' that are associated with labour (Jenkin & Nathanielsz, 1994). Contractures are linked with a modest increase in intra-amniotic pressure, decreased uterine blood flow, decreased fetal PaO_2 and CaO_2, and an increase

in $PaCO_2$, haemoglobin concentration and arterial blood pressure (Llanos *et al.*, 1988). More recently, measurements taken over a 6-hour period have shown that the changes in blood gases that occur with uterine contractures are quite considerable, with variations from 2.5-5.5% in PaO_2 and 3.6-7.0% in SaO_2 (Woudstra *et al.*, 1995). It is not known how much these physiological changes in blood gases affect the fetus. Sleep apnoea in adult humans is a syndrome that also results in repeated reductions in PaO_2, and it is associated with primary hypertension (Tilkian *et al.*, 1976; Kales *et al.*, 1984; Fletcher, 1995). Sleep apnoea has also been observed in pregnant women (Kowall *et al.*, 1989; Schoenfeld *et al.*, 1989; Sherer *et al.*, 1991), although how commonly it occurs during pregnancy is unknown. Schoenfeld *et al.* (1989) reported acid-base changes in the fetus during maternal apnoeic episodes, which they suggested were due to compromised fetal oxygen delivery. They suggested that sleep apnoea during pregnancy may affect fetal growth and well-being. It is possible therefore that repeated acute hypoxaemia in the fetus may result in an increase in blood pressure and reduced fetal growth.

Bearing this in mind and in view of growing interest concerning the implications of intrauterine events for disease in later life, it was my hypothesis that repeated acute hypoxaemia would cause resetting of the peripheral chemoreceptors, resulting in a chronic increase in sympathetic tone and thus vascular resistance, which would in turn cause the baroreceptors to reset to higher blood pressure levels resulting in chronic hypertension (Fig. 4.1).

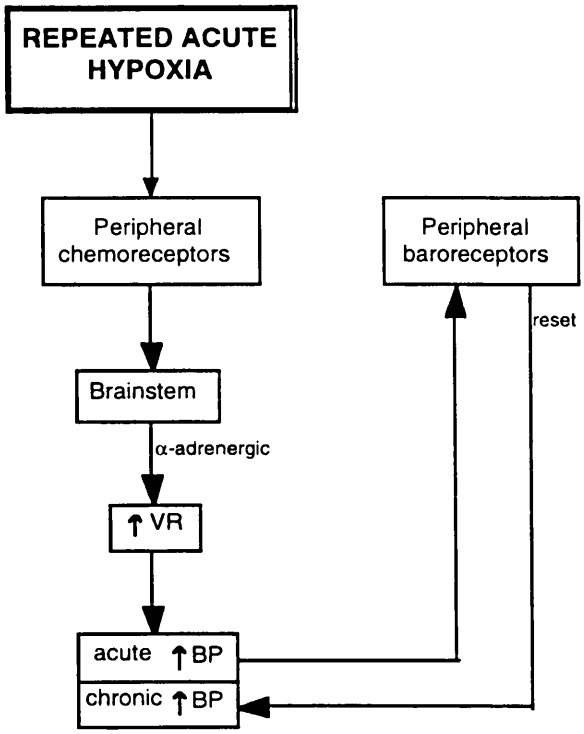

Figure 4.1. Diagram illustrating my hypothesis as to how repeated acute hypoxaemia may result in fetal hypertension.

The work described in the previous chapter was able to show that intrauterine events do impact upon future cardiovascular development. However that study was not able to address the possible mechanisms involved in the development of blood pressure *in utero*, primarily because the rat is not a suitable model in which to do so. The need for further investigation of fetal cardiovascular development was thus highlighted.

So, the aim of the project described in this chapter was to investigate the effect of a repeated acute hypoxic insult on the cardiovascular development and growth of the fetus.

4.2 EXPERIMENTAL DESIGN

4.2.1 Protocol

The ewes used in this experiment were Mules (see section 2.3.1).

14 fetuses between 105 and 109 days gestation were instrumented, as described in Chapter 2 (section 2.3.4), with carotid artery, jugular vein and amniotic catheters, an ECG electrode and a Transonic flow probe around the femoral artery.

Experiments began after the 5-day recovery period. For 14 days, 7 fetuses were subjected to a daily 1 hour episode of acute isocapnic hypoxaemia (as described in section 2.3.8.i) (Hypoxia group, H) and 7 control (C) fetuses were studied for 3 hours in normoxia. Blood samples were taken at intervals during each 3 hour experiment for blood gas, glucose and lactate analysis (see section 2.3.8.i, Fig. 2.9).

4.2.2 Post mortem

At the end of the study a basic post-mortem was carried out, where fetal body and organ weights were recorded, as described in section 2.3.9.i.

4.2.3 Problems

Unfortunately, there were 3 fetuses (2 H and 1 C) for which it was not possible to collect data for the whole of the 14-day monitoring period. In one fetus (92-45) the vascular catheters became blocked on the 11th. experimental day, so no arterial venous, or blood gas values could be obtained after that. The second animal (94-14) had to be put down on the 12th. experimental day, because the ewe went into labour and would have aborted otherwise. In the third fetus (92-43) the catheters pulled out on the 13th. experimental day, so the fetus died. Owing to the fact that these experiments are extremely time-consuming, and since not less than 10 days worth of data had been collected for each of the animals that were lost, it was decided to include them in the analysis.

There were also 7 animals in which the perivascular flow probe failed to work. In 3 animals the flow probes did not work from the beginning of the experiment (presumably due to poor placement). In 1 animal the ewe bit through the cable on the fourth experimental day. In the other 3 fetuses, the vessel slipped out of the probe (determined at post-mortem).

4.3 RESULTS

In all of the following figures the statistics relating to the symbols in the figures are explained in the figure legend. Where analysis was by ANOVA or summary measure, the statistical significance is stated in the text.

4.3.1 Mean arterial blood pressure (MAP) and heart rate (FHR)

Absolute MAP and the increase in MAP were not significantly different between C and H, with both groups showing a significant increase in MAP between 115 and 128 d GA (Fig. 4.2). Although SBP and DBP tended to increase in both groups, the increase was not significant over the 14 days, and nor was there a significant difference in absolute values between groups. There was, however, a significant difference in the slightly elevated SBP ($P < 0.05$) and DBP ($P < 0.05$) trajectory of H compared to C (Fig. 4.2). FHR decreased significantly between 115 and 128 d GA in both C and H fetuses (Fig. 4.2). Absolute FHR values and the decrease in FHR over the study period were the same in the two groups (Fig. 4.1). The rate-pressure product, likewise, was not significantly different between C and H, and it did not change significantly in either group with increasing gestational age (Fig. 4.2).

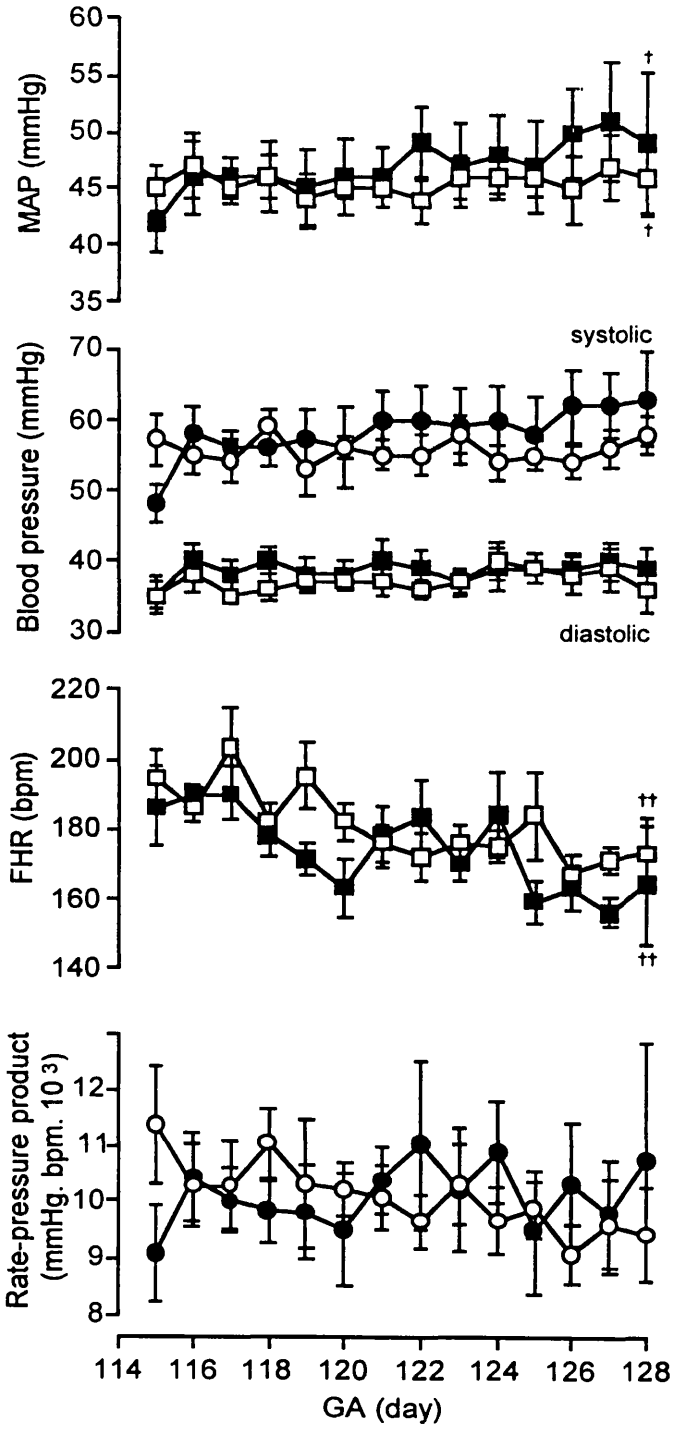

Figure 4.2. Development of MAP, SBP and DBP, FHR, and rate-pressure product in C (o/□) and H (●/■) fetuses. † $P < 0.05$, †† $P < 0.01$, 115 d GA vs. 128 d GA. Values are mean ± S.E.M.

4.3.2. Chemoreflex

The cardiovascular chemoreflex was assessed in H fetuses by looking at the responses to hypoxaemia of heart rate, femoral blood flow and femoral vascular resistance, 5 minutes after onset; and the blood pressure response 15 minutes after onset. None of the 4 parameters showed a significant change from the beginning to the end of the study (Fig. 4.3). The changes in MAP and FHR were quite variable from day to day. It looked as though there was an increase in the flow and resistance responses from 114 d GA to 128 d GA, but there were only 3 animals in which measurements were obtained, so statistical significance was not reached. Thus, there was no significant change in the reflex with increasing gestational age.

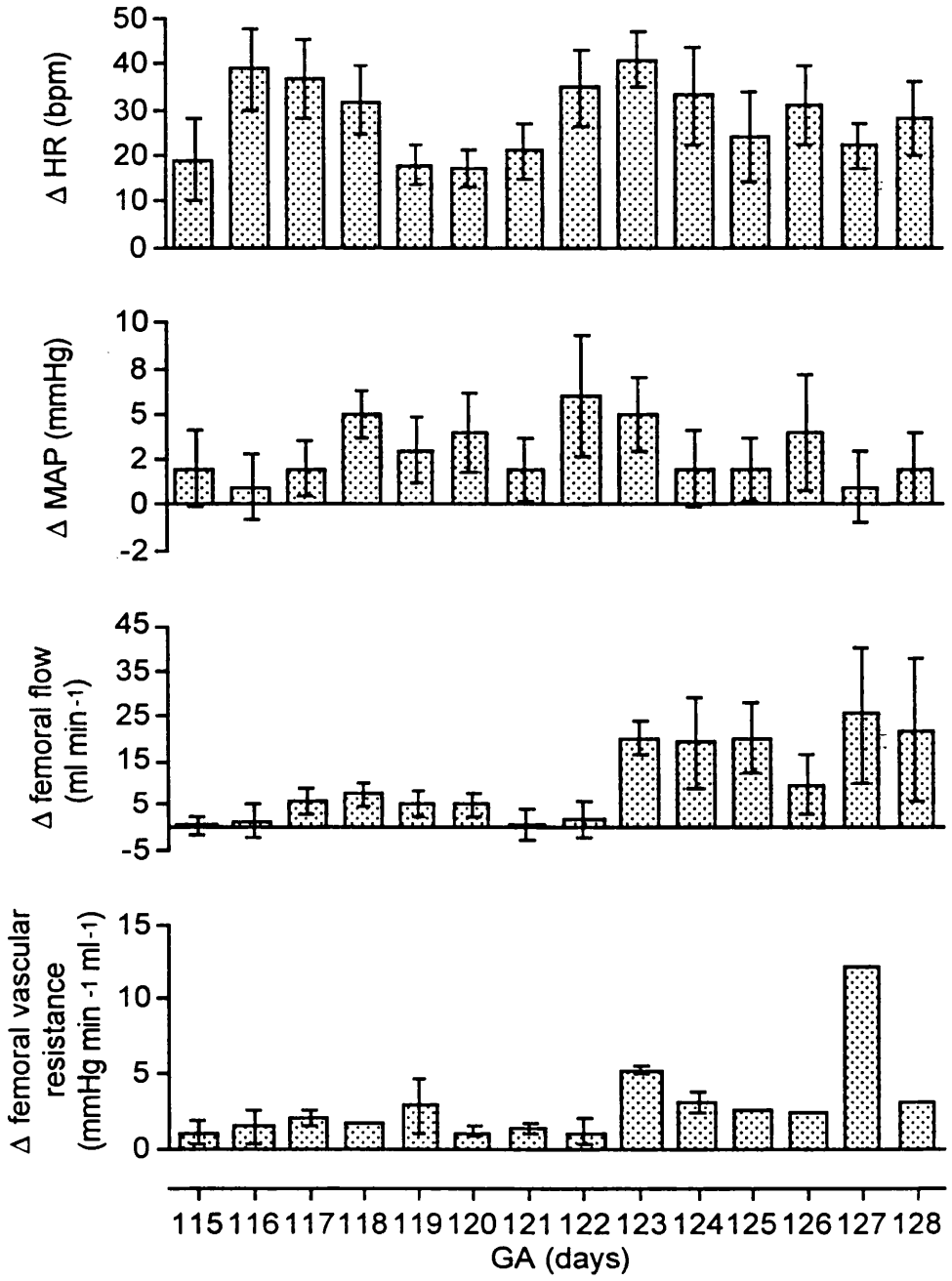

Figure 4.3. The developmental change in the bradycardic (5 min), hypertensive (15 min), femoral flow (5 min) (n=3) and femoral vascular resistance (5 min) (n=3) responses to hypoxaemia in H fetuses. Values are mean ± S.E.M.

4.3.3 Baroreflex

Going on to look at the baroreflex, there was a significant ($P < 0.01$) shift of the curve to the right between 115 d and 128 d GA in both C and H fetuses (Fig. 4.4), suggesting that the baroreceptors had reset by 128 days gestation in both groups. This is not surprising in view of the increase in MAP (Fig. 4.2) that occurred over this period.

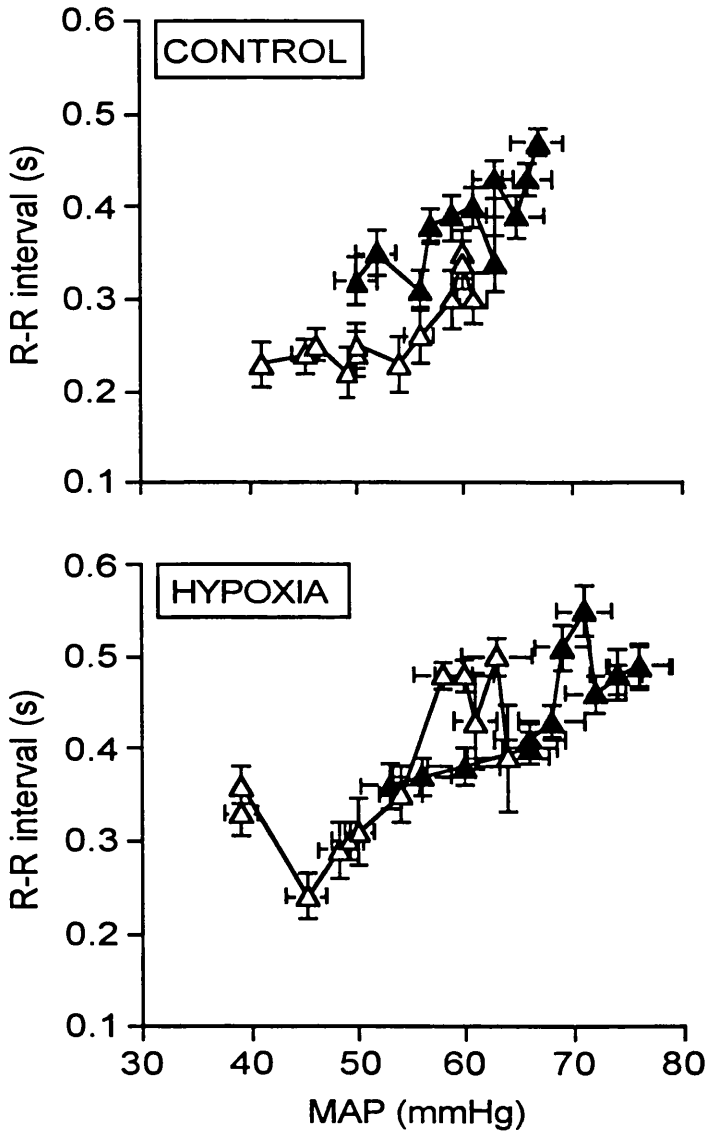

Figure 4.4. Baroreflex curves for C (n = 6) and H (n = 5) fetuses at 115 d GA (Δ) and 128 d GA (▲). Values are mean ± S.E.M.

The slope of the baroreflex curve was not significantly different between 115 and 128 d GA within either group, or indeed between groups. But, there was a tendency for the slope to decrease in both C (P = 0.2) and H (P = 0.2) between 115 and 128 days gestation (Fig. 4.5).

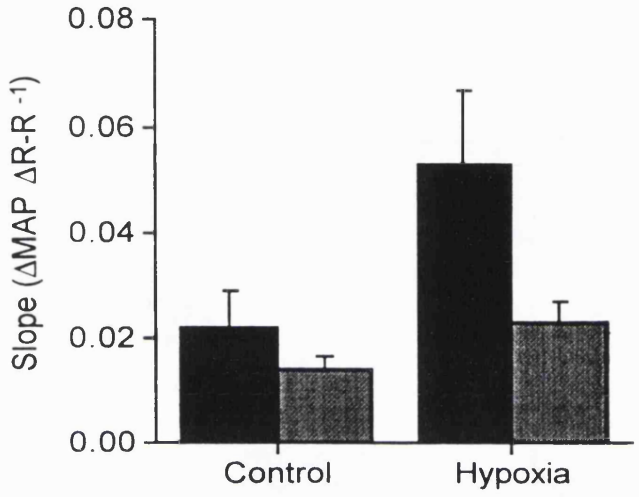

Figure 4.5. Slope of the steep part of the baroreflex curve in C (n=6) and H (n=5) fetuses at 115 d GA (■) and 128 d GA (▣). Values are mean ± S.E.M.

4.3.4 Femoral blood flow and vascular resistance

Unfortunately, I did not obtain flow data for all 14 animals (see section 4.2.3). This was because the flow probes did not work after implantation in some animals, primarily because the vessel slipped out of the probe. I addressed this problem by changing to U-type rather than L-type reflectors (see Fig. 2.6 for illustration of different reflector types).

Femoral flow increased significantly in C (n=4) but there was no significant change in vascular resistance. In H (n=3), femoral flow seemed to increase and vascular resistance to decrease, but neither increase nor decrease were significant. Despite the increase of femoral flow in C, trajectory over the 14 days of the study was similar for both groups. The development of vascular resistance was also similar (Fig. 4.6).

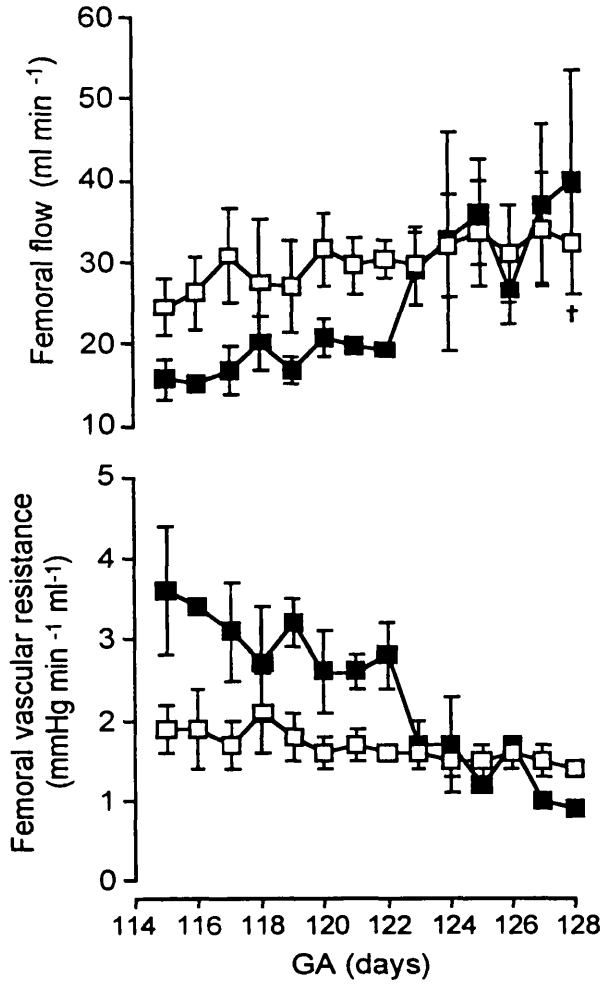

Figure 4.6. Femoral flow and femoral vascular resistance in C (□) (n=4) and H (■) (n=3) fetuses. † $P < 0.05$, 115 d GA vs. 128 d GA for C. Values are mean ± S.E.M

4.3.5 Fetal growth

Fetal growth was unaffected by repeated acute hypoxaemia, as can be seen from body and organ measurements taken at post-mortem (Table 4.1). There was no significant difference in CRL, body, heart, lung or liver weights, though kidney weights in H were found to be significantly smaller than in C. However, when expressed as a percentage of body weight, no difference in organ size was found between the two groups (Table 4.1).

GA for both groups was the same and they were evenly distributed in terms of sex and plurality.

	CONTROL	HYPOXIC
GA (days)	128 ± 1	128 ± 1
Sex	4M, 3F	4M,3F
Twin/Singleton	3T, 4S	3T, 4S
CRL (cm)	44.3 ± 1.4	48.5 ± 1.4
Body weight (g)	3345 ± 536	3964 ± 251
Heart weight (g)	25.4 ± 2.6	33.2 ± 2.7
Lung weight (g)	100.3 ± 14.5	120.2 ± 7.5
Liver weight (g)	122.2 ± 17.1	105.1 ± 8.1
Kidney weight (g)	13.8 ± 0.5	11.8 ± 0.6 *
Heart % BW	0.71 ± 0.05	0.83 ± 0.04
Lung % BW	2.71 ± 0.18	3.05 ± 0.10
Liver % BW	3.40 ± 0.30	2.66 ± 0.14
Kidney % BW	0.43 ± 0.06	0.31 ± 0.02

Table 4.1. Gestational age (GA), number of male (M), female (F), twin (T) and singleton (S) fetuses; actual body, heart, lung, liver and kidney weights, and heart, lung, liver and kidney weights as a percentage of body weight (BW), in C and H fetuses. * $P < 0.05$, C vs. H. Values are mean ± S.E.M

4.3.6 pH, $PaCO_2$ and PaO_2

Figure 4.7 (left-hand side) shows the trend in mean basal values of pH, $PaCO_2$ and PaO_2 over the 14 days of the study. There was no significant increase or decrease over time in any of theses variables. The trends in $PaCO_2$ and pH over the 2 weeks were similar for C and H. PaO_2, however, followed a significantly ($P < 0.001$) higher trajectory in H animals compared to C.

I saw no increase or decrease in basal pH, $PaCO_2$ or PaO_2 over time, as described above, I therefore expressed them as a single mean value (summary measure) for the whole of the 14 days. In so doing I found that $PaCO_2$ and pH were the same for both groups and PaO_2 was significantly higher in H than in C (Fig. 4.7 - right-hand side).

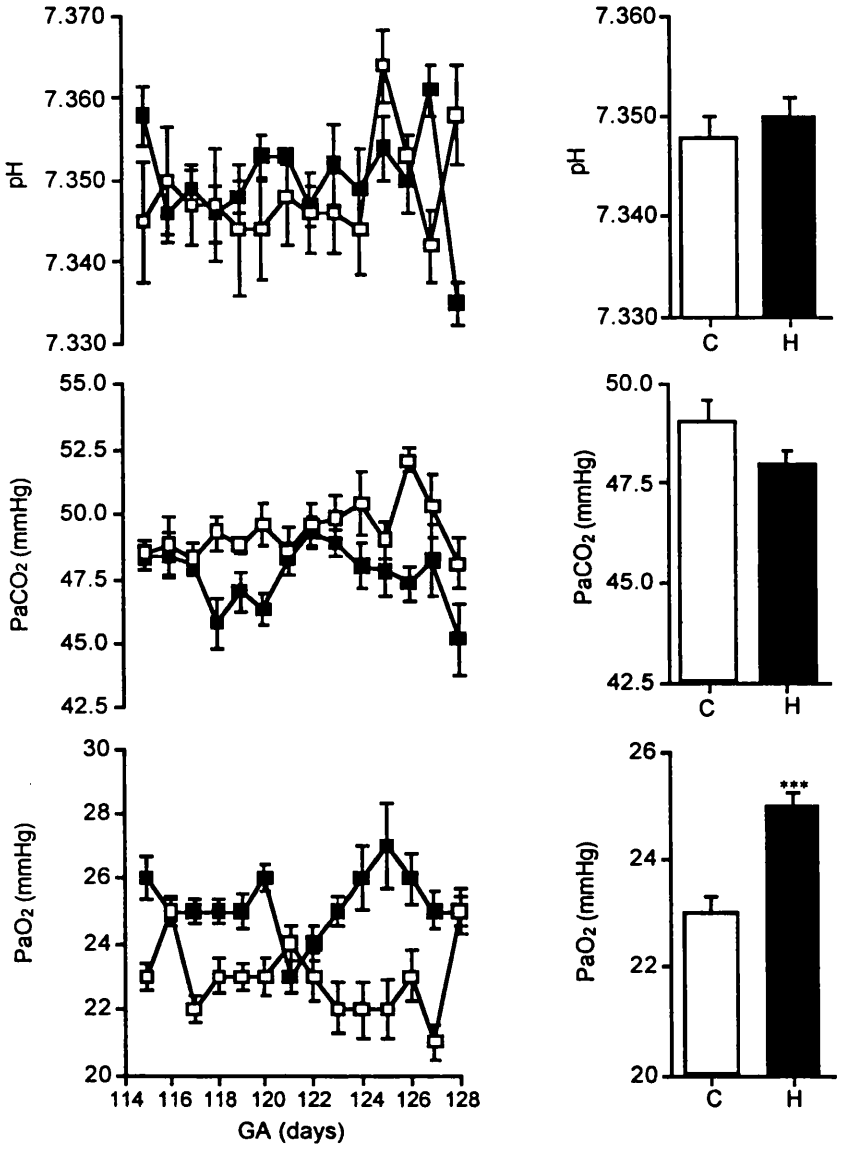

Figure 4.7. pH, $PaCO_2$ and PaO_2 values over the 2-week study period in C (□) and H (■) fetuses (left-hand side), and the mean values for the whole 14 days in each group (right-hand side). *** $P < 0.001$, C vs. H. Values are mean ± S.E.M.

4.3.7 Haematocrit and haemoglobin

Haematocrit and haemoglobin values decreased significantly in H fetuses over the 14 days of the study, and haematocrit values followed a significantly ($P <$ 0.005) lower trajectory throughout than in C (Fig. 4.8). There was a tendency for haematocrit and haemoglobin to decrease in C, but the decrease was not significant. Despite the significant decrease of haemoglobin levels in H, the trajectory was not significantly different to that of C.

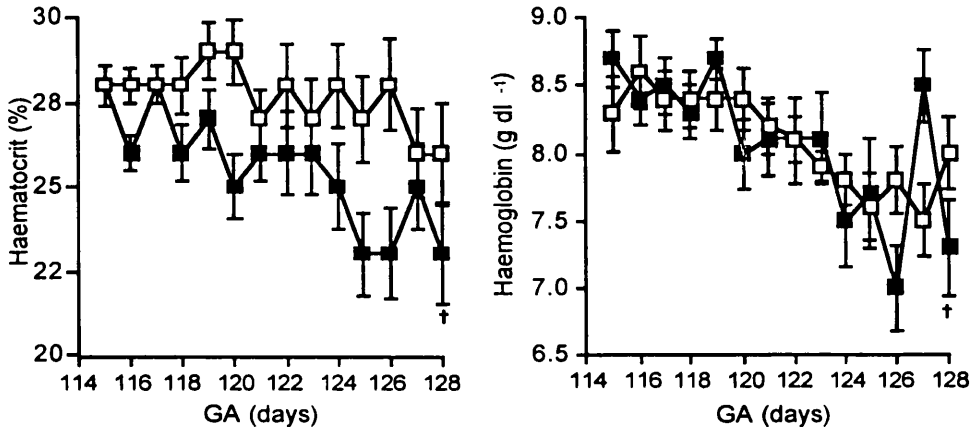

Figure 4.8. Haematocrit and haemoglobin values in C (□) and H (■) fetuses. † $P < 0.05$, Day 1 vs. Day 14 for H. Values are mean ± S.E.M.

4.3.8 Glucose and lactate

Glucose levels were somewhat variable but there was no significant difference in absolute values between groups, and neither was there any significant change in glucose levels from the beginning to the end of the study in either C or H fetuses. Lactate levels looked as though they may have increased between 114 d and 128 d GA, but the increase was not significant for either group. Lactate values in H fetuses did, however, follow a significantly ($P < 0.001$) higher trajectory than in C over the 14 days (Fig. 4.9)

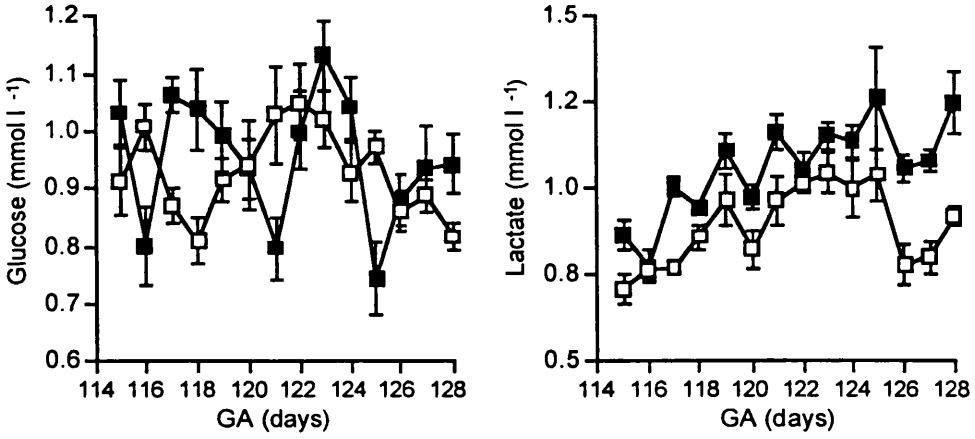

Figure 4.9. Blood glucose and lactate levels in C (□) and H (■) fetuses. Values are mean ± S.E.M.

4.3.9 Cations

The concentrations in blood of Na^+, K^+ and Ca^{++} were similar in C and H and did not change significantly over the 2-week study period. The trajectory for each was not significantly different between groups either (Fig. 4.10).

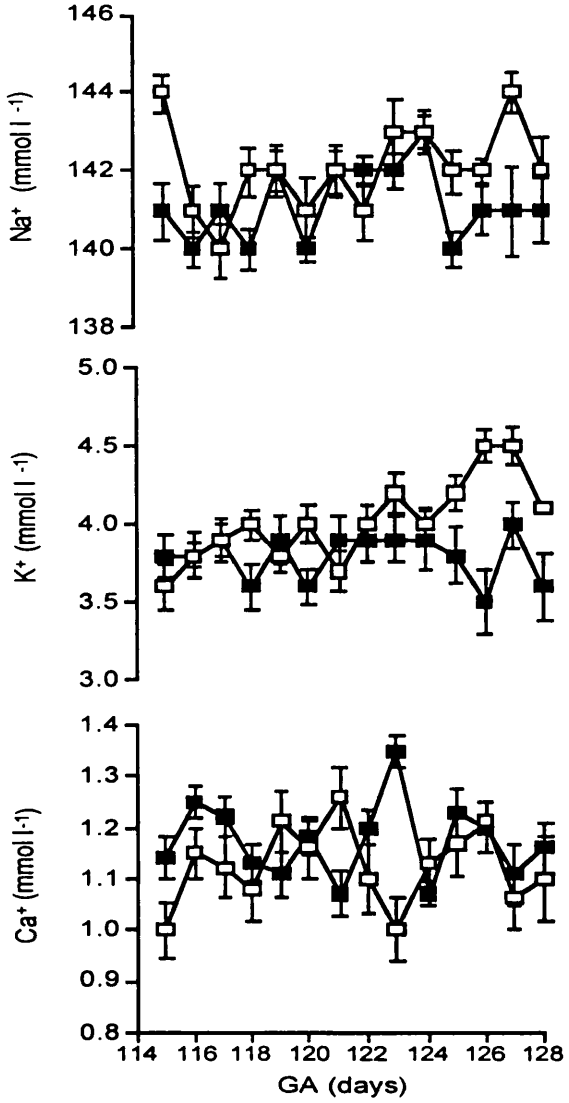

Figure 4.10. Na^+, K^+ and Ca^{++} concentrations in the blood of C (□) and H (■) fetuses. Values are mean ± S.E.M.

4.4 DISCUSSION

The purpose of this study was to investigate the effects of repeated acute hypoxaemia on fetal cardiovascular development. I have found that repeated acute hypoxaemia of this degree and for a duration of 14 days does not have a significant affect on MAP, FHR, and chemo- and baroreflex development. Furthermore, fetal growth, other than the kidney, is not affected.

4.4.1 Hypoxaemia, MAP and FHR

There was a progressive increase in MAP and decrease in FHR over the period of the study (Fig. 4.2), as has previously been observed in the late gestation fetal sheep (Boddy *et al.*, 1974; Kitanaka *et al.*, 1989; Daniel *et al.*, 1989; Mostello *et al.*, 1991; Kamitomo *et al.*, 1994; Gagnon *et al.*, 1994; Murotsuki *et al.*, 1996). The gestational changes of MAP and FHR were the same in both C and H (Fig. 4.2), suggesting that repeated acute hypoxaemia of the degree and duration (14 days) employed in this study does not affect cardiovascular development. However, both SBP and DBP followed a significantly higher trajectory in H than in C, SBP showing a tendency to increase (Fig. 4.2). This may reflect increasing contractility in H fetuses, caused by increased sympathetic tone due to repeated chemoreceptor activation (see section 4.4.3.i later on in this chapter) in response to the repeated hypoxaemia. If this were the case, however, one would expect to see an increase in vascular resistance also. There was no difference between groups in the development of femoral flow, which tended to increase, and femoral vascular resistance, which tended to decrease (Fig. 4.6). The tendency for femoral flow to increase was most probably due to the increasing size of the fetuses. Seeing as femoral vascular resistance decreased with increasing gestational age, the increase in MAP must have been due to increased CVO and/or a rise in vascular resistance in beds other than the femoral, for both C and H.

Adult studies

No previous investigators have looked at the effects of repeated acute hypoxaemia on the development of blood pressure in the fetus, but there are studies in the adult rat. Young male rats, intact and carotid body denervated, were housed in chambers, in which the gas concentrations could be manipulated, and they were exposed to episodic hypoxia over 35 days every 30 seconds for 7 hours/day (Fletcher *et al.*, 1992). This pattern of episodic hypoxia was set up to mimic that seen during obstructive sleep apnoea in humans. It was found that repeated hypoxia caused an increase in MAP over the 35 days and that the increase in pressure was chemoreflexly mediated. Further experiments indicate that peripheral sympathetic nerves play a role in the chronic increase of pressure in response to acute episodic hypoxia (Fletcher, 1995). But the role of adrenal hormones in the chronic elevation of blood pressure in response to acute recurrent hypoxia, and therefore repeated

acute recurrent increased sympathetic output, remains to be elucidated (Fletcher *et al.*, 1995). Thus it remains unclear as to the mechanisms whereby acute increases in blood pressure are translated into a chronic increase. What is clear, however, is that the rise in blood pressure is gradual, as it was found that rats exposed to less than 30 days episodic hypoxia showed no significant increase in MAP (Fletcher *et al.*, 1992). So, coming back to the fetuses in my study, would a prolongation of the protocol have resulted in an effect on blood pressure?

Length of exposure

Perhaps repeated acute hypoxaemia for longer than 14 days would have resulted in hypertension, such as has been observed in the study of Murotsuki *et al.* (1996) where they performed repetitive placental embolization for 21 days. The decrease in PaO_2 for 1 hour daily that I imposed on H fetuses is not dissimilar to the changes in PaO_2 and CaO_2 which are seen in fetuses where fetal placental embolization is carried out on a daily basis (Gagnon *et al.*, 1994; Gagnon *et al.*, 1995; Murotsuki *et al.*, 1995; Murotsuki *et al.*, 1996a; Murotsuki *et al.*, 1996b). In those studies fetuses were injected daily over a period of 10-21 days with non-radioactive microspheres through the descending aorta, so as to decrease fetal arterial oxygen content by 30-35% of the pre-embolization value. Each day when the microspheres were injected, CaO_2 decreased, as was desired, but then there was a partial recovery in fetal oxygenation. Repeated embolization is not directly comparable to my repeated acute hypoxaemia challenge, however, because there is a gradual decrease in basal (pre-embolization) oxygenation levels from the beginning of the study to the end. Whereas, basal PaO_2 values in my H fetuses, although not higher in absolute terms, tracked at a higher level and when expressed as a single mean for the whole 2-week period, were higher compared to C (Fig. 4.7). In seeking to make comparisons between repetitive placental embolization and my repeated acute hypoxia challenge, we must also remember that embolization results in an increase in umbilical-placental resistance, seen as an increase in the umbilical artery resistance index (Gagnon *et al.*, 1994; Murotsuki *et al*, 1996), but in contrast, hypoxaemia for up to an hour in late gestation results in an increase in placental blood flow (Bocking *et al.*, 1988; Rurak *et al.*, 1990). Nonetheless, Gagnon *et al.* (1994) found that the maturational changes in MAP and FHR in their fetuses were not affected by repeated acute embolization over a period of only 10 days. Similarly,

Trudinger *et al.* (1987), Block *et al.* (1989 & 1990) and Carter *et al.* (1996) found that MAP and FHR in their fetuses were unaffected by repetitive embolization for a period of either 8 (Carter *et al.*, 1996) or 9 (Trudinger *et al.*, 1987; Block *et al.*, 1989 & 1990) days in late gestation. Murotsuki *et al.*, (1996a) have found, however, that when embolization is carried out for longer, for a period of 21 days, MAP is significantly elevated by the end of the study and FHR is similar to controls. Taking the evidence so far it seems that the processes leading to alterations in the maturation of MAP and FHR in response to hypoxaemia evolve over a period of more than 14 days.

Severity

This may be because different causes of hypoxaemia produce varying severities of hypoxaemia in the fetus (Table 4.2). However, we can see that similar degrees of hypoxaemia do not necessarily have similar effects on MAP and FHR, e.g. reduced maternal plasma volume and reduced maternal FiO_2 both reduced fetal PaO_2 to about 17 mmHg, which resulted in increased MAP and decreased FHR, and decreased FHR without change in MAP, respectively.

Methods of inducing hypoxaemia

It may be that the method whereby hypoxaemia is produced is also an important factor in determining cardiovascular adaptation and outcome. As I have already mentioned, placental embolization produces an increase in placental resistance, which is contrary to the decrease in placental resistance that occurs in response to hypoxaemia. There have been a number of studies investigating the effects of chronic hypoxaemia, using methods other than repeated embolization, on aspects of fetal development including effects on MAP and FHR. Davis & Hohimer (1991) made late gestation fetal sheep hypoxaemic as a result of fetal anaemia, which was induced by daily haemorrhage of 60-100 ml for 7 days. They found that anaemic fetuses developed higher FHR and lower MAP than controls. It may be important to remember that anaemia may also be accompanied by a decrease in blood viscosity which would affect vascular resistance and hence blood pressure (the effects of altered viscosity are discussed in some detail in section 3.4.1.i). Fetal sheep made hypoxaemic for 14 days, as a consequence of reduced maternal FiO_2, developed a lower FHR than controls by the end of the study, but MAP was not significantly different (Kamitomo *et al.*, 1994).

They also showed a drop in $PaCO_2$ from day 3 onwards, although there was no change in pH. Daniel *et al.* (1989) induced fetal hypoxaemia for 30 days (between about 111 and 141 days gestation) in fetal sheep by preventing the normal expansion of maternal blood volume (20 ml/day) that occurs during pregnancy. They found that hypoxaemic fetuses failed to show the gestational increase in blood pressure but that the decrease in FHR was smaller than that seen in control fetuses. So by the end of their study, hypoxaemic fetuses had a lower systolic blood pressure and higher FHR than control animals. Fetal hypoxaemia for 30 days secondary to chronic maternal anaemia, which was produced by repetitive exchange transfusions such that maternal haematocrit was reduced to 12%, did not affect the developmental decrease of FHR but caused less of an increase in MAP, so hypoxaemic fetuses had a lower MAP than controls by the end of the study (Mostello *et al.*, 1991). It is possible that there may be placental adaptations to maternal anaemia, such as a decrease in resistance, that could contribute to the decreased MAP observed by Mostello *et al.* (1991) in their fetuses. Fetal guinea pigs developed both a lower MAP and FHR by 60-64 days gestation after hypoxaemia for 30 days as a result of uterine artery ligation (Detmer *et al.*, 1991). High altitude hypoxaemia for 90 days (30-120 days gestation) has been shown to cause elevated MAP but no change of FHR in late gestation fetal sheep (Kamitomo *et al.*, 1992). It may be important to note that in the study of Kamitomo *et al.* (1992) the sheep were studied after they had been transported back down to sea-level, and hypoxaemia was maintained by the administration of nitrogen through a maternal tracheal catheter. High altitude is also associated with placental hypertrophy (Krüger & Arias-Stella, 1970) which it has been suggested is the result of structural changes in terms of vascular density and trophoblast volume (Mayhew, 1991). Sheep fetuses that were hypoxaemic for an even longer period, as a result of caruncleclectomy, had lower blood pressures and higher heart rate than controls (Robinson *et al.*, 1983). These effects of chronic hypoxaemia on MAP and FHR are summarised in Table 4.2. Clearly the fetus does not have to be subjected to hypoxaemia for more than 14 days in order for FHR and MAP to be affected, but the method of induction, is also an important factor influencing cardiovascular adaptation and function.

Gestational age

Gestational age at exposure is probably also an important factor. It is known that the cardiovascular responses of the fetal sheep to hypoxaemia before

about 110 d GA (Iwamoto *et al.*, 1989) are different from those of the fetal sheep at more than 110 d GA (Iwamoto *et al.*, 1989) (cardiovascular responses to hypoxaemia are described more fully in section 1.12). This is in support of the idea first put forward by Widdowson & McCance (1975) of there being 'critical periods' during development (discussed in section 1.7.2.i).

Are there any common denominators?

Table 4.2, below, summarises the relationships and complexities discussed above surrounding the cardiovascular responses to chronic hypoxaemia.

Method	Duration (days)	GA (days)	PaO_2 (mmHg)	FHR	MAP	Hct	Body weight
Fetal anaemia [a]	7	124-131	14.8	↑	↓	↓	⇔
Embolization [b]	8-10	120-134	17-19.9	⇔	⇔	⇔	⇔/↓
Reduced FiO_2 [c]	14	124-138	17.1	↓	⇔	↑	↓
Embolization [d]	21	109-129	16.5	⇔	↑	⇔	↓
Maternal anaemia [e]	30	110-138	22	⇔	↓	↑	↓
Reduced maternal plasma volume [f]	30	115-145	17	↑	↓	↑	⇔
High altitude [g]	90	30-120	19.3	⇔	↑	↑	⇔
Carunclectomy [h]	140	0-140	16.4	↑	↓	↑	↓

Table 4.2. Various methods by which chronic hypoxaemia has been produced in the fetal sheep, showing the PaO_2 levels attained and the effect on MAP, FHR, haematocrit and fetal growth.
Data derived from [a]Davis & Hohimer, 1991; [b] Trudinger *et al.*, 1987; Block *et al.*, 1989; Gagnon *et al.*, 1994; Carter *et al.*, 1996; [c] Kamitomo *et al.*, 1994; [d] Murotsuki *et al.*, 1996a; [e] Mostello *et al.*, 1991; [f] Daniel *et al.*, 1989; [g] Kamitomo *et al.*, 1992; [h] Robinson *et al.*, 1983.

It is apparent that there are a number of factors acting together in the determination of fetal cardiovascular development. Perhaps by looking at blood pressure and heart rate responses individually, a pattern will emerge, and the influences which are important in determining the pattern of cardiovascular development will become clearer.

Let us concentrate just on MAP. We find that embolization for 8-10 days and reduced maternal FiO_2 for 14 days does not affect the development of MAP. However, if embolization is extended for 21 days, there is an increase in MAP. Similarly, as a consequence of high-altitude hypoxaemia for 90 days MAP is increased. Conversely, severe fetal anaemia, maternal anaemia and reduced maternal plasma volume all cause fetal MAP to be reduced compared to controls.

Now if we look at the heart rate responses, we can see that embolization, regardless of duration, does not affect the development of FHR. FHR development was also unaffected by maternal anaemia (which did not result in fetal anaemia) and high altitude hypoxaemia. On the other hand, a reduction in maternal plasma volume, carunclectomy and fetal anaemia all resulted in increased FHR compared to controls, whereas reduced maternal FiO_2 resulted in decreased FHR.

However, those fetuses in which both MAP and FHR were unaffected also showed no change in haemoglobin concentrations or body weights compared to control animals (Trudinger *et al.*, 1987; Gagnon *et al.*, 1994; Carter *et al.*, 1996). This suggests an interaction between oxygen carrying capacity (haemoglobin), growth and cardiovascular development. When either oxygen carrying capacity and/or growth are changed, cardiovascular development is affected. In my study of repeated acute hypoxaemia, growth (Table 4.1) was not affected and, although haemoglobin concentration decreased over the 14 days, values were not significantly different between C and H fetuses (Fig. 4.8), so it is perhaps not surprising that neither MAP nor FHR (Fig. 4.2) development were perturbed.

4.4.2 Growth, MAP and FHR

Growth pattern

Studies in the fetal sheep (Bocking *et al.*, 1986; Bocking *et al.*, 1992; Matsuda *et al.*, 1992; Matsuda *et al.*, 1994) and fetal goat (Fujimora *et al.*,

1994), examining the effects of sustained (8-24 hours) hypoxaemia on various endocrine, cardiovascular and behavioural responses, show that, in the absence of acidaemia, all cardiovascular and behavioural variables return to their baseline values by 24 hours of hypoxaemia. Hormone levels, except norepinephrine which remains slightly elevated (Fujimora *et al.*, 1994), also return to pre-hypoxia levels. However, it is known that more persistent hypoxaemia (for a matter of days or longer) produces redistribution of CVO (Block *et al.*, 1984; Bernstein *et al.*, 1988; Kamitomo *et al.*, 1993; Iwamoto, 1993; Jensen & Berger, 1993) which results in asymmetrical growth patterns (Block *et al.*, 1984; Lafeber *et al.*, 1984; Harding *et al.*, 1985; Jacobs *et al.*, 1988a; Bernstein *et al.*, 1988; Kamitomo *et al.*, 1993; Lueder *et al.*, 1995). In my fetuses that were exposed to repeated acute hypoxaemia, growth was not affected (Table 4.1), at least in terms of body weight and organ weights as a percentage of body weight, similar to the findings of Trudinger *et al.* (1987) and Gagnon *et al.* (1994) in their embolization studies.

Kidney and heart

It may be significant, however, that the absolute kidney weights were significantly smaller in H than C fetuses. In fact, looking at both the absolute organ weights and organ weights as a percentage of body weight (Table 4.1), it may be no coincidence that in H there was a tendency for heart weights to be more and kidney weights to be less than in C. This follows the pattern of blood flow distribution that is known to occur in response to hypoxaemia (Block *et al.*, 1984; Bernstein *et al.*, 1988; Kamitomo *et al.*, 1993; Iwamoto, 1993; Jensen & Berger, 1993) i.e. redistribution in favour of the brain, heart and adrenals at the expense of the kidneys, lungs, gastro-intestinal tract and periphery. Does this tendency suggest that there was a redistribution of blood flow in response to repeated acute hypoxaemia? It is interesting that asymmetrical IUGR in the human baby has been found to be associated with a decrease in kidney weight and a reduction in nephron number (Hinchliffe *et al.*, 1992). In fact, babies that were of normal birthweight but died of SIDS have also been found to have a reduced number of nephrons (Hinchliffe *et al.*, 1993). This suggests that renal development may be a more sensitive marker of adverse uterine conditions than altered growth of the other organs and total body weight. It raises the possibility that the reduced kidney weight in PD fetuses was in response to the repeated acute hypoxaemia. Reduced kidney size, if accompanied by a reduction in nephron number, may mean

that there is reduced capacity for the kidney secretion of renin, which would result in perturbation of the renin-angiotensin system and thus blood pressure.

More sensitive markers of altered growth

A more sensitive method of measuring altered growth rates than just gross weight, is to measure changes in cellular growth by assessing DNA synthesis rate in tissues. Gagnon *et al.*, (1995) showed in their chronically hypoxaemic fetuses that, although fetal body and organ weights were not significantly affected by 10 days of hypoxaemia, there was a decrease in DNA synthesis rate in left ventricle and quadriceps muscle. We may speculate that DNA synthesis rate was altered in my H fetuses and that a prolongation of the study would have resulted in alterations in both growth and cardiovascular variables, such as was observed when repeated embolization was continued for 21 days (Murotsuki *et al.*, 1996).

4.4.3 Cardiovascular reflexes

It is possible that had I induced a more severe hypoxaemia in my study, MAP and FHR, as well as growth would have been affected. There are at present no studies that have examined the effect of repeated severe hypoxaemia or asphyxia over a period of days on fetal growth and development, however there are studies on the effects of relatively short repeated acute hypoxaemic or asphyxic insults repeated every few minutes.

4.4.3.i Chemoreflexes

Jensen *et al.* (1985 & 1987) investigated the effects of repeated fetal asphyxia, by occlusion of the maternal abdominal aorta, on cardiovascular variables. They exposed the fetuses to 11 asphyxic episodes within 33 minutes by occluding for 30, 60 or 90 seconds with recovery between each occlusion of 150, 120 or 90 seconds, respectively. In response to each asphyxic episode there was a fall in heart rate which increased when the duration of the asphyxia was increased. The blood pressure response varied with the number of asphxial episodes. They also showed that as the duration of asphyxia increased, and as the mean SaO_2 decreased, there was an increase in the plasma concentrations of adrenaline and noradrenaline (Jensen *et al.*, 1987). This suggests that a longer duration of asphyxia causes increased sympathetic activation, which is mediated in part by the arterial

chemoreceptors i.e. there was increased chemoreceptor activity. Similarly, Mallard *et al.* (1995) found that 4 episodes of asphyxia of 5 minutes duration repeated every 30 minutes caused bradycardia during each occlusion. Blood pressure increased during the first occlusion, but during subsequent occlusions progressively decreased, which they suggested to be indicative of increasing sensitisation of the heart to further insults. This asphyxia is a much more severe insult than that which my fetuses experienced as PaO_2 was reduced to almost 0 mmHg, and was accompanied by acidaemia (pH may decrease to below 7.0). Thus, it is difficult to make comparisons between asphyxic and moderately hypoxic (such as in my study) insults. However, there is the suggestion from the studies of Mallard *et al.* (1995) and Jensen *et al.* (1987) that a greater severity of insult causes alterations in reflex control, at least in the acute situation. Interestingly, in this regard, the human adult shows a pressor response to hypoxia that is positively correlated with the frequency and severity of sleep apnoeas per hour of sleep (Hedner *et al.*, 1992). Giussani *et al.* (1996) investigated the cardiovascular responses to repeated, partial umbilical cord occlusions in the fetal sheep, producing only mild reductions in PaO_2 (reduced from about 21 to about 16 mmHg). They did 12 occlusions for a period of 5 minutes each every 15 minutes. In response to each hypoxic episode there was little change in blood pressure, a rapid and pronounced fall in heart rate, and a rapid but transient decrease in femoral blood flow and increase in femoral vascular resistance. The effect of such an insult as that imposed by Giussani *et al.* (1996) over a period of several days or longer has not been studied, but it is interesting that they also observed a diminution of heart rate and blood flow responses from the first to the last occlusion. This indicated that there was a modification in the magnitude and gain of the chemoreflex i.e. there was a blunting of the response. Thus, it appears that, even in response to a repeated hypoxic insult where the hypoxaemia induced is moderate rather than severe, the chemoreceptors adapt.

However, in my study there was no significant attenuation or potentiation in either the bradycardia, hypertension or vasoconstriction produced in response to hypoxia over the course of the study, i.e. from first to last hypoxic insult (Fig. 4.3). This shows that there was no alteration in the chemoreflex, as it has been shown that the fall in heart rate (Baan *et al.*, 1993) and the increase in femoral vascular resistance, measured from the increase in femoral flow (Dawes *et al.*, 1968), are a useful method of investigating maturation of the

fetal chemoreceptor response. It therefore appears that repeated acute hypoxaemia did not cause resetting of the chemoreceptors. Bearing in mind the findings in the acute situation described above, I wonder why there is no alteration in the chemoreflex of H fetuses. Maybe it is not only the duration and relative severity (Jensen *et al.*, 1987; Hedner *et al.*, 1992) but also the frequency of hypoxaemic episodes that is important in determining adaptation and resetting of the reflex response. It would be interesting to look at individual fetal MAP trajectories in pregnancies where uterine contractures had also been recorded, to see if the developmental increase in MAP correlates with intensity, frequency or duration of contractures.

4.4.3.ii Baroreflex

As there was no difference between H and C fetuses in MAP and FHR, and there was no change in the chemoreflex of H over time, I was not surprised to find that the baroreflex was also not different between the two groups (Figs. 4.4. & 4.5). Both groups showed a similar shift of the baroreflex curve upwards and to the right (Fig. 4.4), and there was no difference between groups in the gain of the baroreflex either at 114 or 128 days gestation. (Fig. 4.5). Although not significant, there was a tendency for the gain of the reflex to decrease in both groups from 114 to 128 days gestation, such as one would expect at this period of development (Blanco *et al.*, 1988).

4.4.4 Blood gases, haemoglobin, haematocrit, glucose, lactate, and ion concentrations

The only significant differences in blood composition between C and H fetuses were in PaO_2 (Fig. 4.9) and in the changing concentration of lactate over time (Fig. 4.9). PaO_2 was higher in H than in C and lactate increased in H but not in C. It is difficult to know why this should have occurred. Is it a response to the repeated acute hypoxaemia? Does it reflect differences in placental function between the 2 groups of sheep?

The higher PaO_2 of H fetuses may have represented a decrease in oxygen consumption, which has been shown to occur in fetal sheep that have decreased growth rates (Fowden & Silver, 1995). Certainly it suggests an alteration in metabolism. Likewise, the somewhat elevated and increasing lactate concentration in H may have been due to altered metabolism. Alternatively, the placental clearance of lactate may have been decreased. Lactate is produced by the fetus as a result of anaerobic glycolysis. As PaO_2

levels were higher in H than in C, this is an unlikely explanation for the higher levels of lactate. The placenta provides enough lactate to account for 25% of fetal oxidative metabolism (Burd *et al.*, 1975) and in situations of inadequate glucose supply the amount of lactate produced may increase. But again, this seems an unlikely explanation for the higher lactate levels in H, as their glucose levels were not low. In response to acute hypoxaemia there is an increase in lactate levels (Comline *et al.*, 1965). It may be that the placenta was unable to clear all the excess lactate from the fetal circulation after each hypoxic insult. This could perhaps have occurred if repeated hypoxia affected the specific lactate carrier system that has been suggested to exist (Fowden, 1994).

The fact that there was no difference between groups in any of the other blood constituents measured - haemoglobin, haematocrit, glucose and ions - shows that the repeated acute hypoxaemia did not cause there to be any change in basal levels in the long-term.

4.5 CONCLUSION

The results of this study show that repeated acute hypoxaemia of a moderate degree over a period of 2 weeks does not affect fetal MAP (despite the suggestion that SBP may have increased slightly) or growth in the late gestation fetal sheep, further emphasising the association of growth with cardiovascular development. Clearly, the fetus is able to withstand an insult such as that presented in this study. Thus, my original hypothesis that repeated acute hypoxaemia would result in perturbations in the development of cardiovascular reflexes and blood pressure is not confirmed.

It is possible that hypoxaemia of greater severity, or perhaps increased frequency, would have produced changes in MAP and FHR, as would perhaps a greater duration of exposure in terms of number of days. However, whilst there seems to have been no effect of repeated acute hypoxaemia on cardiovascular development, it may be that the feto-placental unit successfully adapted to the insult, which may have resulted in alterations in placental structure and function. Placental adaptation is suggested by the early epidemiological findings of Barker *et al.* (1990), where they have found that babies, within the normal range for birth weight but with large placentae, are predisposed to hypertension in later life. The blood pressures of the babies at

birth was not recorded, so it is impossible to say whether those people who went on to develop cardiovascular disease in later life had significantly altered blood pressures at birth.

The question that arises from the results of this study is, what factors may determine the development of MAP in fetal life?

Chapter 5

FETAL CARDIOVASCULAR DEVELOPMENT: RELATION TO GROWTH, BLOOD GASES, GLUCOSE, LACTATE AND THYROXINE

5.1 INTRODUCTION

The findings of Barker *et al.* (1990) and the work described in the previous two chapters of this thesis suggest that the intrauterine environment is important in determining fetal growth and cardiovascular development. Decreased fetal growth is associated with the development of hypertension in adult life (Barker *et al.*, 1990; Campbell *et al.*, 1996; Forrester *et al.*, 1996; 1996; Leon *et al.*, 1996; Taittonen *et al.*, 1996; Zureik *et al.*, 1996; Chapter 3) and has been found to be related to a variety of maternal influences: height, weight (Barker, 1994), haematological status (Beischer *et al.*, 1970; Godfrey *et al.*, 1991 & 1994), smoking (Callan & Witter, 1990), nutrition (Stein & Susser, 1975a & b; Mellor & Matheson, 1979; Mellor & Murray, 1981; DeBarro *et al.*, 1992; Godfrey *et al.*, 1994; Harding & Johnston, 1995; Bauer *et al.*, 1995; Barker, 1996), to mention just a few. It is possible that these maternal influences result in hypoxaemia and hypercapnia, high lactate levels, acidosis, hypoglycaemia and disturbed plasma amino acid profiles, such as have been observed in both human (Economides *et al.*, 1989; Montemagno & Soothill, 1995) and ovine (Robinson *et al.*, 1983) IUGR fetuses. However, the pathophysiology of IUGR is still poorly understood and the factors involved in the development of blood pressure remain to be elucidated.

The results of the previous chapter show that moderate repeated acute isocapnic hypoxaemia does not affect the development of MAP or FHR, or have an impact on growth in the late gestation fetal sheep. Thus, taking those findings into account and bearing in mind the evidence from other studies, the question arises as to what are the determinants of fetal blood pressure development. It was therefore the purpose of the work described in this chapter to examine the data obtained more closely, with particular reference to fetal cardiovascular development and growth.

5.2 EXPERIMENTAL DESIGN

14 fetuses aged 105-109 days gestation were instrumented with carotid artery, jugular vein and amniotic catheters, an ECG electrode and a Transonic flow probe around the femoral artery (see section 2.3.4).

As described in section 4.2, after the 5-day recovery period, fetuses were studied each day for 14 days and were subjected to either a daily episode of

acute isocapnic hypoxaemia (n=7) or recordings were made for 3 hours daily in normoxia (n=7). Blood samples were taken at intervals during each 3 hour experiment for blood gas, glucose and lactate analysis (see Fig. 2.9, section 2.3.8.i).

Prior to experimentation on the first day (114 d GA) and the last day (128 d GA), the baroreflex was assessed in each fetus as described in section 2.3.8.iii. After baroreflex assessment, fetal blood pressure, heart rate and blood flow measurements were allowed to return to basal values before the experiment (3 hours isocapnic hypoxia or 3 hours normoxia) began. Pressures, heart rate, femoral blood flow, blood gases, pH, ion concentrations, haematocrit, haemoglobin, glucose and lactate were measured as described in section 2.3.7.ii.

After 2 weeks, when all experiments had been completed, ewes were killed by an overdose of pentobarbitone and a basic post-mortem was carried out where fetal body and organ weights were recorded, as described in section 2.3.9.i.

5.3 ANIMAL GROUPS

I mentioned in the introduction to this chapter that the findings described here are the result of reconsideration of the data obtained from the animals in the last chapter, which describes the effects of repeated acute hypoxaemia. The fetuses were grouped, irrespective of whether they were C or H, on the basis of the trajectory that their arterial blood pressure (ABP) development followed over the 14-day study period (Fig. 5.1). Those fetuses that showed the increase in ABP expected over this period of gestation (Boddy *et al.*, 1974; Kitanaka *et al.*, 1989; Daniel *et al.*, 1989; Mostello *et al.*, 1991; Kamitomo *et al.*, 1994; Gagnon *et al.*, 1994; Murotsuki *et al.*, 1996) I called the "Pressure Up" (PU) group, and those that showed either no increase or a decrease in ABP I called the "Pressure Down" (PD) group.

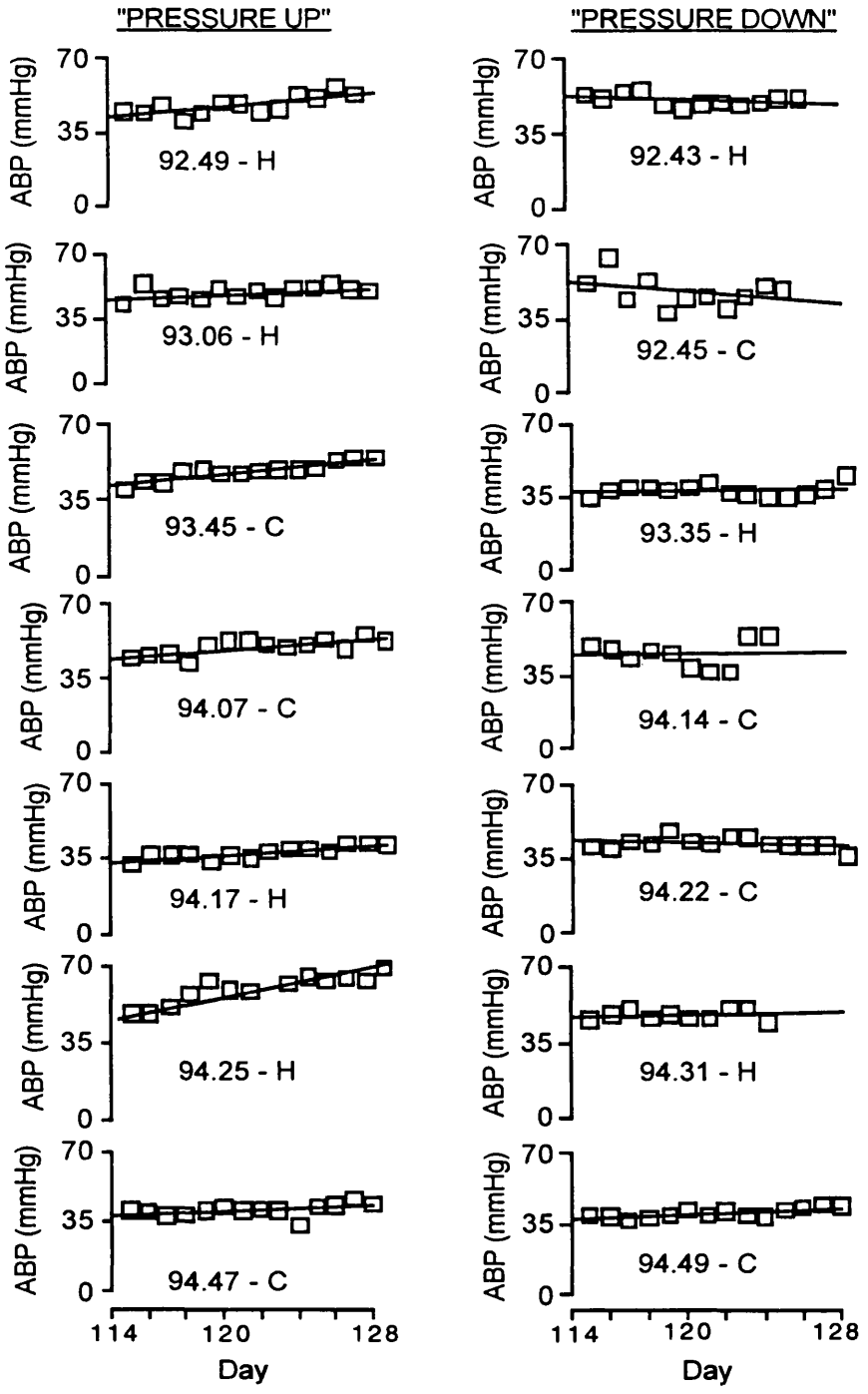

Figure 5.1. Individual ABP points on each day for C and H fetuses, through which a regression line was drawn to determine their ABP trajectories over the 14-day period. This diagram also shows the blood pressure trajectories of the fetuses in the two groups PU and PD.

5.4 RESULTS

5.4.1 MAP and FHR

There was a tendency for MAP to decrease from Day 1 to Day 14 in PD fetuses ($P < 0.06$, 114 d GA vs. 128 d GA) and a significant increase in PU fetuses (Fig. 5.2). The upward trend of MAP in PU was significantly different to the downward trend seen in PD ($P < 0.01$). Similarly, both systolic (SBP) and diastolic (DBP) pressures increased significantly over the 14 day period in PU, but neither changed significantly in PD fetuses (Fig. 5.2). The increasing trajectory in PU was significantly different for both systolic ($P < 0.005$) and diastolic ($P < 0.01$) pressures in PU compared to PD fetuses.

FHR decreased significantly over the course of the study in both groups, and the decrease over time was the same in both groups (Fig. 5.2). The rate-pressure product, an indicator of myocardial oxygen consumption (Kitamura *et al.*, 1972), remained unchanged in PU fetuses throughout the study, but decreased significantly in PD (Fig. 5.2). Thus, the rate-pressure product trajectories were significantly different ($P < 0.001$) between groups.

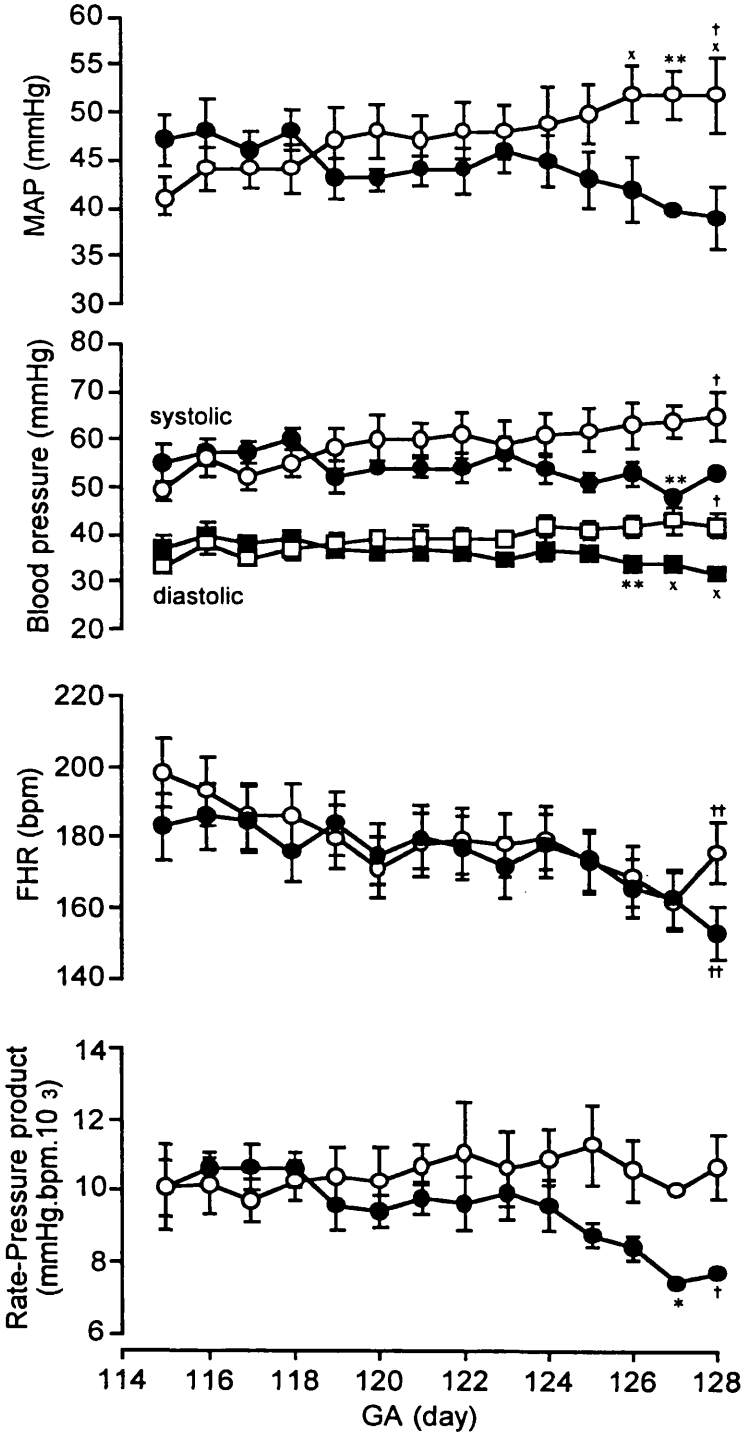

Figure 5.2. The trend in basal MAP, SBP and DBP, FHR and rate-pressure product for PU (o/□) and PD (●/■) fetuses. ^{x} $P < 0.05$, * $P < 0.01$, ** $P < 0.005$, PU vs. PD; † $P < 0.05$, †† $P < 0.01$, 115 d GA vs. 128 d GA. Values are mean ± S.E.M. (See also Appendix 8).

5.4.2 Cardiovascular reflex responses

5.4.2.i Baroreflex

In keeping with the increase in MAP seen in PU, there was a significant ($P < 0.05$) shift of their baroreflex curve upwards and to the right (Fig. 5.3) over the 14-day period, i.e. the baroreceptors of this group had reset. The baroreflex curve of the PD group, on the other hand, showed no shift to the right. It was, however, displaced upwards, consistent with the fall in FHR.

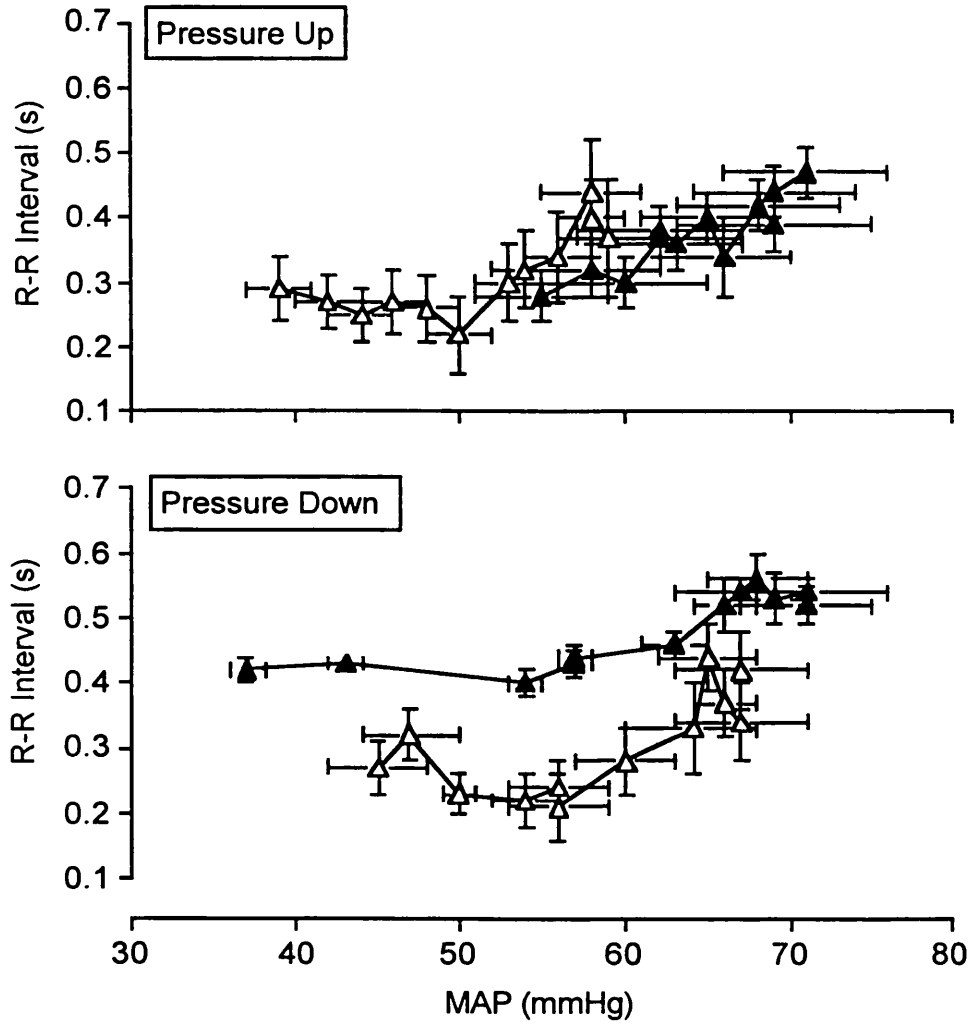

Figure 5.3. Baroreflex curves for PU (n = 5) and PD (n = 4) fetuses at 115 d GA (Δ) and 128 d GA (▲). Values are mean ± S.E.M.

There was a tendency (P = 0.07) for the slope of the baroreflex curve to decrease from 114 d GA to 128 d GA in PU, suggesting a decrease in the gain of the reflex in this group. But, in PD fetuses the slope at 128 d GA was similar to that at 114 d GA, suggesting that there was no change in gain (Fig. 5.4).

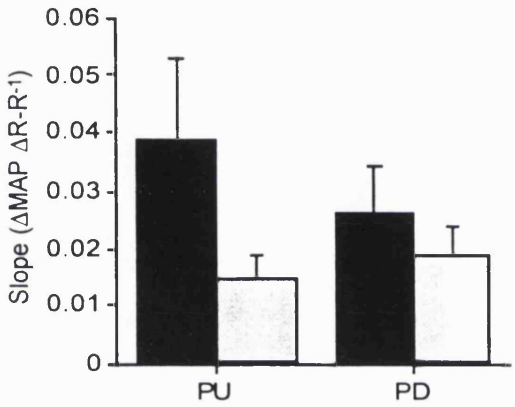

Figure 5.4. Slope of the steep part of the baroreflex curve in PU and PD fetuses at 115 d GA (■) and 128 d GA (▨). Values are mean ± S.E.M.

5.4.2.ii Chemoreflex

The hypertensive response to hypoxaemia was greater in PD fetuses than PU group (Fig. 5.5). This suggests that the magnitude of the chemoreflex was greater in PD than in PU fetuses. Furthermore, the response looked as though it increased over the course of the study in PD fetuses, but the increase was not significant. In contrast, the level of response did not change in PU (Fig. 5.5).

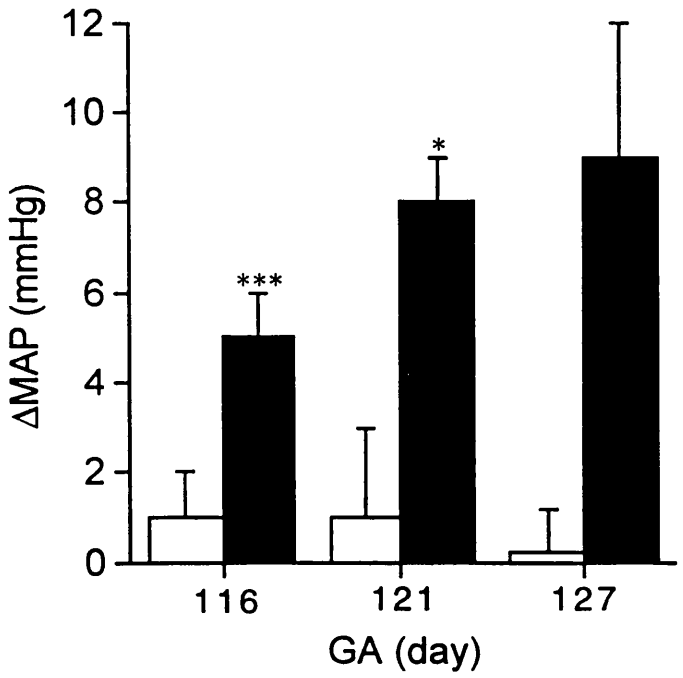

Figure 5.5. The increase in MAP over basal levels (ΔMAP) after 15 min hypoxia in PU (n=5) (□) and PD (n=6) (■) fetuses at increasing ages during the study. * $P < 0.01$, *** $P < 0.001$, PU vs. PD. Values are mean ± S.E.M.

5.4.3 Femoral flow and femoral vascular resistance

Femoral flow increased to significantly higher levels in PU than PD fetuses, there being no significant change in flow in PD. The blood flow trend was significantly different between groups ($P < 0.001$) (Fig. 5.6). Both groups showed a decrease in femoral vascular resistance, although the decrease did not reach significance (PU: $P = 0.25$; PD: $P = 0.10$). The downward trajectory was similar for both PU and PD.

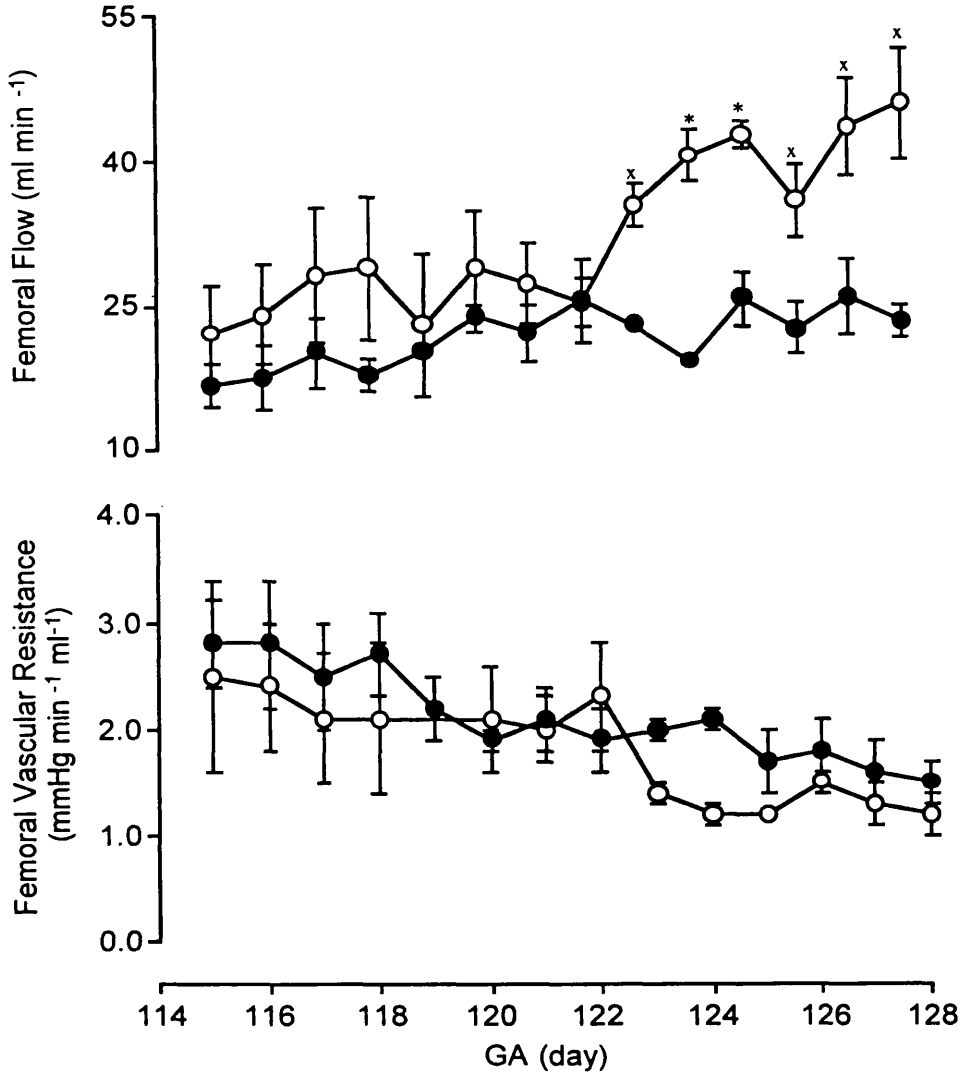

Figure 5.6. Basal femoral flow and femoral vascular resistance in PU (o) (n = 4) and PD (•) (n = 3) fetuses. ^{x} $P < 0.05$, * $P < 0.01$. PU vs. PD. Values are mean ± S.E.M.

5.3.2 Weights

PD fetuses were smaller than PU fetuses, as can be seen by the fact that they tended ($P < 0.07$) to have a shorter CRL and they were significantly lighter (Table 5.1). Heart and kidney weights of PD were significantly less than those of PU fetuses and there was a tendency for their lungs ($P < 0.07$) and

livers ($P < 0.15$) also to be lighter than those of PU fetuses (Table 5.1). When organ weights were expressed as a percentage of body weight there was no significant difference between the two groups for any of the organs (Table 5.1), indicating that PD fetuses were proportionately smaller than PU fetuses.

	PRESSURE UP	PRESSURE DOWN
Hypoxia/Control	4H, 3C	3H, 4C
Sex	4M, 3F	5M, 2F
Twin/Singleton	3T, 4S	3T, 4S
CRL (cm)	48.4 ± 1.4	44.4 ± 1.4
Body weight (g)	4000 ± 218	2942 ± 297 x
Heart weight (g)	34 ± 2	22 ± 2 **
Lung weight (g)	121 ± 8	91 ± 13
Liver weight (g)	125 ± 12	99 ± 11
Kidney weight (g)	14 ± 0.3	12 ± 0.7 **
Heart % BW	0.86 ± 0.03	0.76 ± 0.03
Lung % BW	3.03 ± 0.09	3.02 ± 0.19
Liver % BW	3.11 ± 0.19	3.41 ± 0.28
Kidney % BW	0.36 ± 0.01	0.43 ± 0.05
GA (days) on Day 1	114 ± 1.2	113 ± 1.1
GA (days) on Day 14	129 ± 1.5	127 ± 0.6

Table 5.1. Number of hypoxia (H), control (C), male (M), female (F), twin (T) and singleton (S) fetuses and body weight, CRL, organ weights, organ weights as a percentage of body weight, and gestational age (GA) at the start (115 d GA) and end (128 d GA) of the study period in PU and PD fetuses. x $P < 0.05$, ** $P < 0.005$, PU vs. PD. Values are mean ± S.E.M.

It can be seen from Table 5.1 that there was no bias in either group with regard to exposure to repeated acute hypoxia, sex, plurality, or gestational age.

5.4.4 Blood gases, haematocrit and haemoglobin

Some notable differences in blood composition were observed between the two groups of fetuses. There was a significant downward trend in total haemoglobin ($P < 0.001$), PaO_2 ($P < 0.05$), SaO_2 ($P < 0.001$), CaO_2 ($P < 0.001$) and haematocrit ($P < 0.001$) in PD fetuses compared to PU fetuses over the period of the study. From about 122 d GA onwards levels of all four variables were significantly lower in PD than in PU animals (Fig. 5.7). SaO_2 tended to decrease and at 122 d GA and 124 d GA percentage saturation was significantly lower in PD fetuses. However, by 128 d GA values were the same as those in PU fetuses (Fig. 5.7). $PaCO_2$, pH and BE_b were the same for both groups and did not change over the course of the study (Fig. 5.7).

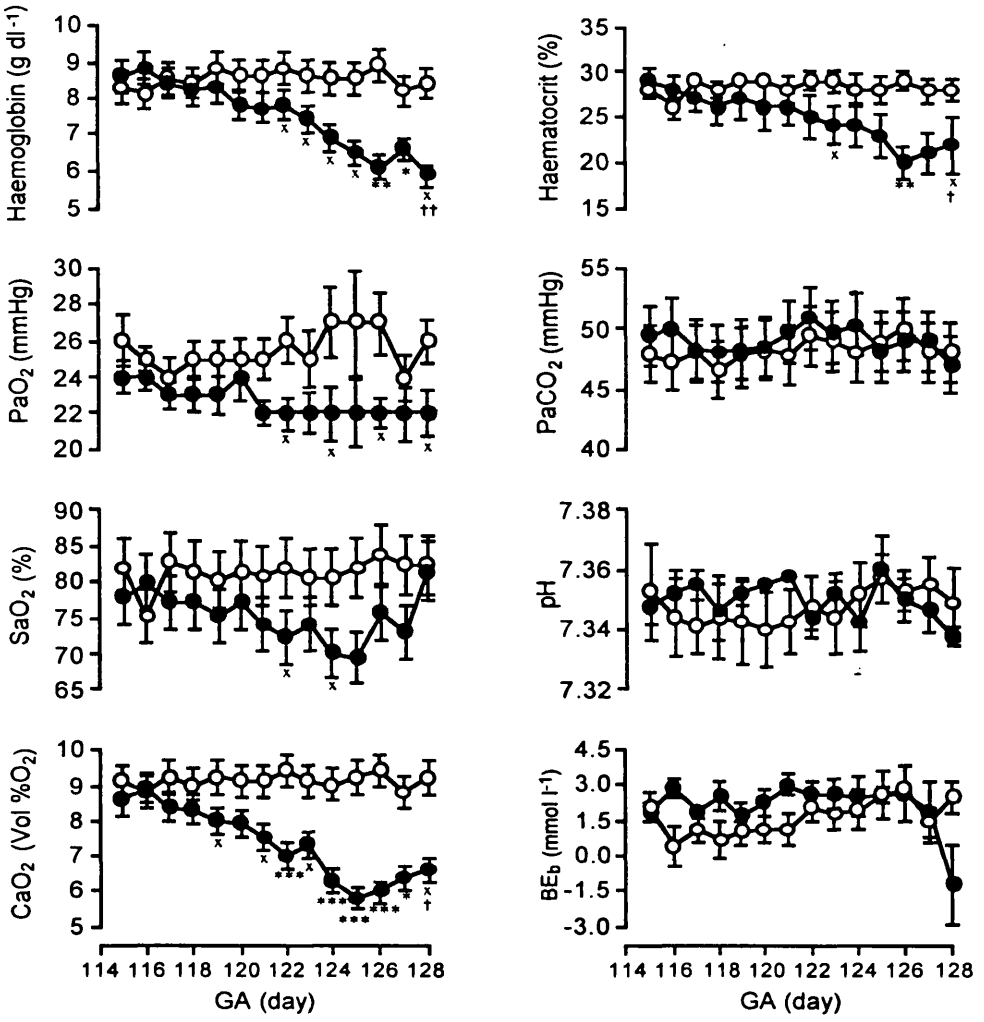

Figure 5.7. Basal levels of total haemoglobin, PaO_2, SaO_2, CaO_2, haematocrit, $PaCO_2$, pH and BE_b in PU (o) and PD (•) fetuses. ^{x} $P < 0.05$, * $P < 0.01$, ** $P < 0.005$, *** $P < 0.001$, PU vs. PD. † $P < 0.05$, †† $P < 0.01$, 115 d GA vs. 128 d GA. All values are mean ± S.E.M.

5.4.5 Ions

The concentrations of Na^+, K^+ and Ca^{++} ions did not change significantly over the period of the study and there were no significant differences between the two groups (Fig. 5.8). Likewise, the change in concentration of the three ions in response to hypoxaemia was the same for both groups (Fig. 5.9).

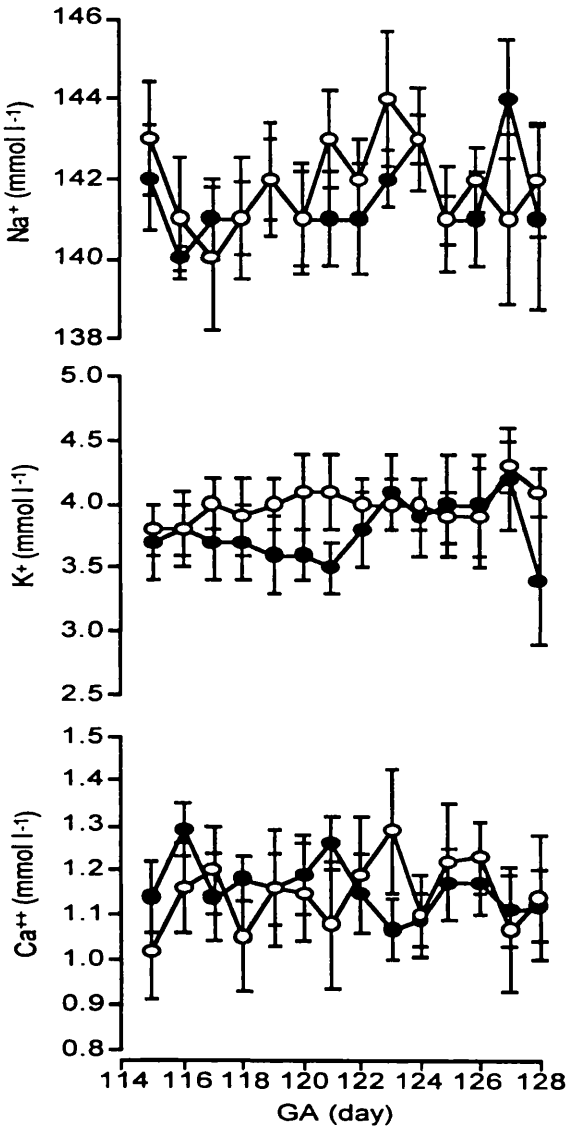

Figure 5.8. The concentrations of Na^+, K^+ and Ca^{++} in the blood of PU (o) and PD (●) fetuses over the 14-day study period. Values are mean ± S.E.M.

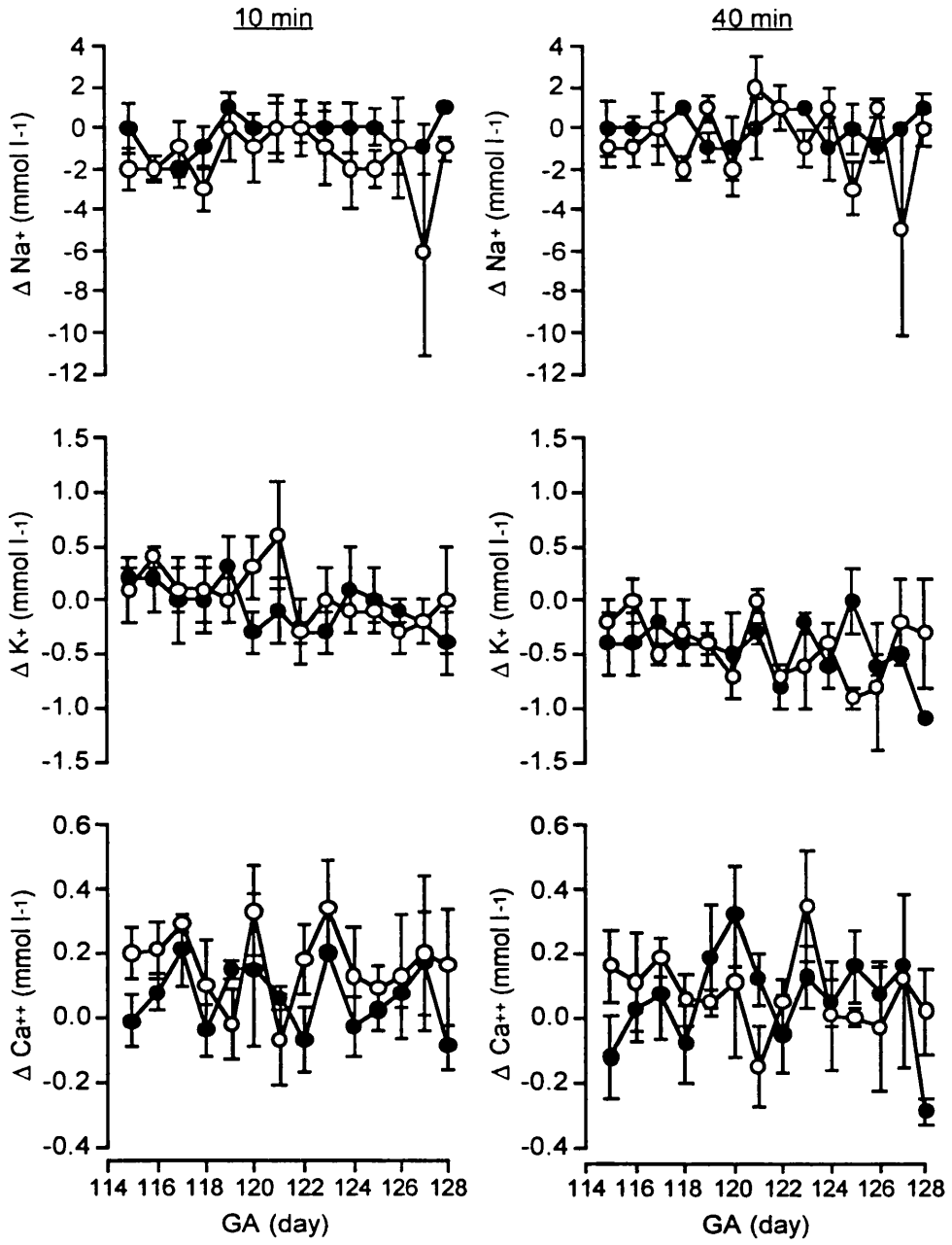

Figure 5.9. The change in blood concentrations of Na^+, K^+ and Ca^{++} 10 min (left-hand side) and 40 min (right-hand side) after the onset of hypoxaemia in PU (o) and PD (●) fetuses, over the 14-day study period. Values are mean ± S.E.M.

5.4.6 Glucose and lactate

Blood glucose concentration tended to slope downwards with increasing gestational age in PD fetuses, which was significantly different ($P < 0.01$) to the trend seen in PU (Fig. 5.10). There was, however, no significant difference in blood glucose concentration at 128 d GA compared to 114 d GA within either group. However, when expressed as a single mean value for the whole 14 days, the concentration was significantly lower in PD than PU fetuses (Fig. 5.10 bar graph).

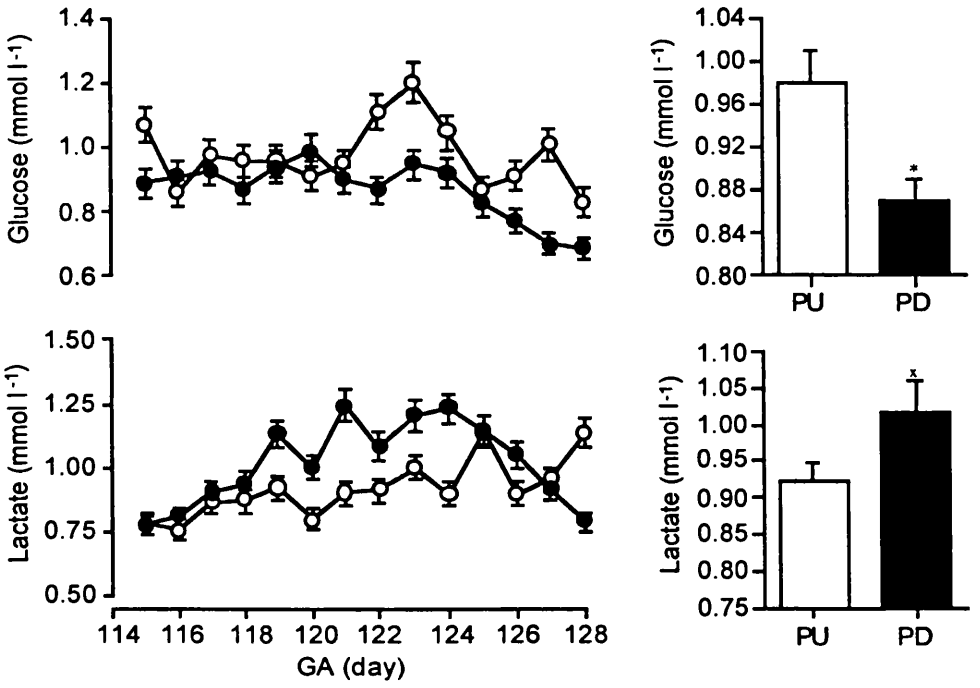

Figure 5.10. Blood glucose and lactate concentrations in PU (o/□) and PD (●/■) fetuses. The graphs on the left show the trend over the 2-weeks of the study, and the bar graphs on the right show concentrations over the 2 weeks expressed as a single mean value. ^{x} $P <$ 0.05, PU vs. PD; * $P < 0.01$, PU vs. PD. Values are mean ± S.E.M.

The trend in lactate levels was not significantly different between groups and there was no significant change in concentration between 115 d and 128 d GA in either group (Fig. 5.10). But, looking at the concentration of lactate

expressed as a single mean value for the whole duration of the study, it was significantly higher in PD fetuses than in PU (Fig. 5.10 bar graph).

5.4.7 Thyroxine (T_4)

T_4 levels in PD fetuses declined in a manner reminiscent of their haemoglobin and CaO_2 levels (Fig. 5.7). T_4 levels at 128 d GA were not significantly different from those at 115 d GA for either group, and neither were levels between groups significantly different, though by 128 d GA differences between PU and PD were approaching significance ($P < 0.08$). Likewise, the slightly upward trajectory seen in PU was not significantly different to the slightly downward trajectory in PD, though there was definitely a tendency for them to differ ($P = 0.08$) (Fig. 5.11).

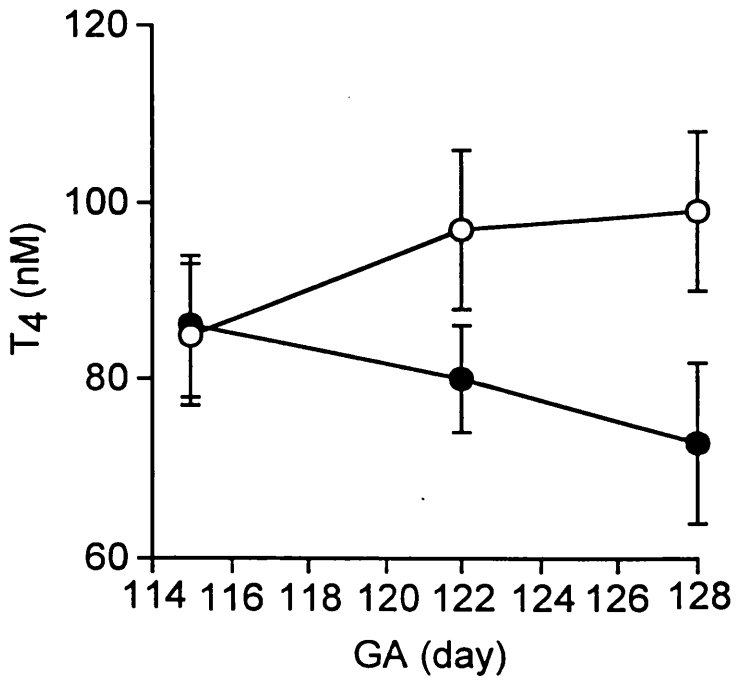

Fig. 5.11. T_4 concentrations in PU (o) and PD (●) fetuses, as measured at 115 d, 122 d, and 128 d GA.

5.4.8 Relationship of several variables to fetal body weight

Having found that PD fetuses were smaller than PU (Table 5.1) and in view of the suggested association of blood pressure with growth, it seemed that some valuable information may be gained by looking at the relationship of several different variables with body weight. Fetal body weight at post-mortem was therefore correlated to variables measured on that day (Fig. 5.12).

I found that there was no good correlation of fetal body weight with most of the variables looked at except for slope of the baroreflex curve and femoral blood flow. Body weight showed a significant ($r=0.666$, $P < 0.05$) negative correlation with baroreflex gain (slope), and a significant ($r=0.906$, $P < 0.05$) positive correlation with femoral blood flow (Fig. 5.12).

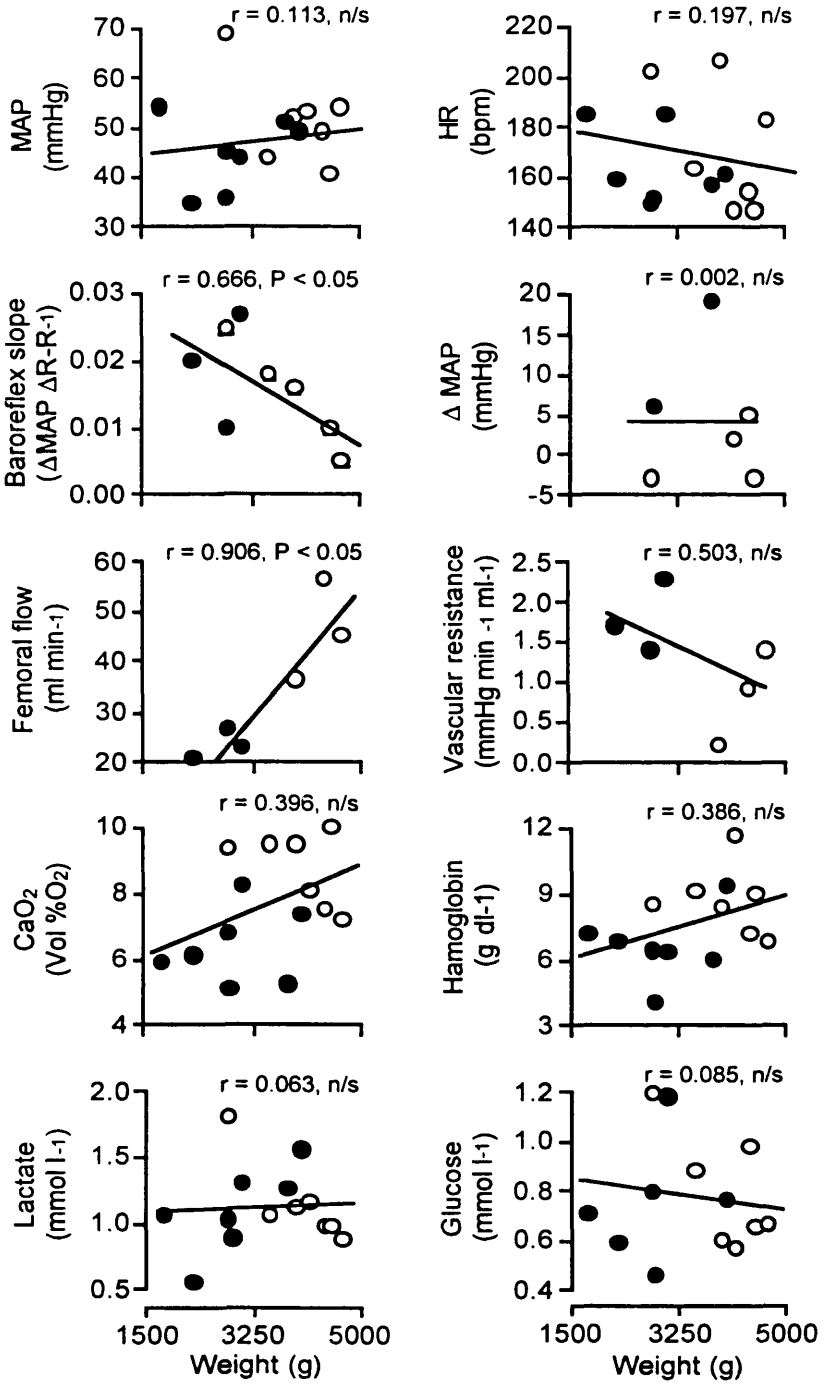

Figure 5.12. Correlation of weight in PU (o) and PD (●) fetuses with MAP, FHR, slope of the baroreflex curve, pressor response to hypoxia, femoral flow, femoral vascular resistance, CaO_2, haemoglobin, glucose and lactate. n/s denotes not significant. Values are mean (S.E.M. omitted for clarity).

5.5 DISCUSSION

The principal finding of this study was that late-gestation fetal sheep fall into two groups, those that show an increase in blood pressure with advancing gestational age and those that do not. Furthermore, there were differences between the 2 groups that extended beyond that seen in blood pressure development.

5.5.1 Cardiovascular development of PU and PD fetuses

5.5.1.i Blood pressure and FHR

PU fetuses

This group of fetuses were identified on the basis of a rise in MAP. It is no surprise that such fetuses showed a significant increase in MAP over the two weeks (Fig. 5.2). The increase of MAP and decrease of FHR observed in PU is expected during this stage of gestation (Boddy *et al.*, 1974; Kitanaka *et al.*, 1989; Daniel *et al.*, 1989; Mostello *et al.*, 1991; Kamitomo *et al.*, 1994; Gagnon *et al.*, 1994; Murotsuki *et al.*, 1996a). SBP and DBP increased in parallel in PU with advancing gestational age (Fig. 5.2), so both contributed to the increase in MAP. SBP, the maximum pressure reached during the ejection phase of the cardiac cycle, is determined primarily by cardiac performance (which is dependent on preload, afterload, heart rate and contractility) and aortic compliance, and to a lesser degree by total peripheral resistance. Thus, changes in SBP give us some information as to changes in these variables. DBP is determined by total peripheral resistance and heart rate, and is thus able to give us informtion regarding them.

Blood pressure is determined by cardiac output and peripheral vascular resistance. In PU, the decrease in femoral vascular resistance (Fig. 5.6) suggests that their increase in MAP must have been due to increased CVO. Although in PU the rate-pressure product, a correlate of myocardial oxygen consumption (Kitamura *et al.*, 1972; Jorgensen *et al.*, 1973; Nelson *et al.*, 1974), did not increase significantly (Fig. 5.2), it did rise a little, which suggests an increase in CVO. This is supported by the observation in newborn lambs that myocardial oxygen consumption increases with increased CO (Fisher, 1989). The fact that heart rate decreased and systolic pressure increased (Fig. 5.2) suggests that the proposed increase in CVO was due to increasing contractility of the heart. The increasing pressure and decreasing

resistance together accounted for the rising femoral blood flow (Fig. 5.6), as flow is determined by pressure and resistance. However, growth of the fetus (Dawes *et al.*, 1968; Bendeck & Langille, 1992) and increasing tissue metabolism, as seen by the increase in oxygen delivery (Bendeck & Langille, 1992), also account for the rise in flow.

PD fetuses

It is interesting that MAP, SBP and DBP did not increase in PD fetuses (Fig. 5.2). From this observation alone it would seem that the cardiovascular development of PD was not normal, even though FHR decreased as expected (Boddy *et al.*, 1974; Kitanaka *et al.*, 1989; Daniel *et al.*, 1989; Mostello *et al.*, 1991; Kamitomo *et al.*, 1994; Gagnon *et al.*, 1994; Murotsuki *et al.*, 1996a). The decreasing FHR with increasing gestational age would have meant that the diastolic interval was also increasing, so there would have been increasing time during which intracellular Ca^{++} could be sequestered. On this basis one would expect that contractility increased. Rate-pressure product decreased in PD, suggesting that myocardial oxygen consumption decreased. Therefore, the proposed increase in contractility must have been negated, in energy terms, by the decrease in heart rate. Thus, assuming that contractility did increase, the fact that SBP did not increase in PD but decreased, suggests that preload must have decreased. The possibilities that arise from this are, either a decrease in atrial pressure, and/or an increase in ventricular compliance. A decrease in atrial pressure suggests a decrease in venous return to the heart i.e. a decrease in central venous pressure. It may be, therefore, that the failure for MAP to increase in PD fetuses was due to a decrease in central venous pressure and/or structural alterations in the ventricle walls resulting in increased compliance.

The pattern of MAP and FHR development seen in PD is not dissimilar to that observed during a 30 day study in fetal sheep where the ewes were made anaemic (Mostello *et al.*, 1991) It was found that those fetuses whose mothers were anaemic had significantly lower MAP than controls and FHR was not different. The lower MAP, SBP and DBP of PD is also similar to my findings in the neonatal rat, where anaemic pups had lower SBP than controls (Chapter 3). This raises the possibility that haematological status was somehow important, particularly as haemoglobin and haematocrit decreased in PD (Fig. 5.7). But, Mostello *et al.* (1991) found that although the ewes in their study were anaemic, the fetuses were not, in fact they observed a

tendency for fetal haematocrit to be higher than in controls. However, they did observe a decrease in fetal PaO_2 and CaO_2, an observation that I also made in PD fetuses.

In PD fetuses, the decreasing haemoglobin concentration and lower PaO_2 together caused CaO_2 to decrease (Fig. 5.7). Could the significantly lower and decreasing oxygenation of PD fetuses have caused their lower blood pressure? There are three mechanisms whereby this is possible. 1) The decreased CaO_2 in PD may have resulted in a decrease in fetal oxygen consumption and hence growth, such as has been observed before in the fetal sheep (Gu *et al.*, 1985; Mostello *et al.*, 1991; Owens *at al.*, 1987a & b). Smaller fetal size may explain the lower blood pressure through unknown mechanisms associating growth and blood pressure. This seems a possibility because, in fetuses made anaemic by exchange transfusion, growth was preserved, due to maintained oxygen extraction, thus they were not smaller than controls. Not only were they of similar size to control fetuses, but MAP was not significantly decreased either (Papparella *et al.*, 1994). This suggests that if growth is maintained then blood pressure development remains normal. (The association of growth with blood pressure is discussed more fully later in this chapter, section 5.5.3.iii). 2) Alternatively, the lower CaO_2 (Busse *et al.*, 1993; Hudlicka, 1991), and lower haemoglobin (Martin *et al.*, 1986; Jia *et al.*, 1996), of PD fetuses may have caused an increase in the production of paracrine vasodilators such as NO. 3) Possibly the lower oxygen tension in PD caused angiogenesis, which would result in decreased resistance and thus decreased blood pressure (the relation of angiogenesis and blood pressure was discussed more fully in the previous chapter, section 3.4.1.i).

Looking at the available evidence, the failure for MAP to increase in PD appears to be due to both decreasing vascular resistance (Fig. 5.6) and decreasing CVO, as evidenced by the decreasing systolic pressure (contractility), decreasing heart rate, and decreasing rate-pressure product (Fig. 5.2). The failure for flow to increase (Fig. 5.6) would have been due to the decrease in both pressure and vascular resistance.

Further analysis of cardiovascular function and development e.g. assessment of the cardiovascular reflexes may give a more detailed insight into the possible causes and mechanisms of the altered MAP development in PD fetuses.

5.5.1.ii Baroreflex

Ordinarily, as MAP increases throughout the last third of gestation there is resetting of the baroreceptors, observed as a decrease in discharge frequency for a given pressure, as gestational age increases. This is seen as a shift to the right of the baroreflex curve and a decrease in its slope (Blanco *et al.*, 1988). My PD fetuses failed to show either a shift of the baroreflex curve to the right (Fig. 5.3) or a decrease in slope (Fig. 5.4). This is perhaps not surprising since there was no rise in their MAP during the study. However, it does suggest that baroreflex development of PD fetuses was not normal.

Baroreceptor resetting occurs as a result of stretch and activation of the baroreceptors caused indirectly by an acute or chronic changes in arterial blood pressure. There are several mechanisms whereby resetting may occur, including changes in the mechanical properties of the vessel wall (e.g. decreased compliance), mechanisms involving changes in the extracellular concentration of ions and activity of Na^+K^+-ATPase, changes in the release of paracrine (e.g. PGI_2, endothelin) and endocrine (e.g. angiotensin II) factors, and perturbation of higher centres involved in the reflex pathway.

Mechanical factors

The baroreceptors have a three-dimensional structure so they respond to deformation of the vessel wall in any direction (Kircheim, 1976). Thus, either an increase or a decrease in diameter of the vessel wall will cause them to be stimulated.

The perinatal period is characterised by an increase in peripheral blood flow (Dawes *et al.*, 1968; Bendeck & Langille, 1992), as observed in PU (Fig. 5.7). As mentioned above, this increase is due, in part, to growth of the fetus (Dawes *et al.*, 1968; Bendeck & Langille, 1992) and increasing tissue metabolism (Bendeck & Langille, 1992). An increase in blood flow results in an increase in vessel diameter (Berry *et al.*, 1976). I demonstrated a positive correlation between fetal body weight and femoral blood flow at 128 d GA (Fig. 5.12). It can also be seen from this correlation curve that PD fetuses were the smaller fetuses with lower femoral blood flow rates. Thus, the lower femoral blood flow of PD fetuses was presumably partly due to their decreased growth rate, so the diameters of their blood vessels would have been smaller than PU. Diameter of a vessel will determine tension in the vessel wall, as described by Laplace's law:

$$T = \frac{Pr}{2h}$$

where T is the tension in the vessel wall, P is the internal pressure, r is the radius of the vessel, and h is the thickness of the vessel wall. So an increase in vessel diameter will result in greater tension in the vessel wall which means that stretch will be increased, and vice versa. Increasing pressure causes the diameter of the carotid sinus to increase (Kircheim, 1976), which will also result in increased flow. Bendeck & Langille (1992) demonstrated a dramatic increase in blood flow to the cerebral hemispheres and cerebellum between 120 d and 140 d GA. This must mean that there was increased blood flow through the carotid arteries, as the majority of blood flow to the brain is supplied via those vessels. So, carotid artery diameter must also increase causing stretching of the vessel wall and baroreceptor stimulation, which may result in resetting. This may have been a mechanism for the resetting of the baroreceptors in PU fetuses. Similarly, the reduced growth of PD is a possible cause for the failure of baroreceptor resetting to occur in PD between 115 d and 128 d GA, as less stretch would have been imposed on the baroreceptors. The relationship between growth and resetting of the baroreflex is demonstrated in my fetuses by the significant inverse correlation of slope of the baroreflex at 128 d GA with fetal body weight (Fig. 5.12). This fits well with the fact that gain of the reflex decreases with increasing GA (Blanco *et al.*, 1988; Wakatsuki *et al.*, 1992), because there is obviously a strong positive correlation of fetal weight with increasing age. PD fetuses tended to lie to the left of the graph, i.e. at lower weight and higher gain (Fig. 5.12), which is consistent with failure of baroreceptor resetting to occur. There is therefore a strong suggestion that vascular growth is an important mechanism involved in chronic resetting of the fetal baroreflex.

Baroreceptor resetting may also occur as a result of changes in the actual structural properties of the vessel wall (Landgren, 1952; Andresen, 1984) e.g. changes in compliance, as well as the changes in shape just described. Chronic increases in both flow and pressure cause vascular remodelling (Langille, 1993; Struijker-Boudier *et al.*, 1995). Increased flow is associated with increased vessel diameter and increased pressure with increased wall thickness, both occurring as a result of changes in the media of the vessel i.e. changes in smooth muscle cells (Langille, 1993). Resetting of the baroreflex that occurs normally during late gestation may, in part, be the result of

changes in vascular structure that also take place at that time. Ordinarily, in the fetal sheep there is a mean increase in elastin and collagen content of the abdominal and thoracic aorta between 120 and 140 d GA (Bendeck & Langille, 1991). These altered proportions of smooth muscle, elastin and collagen will result in changes in vascular compliance, and thus baroreceptor resetting. Failure of blood flow and/or pressure to increase, such as was the case in PD, will mean that the flow and pressure related changes in vascular structure do not occur in the normal way, and baroreceptor resetting will therefore be affected.

Histological analysis would have helped determine whether changes in the size and structure of the vessels contributed to the differences in baroreflex function between PU and PD fetuses.

Endocrine factors

Angiotensin II is known to affect resetting of the arterial baroreflex (Ismay *et al.*, 1979; Lumbers *et al.*, 1979; Campagnole-Santos *et al.*, 1992; Segar *et al.*, 1994). It has also been shown in the fetal sheep that in response to hypotension there is an increase in plasma renin activity (Chen & Wood, 1992), which will result in an increase in circulating concentrations of angiotensin II. Angiotensin II does not act directly on the baroreceptors, but is thought to exert its effects within the central nervous system resulting in inhibition of vagal discharge to the heart (Ismay *et al.*, 1979; Lumbers *et al.*, 1979; Campagnole-Santos *et al.*, 1992). It has been suggested, however, that interruption of the baroreflex by angiotensin II is less in the fetus than the adult (Ismay *et al.* 1979), and it has been shown more recently that there is no significant contribution of angiotensin II to resetting of the baroreflex in the fetus (Segar *et al.*, 1994). This was shown by recording fetal renal sympathetic nerve activity and heart rate during i.v. administration of the angiotensin-converting enzyme (ACE) inhibitor enalaprilat to the fetus. It was found that there was no significant change in either, and there was also no change in the baroreflex curve midpoint or sensitivity. Segar *et al.* (1994) proposed that this may be due to differences between the fetus and the more mature animal in expression of the angiotensin II receptor subtypes, AT_1 and AT_2. It therefore seems unlikely that any differences that there may have been between PU and PD fetuses in circulating angiotensin II levels would have contributed to the differences seen in their baroreflexes.

Ionic factors

Selective inhibition of the Na^+-K^+ pump using ouabain, and extracellular solutions containing no K^+, cause an increase in threshold and decrease in sensitivity of the baroreceptors (Saum *et al.*, 1976). This shows clearly that alterations in cellular Na^+ and K^+ gradients affect the functioning of the baroreceptors, which may alter their resetting. Ca^{++} has also been implicated as having a possible role in the adaptation of baroreceptors (Hamill & McBride, 1994). Hair cell adaptation is dependent on Ca^{++} influx, which, it is thought, may cause adaptation by disconnecting actin from myosin in a model proposed by Hamill & McBride (1994). I measured blood concentrations of Na^+, K^+ and Ca^{++} in my fetuses, but there was no difference between groups and no significant change with increasing age (Fig. 5.8.). Therefore, ion balance was probably not a significant factor in the differences seen the baroreflexes of PU and PD fetuses.

Paracrine factors

Endothelial factors are also implicated in resetting of the baroreceptors. PGI_2 has been shown to cause increased activity of the baroreceptors, seen as a shift of the baroreflex curve to the left, i.e. it suppresses acute baroreceptor resetting (Chen *et al.*, 1990, Chapleau *et al.*, 1991). This is thought to be due to increased sensitisation of the baroreceptor nerve endings. It has also been observed that the carotid sinus of chronically hypertensive rabbits has a decreased capacity to increase PGI_2 formation acutely in response to arachidonic acid, although basal intra-sinus levels of PGI_2 are higher than in normotensive controls (Xie *et al.*, 1990). It was speculated that this may be due to morphological and functional alterations in the endothelial cells, which are the primary site of PGI_2 production in response to acute stimuli, as opposed to smooth muscle cells, whose production of PGI_2 in response to acute stimuli is limited. It was further speculated that perhaps the elevated basal levels of PGI_2 in hypertensive rabbits were due to vascular hypertrophy and thus increased wall (smooth muscle cell) mass. So, hypertension in the adult animal is associated with elevated basal levels but a reduced capacity for acute production of PGI_2, which is thought to sensitise the baroreceptors to alterations in pressure, thus suppressing acute baroreceptor resetting. It was not reported whether gain of the reflex was altered by PGI_2. These findings lead to the possibility that basal intra-sinus endothelial production of PGI_2 increased in PU fetuses as gestation advanced, in a manner similar to that

suggested above in hypertensive rabbits, leading to resetting of the baroreflex between 115 d GA and 128 d GA (Fig. 5.3). In PD fetuses, because their MAP was lower than PU and did not increase with time, presumably intra-sinus basal PGI_2 production was lower than in PU. This idea fits with the increase in blood flow seen in PU and the decrease in PD (Fig. 5.6), as factors known to modulate the release of endothelial substances include mechanical stimuli, such as stretch and shear stress. However, oxygen also has a modulatory effect on PGI_2 synthesis, and, in the systemic circulation, lower oxygen tensions cause an increase in PGI_2 synthesis (Shaul *et al.*, 1992). However, PD had lower oxygen levels than PU (Fig. 5.7). Thus, if basal intra-sinus levels of PGI_2 were indeed higher in PU fetuses compared to PD, it may be that increased flow stimulates the release of PGI_2 to a greater extent than low levels of oxygen. Unfortunately the oxygen tensions that Shaul *et al.* (1992) exposed their arterial segments to were unphysiological and not representative of fetal oxygenation levels *in vivo*. They exposed the vessels to 3 different PO_2 levels, 680 mmHg, 150 mmHg, and 40 mmHg, and only at 40 mmHg were mesenteric arterial segments stimulated to produce PGI_2. Fetal PaO_2 in the umbilical artery is about 20 mmHg (Rurak, 1994), therefore it is difficult to determine from the results of Shaul *et al.* (1992) whether the PaO_2 differences between PU and PD fetuses (PU: about 26 mmHg; PD: about 22 mmHg) would have had a significant effect on PGI_2 synthesis.

Endothelin (ET), also produced locally by the endothelium, is suggested to suppress baroreceptor activity in adult dogs, particularly at high carotid sinus pressures (above 100 mmHg), suppression depending on ET concentration and the duration of exposure (Chapleau *et al.*, 1992). Perhaps ET production increased in PU fetuses with their increase in blood pressure (Fig. 5.2), but did not in PD, in which blood pressure did not increase (Fig. 5.2). Fetal MAP at the age studied here is about 45 mmHg, so this seems an unlikely mechanism for resetting of the baroreflex in the fetus. Moreover, chronic hypoxia is associated with an increase in ET_A and ET_B receptor mRNA levels in the adult rat (Li *et al.*, 1994) and, although systemic ET-1 levels were not elevated, this suggests increased ET-1 activity. On the strength of this finding, one would expect ET levels to be higher not lower in PD fetuses compared to PU, as PD fetuses were comparatively more hypoxaemic (Fig. 5.7).

Higher centres

Interference of neural pathways in the brainstem involved in the baroreflex would result in changes in the baroreflex. ANP is thought to be a transmitter in the NTS, involved in the baroreflex, where together with glutamate it causes a decrease in both heart rate and blood pressure (for a more detailed description see section 1.10.1.i, Fig. 1.15). Thus, alterations in circulating levels of ANP may alter the baroreflex. Hypoxia has been shown to stimulate the release of ANP (Lew & Baertschi, 1988), therefore it is conceivable that PD fetuses may have had elevated levels of ANP.

Clearly baroreflex development of PD fetuses was not normal. It is possible that this could result in long-term effects beyond birth, such as the development of hypertension.

5.5.1.iii Chemoreflex

The fetal chemoreflex can be assessed by examining the blood pressure response to hypoxaemia (Dawes *et al.*, 1968; Boddy *et al.*, 1974; Marshall, 1987; Itskovitz *et al.*, 1991).

The typical response to hypoxaemia in the fetus includes rapid bradycardia and vasoconstriction, and hypertension of slightly later onset. Hypertension results, in part, from chemoreceptor stimulation which increases sympathetic efferent activity and causes peripheral vasoconstriction (described more fully in section 1.12.1). Thus, the change in MAP in response to a hypoxic challenge, such as is illustrated in figure 5.5, will give some indication as to chemoreflex function. However, by 15 minutes, the rise in blood pressure is also caused by catecholamine induced vasoconstriction as a result of hypoxia induced stimulation of the adrenal medulla (see section 1.12.3). It is therefore a possibility that the higher blood pressure in PD after 15 minutes of hypoxaemia was due to greater release of catecholamines from their adrenals than from those of PU fetuses. Nonetheless, the greater increase in blood pressure of PD fetuses seen after 15 minutes of hypoxaemia (Fig. 5.5) raises the possibility that the magnitude of the chemoreflex response was greater in PD than PU fetuses. Also, the increase in this pressor response to hypoxaemia with advancing gestational age in PD fetuses (Fig. 5.5) suggests that there was augmentation of the chemoreflex in PD with time. Bearing in mind that PD fetuses were hypoxaemic compared to PU (Fig. 5.7), this finding is comparable with that of Bennet & Hanson (1994) where fetuses

made chronically hypoxic by uterine artery occlusion displayed enhanced reflex bradycardia and more pronounced vasoconstriction of the femoral circulation than controls. Similarly, Boekkooi *et al.* (1992) concluded that the fetal chemoreflex response to hypoxaemia (as opposed to hypercapnia) is accentuated when there is a decreased resting SaO_2. In the llama fetus at 0.6-0.7 gestation there is an intense vasoconstriction in response to acute hypoxaemia, suggesting that the cardiovascular responses to hypoxaemia may be increased in this species, which is adapted to high altitude hypoxia (Giussani *et al.*, 1996b). It is interesting with regard to my studies that basal CaO_2 was significantly lower in PD fetuses. Recently Prabhakar & Kou (1994) showed that sympathectomy by chemical means (α_2-adrenergic antagonist) and by denervation results in potentiation of chemoreceptor activity in response to 30 minutes of hypoxia. This raises the possibility that there was reduced sympathetic stimulation of chemoreceptor tissue in the carotid bodies of PD fetuses. This could perhaps occur if there were a reduced number of α_2-adrenoceptors.

It appears that as well as altered baroreflex functioning, PD fetuses also had perturbed chemoreflex development, the consequences of which could be serious. It has been suggested that those infants at high risk for SIDS may have abnormalities in the postnatal adaptation of their chemoreflexes (Blanco, 1994).

5.5.2 Two different groups of fetuses?

Clearly MAP development in PD fetuses followed a different trajectory to that of PU fetuses. Clearly also there were other aspects of cardiovascular development that differed between the two groups. Femoral flow developed differently in PD fetuses than PU; the baroreflex of PU fetuses reset to higher pressures and there was a decrease in gain, whereas the baroreflex curve of PD fetuses did not shift to the right and there was no appreciable change in the gain; PD fetuses showed a greater hypertensive response to hypoxia than PU, suggesting augmentation of the chemoreflex in PD. These differences between the two groups are summarised in Table 5.2 below.

PU	PD
↑ MAP	↓ MAP
↓ FHR	↓ FHR
↑ FF	= FF
= VR	= VR
Baro: resetting	Baro: no resetting
Chemo: maintained	Chemo: augmented

Table 5.2. Summary of the differences in cardiovascular development between PU and PD fetuses. ↑ denotes an increase, ↓ denotes a decrease, and = denotes no significant change, over the 2 weeks of the study.

Thus, PU and PD fetuses were certainly different from each other in terms of their cardiovascular development. PU fetuses showed the normal cardiovascular development that is expected at this time of gestation, but PD fetuses did not. Mounting evidence supporting the idea that altered fetal development results in long-term changes in physiology (Barker, 1994) means that PD fetuses may represent a group that are at high risk for disease in later life. This leads to 2 important questions:

1) Why did MAP fail to increase in PD fetuses?

2) What are the consequences for the mature animal of this abnormal fetal cardiovascular development?

The results of this study are able to address the first question, and possible causes and mechanisms will be discussed below. However, the second question is one that goes beyond the scope of the work presented in this chapter.

5.5.3 Growth

PD fetuses were smaller than PU fetuses (Table 5.1) which indicates that there were differences between PU and PD fetuses in terms of growth rate, as well as those in cardiovascular development described above.

PD fetuses were proportionately smaller than PU fetuses, which may have been the result of limited nutrient supply early in intrauterine life. It has been suggested that nutritional deprivation early in life tends to result in symmetrical growth retardation, as opposed to asymmetrical growth retardation, which it is proposed is the result of nutrient deprivation later on in gestation (discussed fully in section 1.7.1). The history of the ewes used in this project is not known, so we may only speculate that PD fetuses were exposed to a period of reduced substrate supply early on in gestation. The blood composition of the fetuses over the period of the study may, however, give us further clues as to how and why growth was impaired in PD fetuses.

5.5.3.i Oxygen supply

Haemoglobin and haematocrit levels decreased progressively over the 2-weeks of the study to levels significantly lower than those seen in PU fetuses (Fig. 5.7). The reason for this is not obvious, however, it may be that for some reason PD fetuses responded poorly to the repeated blood sampling, or they may have had a reduced rate of red cell production. Although the same amount of blood (7 ml/day) was removed from both groups of fetuses, because PD fetuses were smaller then PU, the proportion of blood removed from PD may actually have been greater. It is generally accepted that blood volume of both the human and the ovine fetus in late gestation is 105-115 ml/kg (Brace, 1993). This being so, PU had a blood volume of about 440 ml and PD about 319 ml, so the percentage of blood volume removed from each group on a daily basis was 1.6% and 2.2% respectively. This represents a 38% greater proportion of blood removal from PD daily than PU. It is possible, therefore, that the progressive anaemia in PD was a direct result of blood sampling. This seems unlikely, however, as 2.2% is a relatively tiny volume of blood to remove.

With regard to the reduced growth of PD fetuses, it is interesting that their lower blood pressure and lower haemoglobin levels are similar to my previous observations in anaemic rats, presented in Chapter 3, and also to the findings of Davis & Hohimer (1989) in anaemic fetal sheep. At 20 days of age my anaemic rat pups were smaller than controls, and had lower systolic blood pressures. Likewise, the anaemic late gestation sheep fetuses studied by Davis and Hohimer (1989) also tended to be lighter than controls (and had a lower MAP). This raises the possibility that reduced oxygen supply as a direct result of anaemia was the cause of the reduced growth of PD fetuses.

However, PD showed a progressive decrease in PaO_2, SaO_2 and CaO_2, as well as haemoglobin concentration and haematocrit (Fig. 5.7). SaO_2 tended to decrease up to 125 d GA then returned to control levels by 128 d GA (Fig. 5.7). The lower SaO_2 in PD fetuses would have been the result of the lower PaO_2. CaO_2, the amount of oxygen bound to haemoglobin in the blood, was lower in PD, which was the result of the lower PaO_2, SaO_2 and haemoglobin observed in these fetuses. It therefore appears that oxygen availability was reduced in PD fetuses, and that this was the result of both lower haemoglobin levels and lower PaO_2. The lighter body weight of PD fetuses (Table 5.1) may be a reflection of their adaptation to this oxygen deficiency by slowing growth rate in order to decrease oxygen requirement. Such a decrease in growth has been observed previously in fetal sheep exposed to decreased oxygen supply during late gestation (Creasy *et al.*, 1972; Jacobs *et al.*, 1988; Block *et al.*, 1990; Murotsuki *et al.*, 1996a).

Interestingly, Fowden & Silver (1995) found that thyroidectomised fetuses had lower haemoglobin levels than intact controls, which they suggested may reflect an influence of thyroid hormones on haemoglobin synthesis. The decreasing T_4 levels in PD fetuses (Fig. 5.11) were remarkably similar to the pattern of decline in haemoglobin concentration (Fig. 5.7), so perhaps the two were related. Fowden & Silver (1995) also found that decreased levels of thyroid hormones resulted in a decrease in fetal oxygen consumption and decreased fetal growth. Thus, the decreasing level of T_4 in PD may have accounted not only for their decreasing haemoglobin and oxygen levels (Fig. 5.7), but may also have been growth limiting by reducing oxygen consumption.

Alternatively, PaO_2, SaO_2 and CaO_2 in PD may have been lower due to placental insufficiency. Reduced oxygen levels are seen in fetuses that are small as a result of carunclectomy (Harding *et al.*, 1985; Robinson *et al.*, 1983; Owens *et al.*, 1986; Owens *et al.*, 1987a & b), placental embolization (Creasy *et al.*, 1972; Block *et al.*, 1990; Murotsuki *et al.*, 1996a), and reduced utero-placental blood flow caused by uterine artery ligation (Detmer *et al.*, 1991) and uterine artery occlusion (Lang *et al.*, 1993). If placental function was impaired in PD, however, it is odd that oxygen levels were similar to those of PU at the beginning of the study. Also, placental insufficiency does not explain the decrease in haemoglobin levels as, ordinarily, when oxygen levels are low there is a compensatory increase in

haemoglobin. Maybe PD fetuses experienced substrate deprivation early in gestation which altered placental and fetal growth in PD and sensitised the fetuses to stress later on, so they responded poorly to the blood sampling. This is feasible as it has been shown in fetal sheep that a periconceptual nutritional insult resulted in slowed fetal growth during late gestation, and that when those fetuses were undernourished for 10 days again in late gestation, fetal growth pattern was more profoundly affected than it was in fetuses that did not experience an early gestation insult (Harding, 1995; Harding & Johnston, 1995). It has also been shown that a periconceptual nutritional insult results in reduced fetal weight and increased placental weight (DeBarro *et al.*, 1992), suggestive of alterations in placental function. It is a possibility, therefore, that placental function was indeed impaired in PD, resulting in slower growth rate and increased susceptibility to adverse stimuli in late gestation, though it is impossible to tell from the results of this study.

5.5.3.ii Glucose and lactate

Glucose and lactate concentrations did not change significantly between 114 d and 128 d GA in either PU or PD fetuses (Fig. 5.10). This is consistent with observations in human fetuses where neither glucose nor lactate levels change significantly with increasing gestational age (Montemagno & Soothill, 1995). However, there was a downward trend of glucose concentration in PD fetuses that was significantly different to the trend seen in PU, and, when expressed as a mean for the whole 14 days, glucose concentration was significantly lower in PD (Fig. 5.10). This lower glucose concentration in PD was accompanied by a higher lactate concentration (Fig. 5.10). These differences in glucose and lactate levels are not unlike those seen in small fetuses where caruncleclectomy was performed prior to pregnancy (Robinson *et al.*, 1983; Harding *et al.*, 1985; Owens *et al.*, 1987b). Furthermore it is evident that reduced glucose supply reduces fetal growth, from the almost 50% reduction in fetal growth rate observed within 3 days of the onset of maternal undernutrition (Mellor & Murray, 1981). On the basis of these findings, it is likely that reduced glucose supply was partially responsible for the reduced growth of PD fetuses. In the fetal sheep amino acids are the most important nutrient for the provision of carbon and energy for tissue accretion (Fowden, 1995). It is interesting, therefore, that in guinea pigs it was found that concentrations of the glucose analogue ^{3}H-methylglucose were relatively normal in IUGR fetuses, whereas concentrations of the amino acid analogue

^{14}C-aminoisobutyric acid are lower (Jansson & Persson, 1990). It may be that a reduction in amino acid supply to PD fetuses was also a contributory factor to their reduced growth. I did not, however, measure levels of any amino acids, so this is purely speculation.

IUGR in human babies is associated with low glucose levels, low plasma concentrations of essential amino acids, and high lactate levels (Montemagno & Soothill, 1995). Raised lactate levels have also been seen in growth retarded fetal sheep exposed to hypobaric hypoxia during late gestation, although glucose levels remained unaffected (Jacobs *et al.*, 1988b). It is difficult to determine exactly why lactate concentration was higher in PD fetuses than PU. The possibilities are that there was increased placental production of lactate to be used by the fetus as an alternative substrate to glucose, increased fetal production of lactic acid from pyruvic acid as a result of anaerobic glycolysis, or perhaps gluconeogenesis was impaired resulting in decreased utilisation of lactate. Nonetheless, increased lactate levels are associated with fetal stress and impaired growth.

5.5.3.iii Growth and blood pressure

Is there an association between the pattern of blood pressure development seen in PD and their reduced growth?

Blood pressure increases throughout life but it does not increase linearly, there being certain times during an individual's normal development when pressure increases at an accelerated rate. During fetal life MAP increases in late gestation (see section 1.7.2.i) at a time when there is also rapid growth (see section 1.5). Then, after birth, blood pressure rises dramatically due to removal of the low resistance placental circulation, and later on there is another increased rate of rise in blood pressure during adolescence. The pubertal increase in blood pressure occurs concomitantly with the adolescent growth spurt. It has been suggested that physical growth during puberty contributes more to the change in systolic blood pressure than does the stage of sexual maturation (Vartiainen *et al.*, 1986), and more recently there has been shown to be a significant positive correlation between the velocity of weight increase and the increase in blood pressure (Berkey *et al.*, 1991; Akahoshi *et al.*, 1996). Thus, there is a definite association between blood pressure development and growth, but whether the two are causally related and possible mechanisms that may link them are not known.

It is interesting that the analysis of Berkey *et al.* (1991) gave some support to their hypothesis that "adolescents whose pressures are more susceptible to elevation during stress may be those who subsequently develop hypertension in adulthood". They described the adolescent growth spurt as a type of physical stress and using a mathematical model predicted that those boys whose systolic peak was greatest at 14 years of age (the time of the growth spurt) would have higher systolic blood pressures when they were 38 years old . If we now also remember the findings of Harding & Johnston (1995), where fetal sheep exposed to nutritional deprivation in early gestation were more profoundly affected by stress in late gestation, the notion that growth and blood pressure are linked seems viable.

A possible candidate for the link between blood pressure development and growth is cortisol, as it has effects on both. Increased cortisol concentrations cause a rise in blood pressure in immature (103-120 d GA) fetal sheep (Tangalakis *et al.*, 1992), which is probably due to peripheral vasoconstriction caused by the permissive effects of cortisol on the catecholamines (see section 1.10.2.iv), and/or due to an increased vascular responsiveness to AII (Tangalakis *et al.*, 1992). Cortisol is also known to reduce growth postnatally by mechanisms that are as yet not fully understood, but involve antagonism of the actions of insulin, and down-regulation of tissue IGF production (Fowden, 1995). Cortisol also has catabolic effects in most target tissues, providing amino acids for the production of glucose by the liver.

There are various reasons why fetal cortisol levels may become elevated:

1) Hypoxaemia. Hypoxaemia causes the adrenal cortex to increase cortisol output (Jackson *et al.*, 1989) as a consequence of elevated ACTH levels, in response to both acute (Jackson *et al.*, 1989) and chronic (Murotsuki *et al.*, 1996b) hypoxaemia. It has also been suggested that the carotid chemo- and baroreceptors may be involved in the release of cortisol in response to acute hypoxaemia, and that the carotid sinus nerve modulates sensitivity of the adrenal cortex to ACTH during acute hypoxaemia (Giussani *et al.*, 1994b).

2) Alterations in the development of the HPA axis. Perturbations in the HPA axis may affect the cortisol feedback inhibition of ACTH synthesis by causing changes within the corticotroph, e.g. altered expression of POMC, increased

CBG synthesis, altered expression of pituitary glucocorticoid receptors (Matthews *et al.*, 1995).

3) Altered levels of placental steroid conversion. Placental 11-β-HSD catalyses the conversion of cortisone (rat) and cortisol to inert 11-keto products, so protecting the fetus from excess maternal glucocorticoid exposure. Thus, if for some reason placental levels of this enzyme are decreased, there will be an increased level of circulating cortisol in the fetus. It has been suggested that a dysfunctional placental glucocorticoid barrier may be involved in the association between low birth weight and hypertension (Edwards *et al.*, 1993). (The evidence relating 11-β-HSD to fetal growth retardation and blood pressure development is discussed in section 7.4.2.ii).

4) Altered progesterone concentrations. Progesterone, synthesised by the placenta during fetal life (Page, 1993; Wooding & Flint, 1994), is a substrate for cortisol synthesis. Thus, it is conceivable that if progesterone levels are elevated cortisol levels may be elevated too. Alterations in placental structure could perhaps result in altered production of progesterone.

PD fetuses were hypoxaemic compared to PU (Fig. 5.7), so it is possible that their cortisol levels were elevated. I have also suggested that placental growth of PD fetuses may have been altered, thus levels of 11-β-HSD and progesterone may not have been normal either. Unfortunately, I did not measure cortisol concentrations in these animals and neither did I make any placental measurements.

5.5 CONCLUSION

The results of this study lead to two conclusions.

1) Blood pressure can follow a different trajectory in different fetuses, and the pattern of blood pressure development is associated with changes in the development of the cardiovascular reflexes.

2) There is the suggestion that the pattern of fetal blood pressure development is related to growth. But, the reasons for deviations from the norm in growth and blood pressure development are not clear. A strong possibility is that nutrition affects the balance between fetal and placental growth and interaction.

5.6 SUMMARY

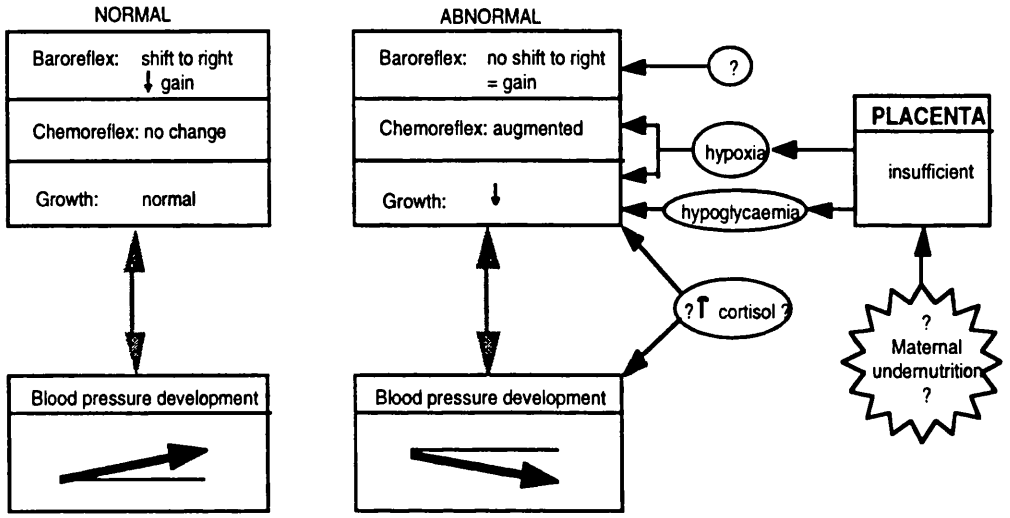

Figure 5.13. Diagram summarising the findings and possible mechanisms involved in fetal cardiovascular development.

Chapter 6

FETAL CARDIOVASCULAR DEVELOPMENT, GROWTH AND BEHAVIOUR: THE EFFECT OF PERICONCEPTUAL UNDERNUTRITION

6.1 INTRODUCTION

The work described in the last chapter led to the conclusion that maternal nutrition may have caused placental insufficiency and thus impaired fetal growth, which was associated with abnormal cardiovascular development. This is supported by epidemiological evidence (Barker, 1994 & 1996) that cardiovascular disease has intrauterine origins associated with poor maternal nutrition. However, the nutrition-dependent effects on fetal growth and development are not simple. Timing, duration, severity, and effects on placental growth, structure and function are important considerations.

The exact time during fetal development at which an insult occurs is important in terms of outcome. McCance & Widdowson (1974) showed that subsequent rates and patterns of growth, and the eventual size achieved at maturity is determined by nutritional plane at particular times during development. This is because there are 'critical' periods during an organism's development (Widdowson McCance, 1975; Lucas, 1990; Harding & Johnston, 1995), when particular organs and axes are undergoing growth, differentiation and specialisation, and perturbation during such a period will result in an altered end-point. Furthermore, such alterations may be irreversible and may have long-term consequences. This is demonstrated by the different phenotypic outcomes that result, dependent on when in gestation stress was encountered. Primary (symmetrical) growth restriction is thought to be the result of factors acting early in gestation and secondary (asymmetrical) growth restriction is thought to result from events occurring in late gestation (Barker, 1994; Owens *et al.*, 1995).

McCance & Widdowson (1974) and Osofsky (1975) both describe studies in animals illustrating that increased severity and exposure to undernutrition result in more severe growth retardation, asymmetrical in form. Studies in humans are, however, less clear-cut. The Dutch Hunger Winter of 1944/1945 (famine conditions taken as 1500 calories/day or less) resulted in significantly reduced birthweight of offspring exposed during late gestation (Stein & Susser, 1975a) as did the more severe Leningrad famine (1941-1944), where a ration of only 300 calories a day was permitted (Antonov, 1942). There was, however, no clear indication that birtweights were more severely affected in Leningrad than in Holland. This may be because there is a minimum maternal body condition, relating to food reserves, below which a

mother is either unable to conceive at all or to sustain a pregnancy. Placental growth is also affected by maternal nutritional status, as evidenced by both increases (DeBarro, 1992; Godfrey *et al.*, 1995) and decreases (Stein & Susser, 1975a, Dwyer *et al.*, 1992) in placental weight. Both are thought to represent placental insufficiency.

Fetal and placental growth are related, and impaired fetal growth is associated with poor developmental outcome (Osofsky, 1975). It was thus my hypothesis that maternal undernutrition would result in alterations in placental structure and function, such that the transfer of oxygen, glucose and amino acids to the fetus are impaired, resulting in reduced fetal growth and altered fetal blood pressure development. The results of the previous chapter suggested that placental insufficiency, perhaps as a result of maternal undernutrition, results in fetal growth impairment and that the pattern of blood pressure development is related to growth. The main period of placental growth and development is during early pregnancy (Winick *et al.*, 1967; Owens *et al.*, 1995), and a previous study (DeBarro *et al.*, 1992) showed that periconceptual undernutrition of the ewe resulted in placental hypertrophy and fetal growth retardation. The aim of this study was, therefore, to investigate the effect of nutritional plane in early pregnancy on fetal growth and cardiovascular development, and on placental size and gross morphology.

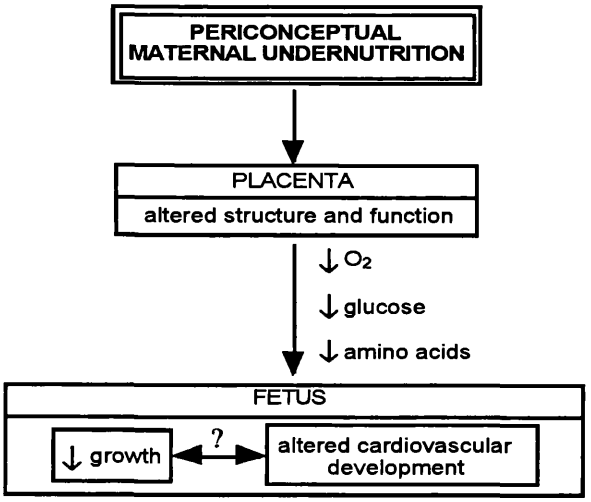

Figure 6.1. Hypothesis suggesting that periconceptual maternal undernutrition results in perturbed fetal cardiovascular development.

6.2 EXPERIMENTAL DESIGN

6.2.1 Ewes

Ewes were all from the same flock of Mule crosses - Border Leicester x Cheviot. Only those animals of similar frame size were enrolled in the study. They were divided into two groups, Heavy (HE) and Light (LI), on the basis of liveweight and body score at mating. Low weight and score were taken to be an indication of poor nutrition, the ewes having been routinely screened for disease and certified by a veterinary surgeon as being healthy. Body weight and condition score in sheep are reliable and widely used measures of nutritional status, and are used routinely in good flock management in agricultural practice (Russel, 1991). The ideal situation for this study would have been one where ewes of the same weight and body score were selected, then significant weight loss induced in those ewes designated as LI prior to mating, such that at mating they had significantly lower BWs and BCSs than HE ewes. In order to be able to control nutritional intake closely to achieve such an end needs ewes to be housed individually and fed a known quantity each day. Unfortunately, at the time this experiment was carried out, access to such facilities was not available. The ewes were weighed at mating then at 30, 60 and 90 days of gestation, and blood samples were taken at 0, 30 and 60 days gestation. Only singleton-bearing ewes were included in the study.

6.2.2 Fetal instrumentation

6 HE and 6 LI ewes were randomly selected and their fetuses instrumented at 103-106 days gestation with arterial, venous and amniotic catheters and an ECG electrode. Crown-rump length (CRL), abdominal circumference (AC) and femur length were also recorded during surgery.

6.2.3 Experimental protocol

After a 5-day recovery period, the fetuses were monitored until about 129 days gestation. On the first and the last study days fetal cardiovascular reflexes were measured. The chemoreflex was studied by exposing the fetus to an acute isocapnic hypoxic challenge for 1 hour (as described in section 2.3.8.i), and the baroreflex was assessed by elevation of blood pressure after a bolus i.v. injection of phenylephrine (as described in section 2.3.8.iii). Fetal and maternal blood gases, blood glucose and lactate levels were measured daily.

6.2.4 Post mortem

At about 129 days gestation an extensive post-mortem was carried out (as described in section 2.3.9.ii). Fetal and maternal body weight; fetal CRL, AC and femur length; and fetal organ weights were recorded. Individual placentome weight, depth, diameter and morphology (see section 2.3.9.ii and Fig. 2.11) were also recorded.

6.2.5 Problems

Unfortunately, out of the 12 fetuses included in this project, only 6 retained fully functioning flow probes. Therefore, I have only been able to present data relating to blood flow and vascular resistance for those 6 animals (Fig. 6.7). Of the 6 flow probes that did not remain patent, 2 failed to work from the very beginning after implantation, in 1 animal the vessel slipped out of the probe, in 2 animals the ewes bit through the leads, and in 1 other animal the probe ceased functioning properly for an unknown reason.

6.3 RESULTS

The statistics relating to the symbols in each of the graphs below are described in the figure legend. For those trends that were analysed by ANOVA, the statistical significance is stated in the text.

6.3.1 Maternal weight

Body weight at mating was significantly different between the 2 groups of ewes (Fig. 6.2), HE ewes being some 10 kg heavier than LI ewes. Over the course of gestation, HE ewes showed a slight significant increase in weight, whilst LI ewes steadily increased in weight such that at 129 d GA they were much more heavy than they were at mating (Fig. 6.2). The increase in body weight was greater in LI than HE ewes, so by the end of the study there was no significant difference in body weight between the two groups of ewes (Fig. 6.2). The weight trajectories of the 2 groups of ewes follow a similar

pattern to those reported by DeBarro *et al.* (1992) in their experiment where the nutritional intake of ewes was manipulated such that they either received food *ad lib* or intake was reduced.

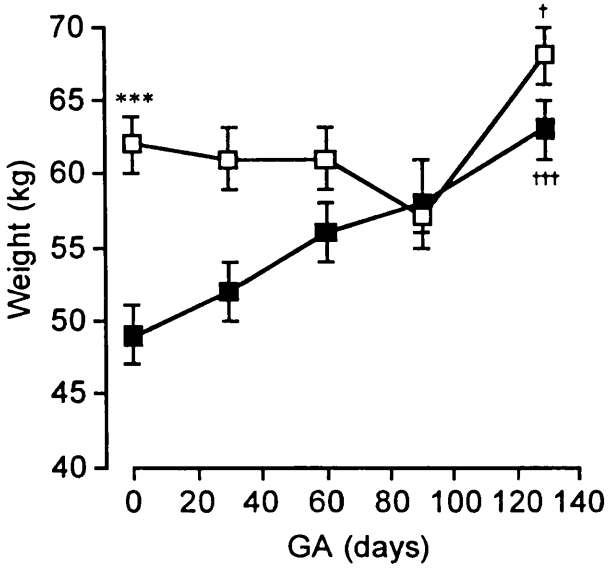

Figure 6.2. Mean body weights of HE (□) and LI (■) ewes. *** $P < 0.001$, HE vs. LI; † $P < 0.05$, ††† $P < 0.001$, 112 d GA vs. 129 d GA. Values are mean ± S.E.M.

6.3.2 Cardiovascular variables

6.3.2.i MAP. SBP and DBP, FHR, and rate-pressure product

MAP in LI fetuses followed a significantly ($P < 0.05$) different trajectory to that of HE fetuses (Fig. 6.3). MAP tended to be higher in LI fetuses, but there was no significant difference in absolute values at any age between the 2 groups. It is notable, though, that HE fetuses showed the increase in MAP ($P = 0.12$, 112 d GA vs. 129 d GA) that is expected during this period of gestation, whereas LI fetuses showed no increase in MAP (Fig. 6.3). Thus, the development of MAP was different between HE and LI fetuses.

The pattern of development of SBP was similar to that of MAP. There was an upward trajectory of SBP in HE but not in LI. The differences in these

trajectories were significantly different ($P < 0.05$) between the two groups (Fig. 6.3). Also, SBP in HE fetuses tended to rise, but there was no rise in LI fetuses. DBP did not change significantly over the course of the study in either group, and there was no significant difference in SBP between groups (Fig. 6.3).

The pattern of FHR development, i.e. its trajectory, was not significantly different between the 2 groups of fetuses (Fig. 6.3). It decreased significantly in both groups between 112 d GA and 129 d GA (Fig. 6.3).

The rate-pressure product tended ($P = 0.08$) to decrease in HE animals, but there was no significant change in LI fetuses between 112 d and 129 d GA (Fig. 6.3). There was no significant difference between the 2 groups in terms of pattern of development.

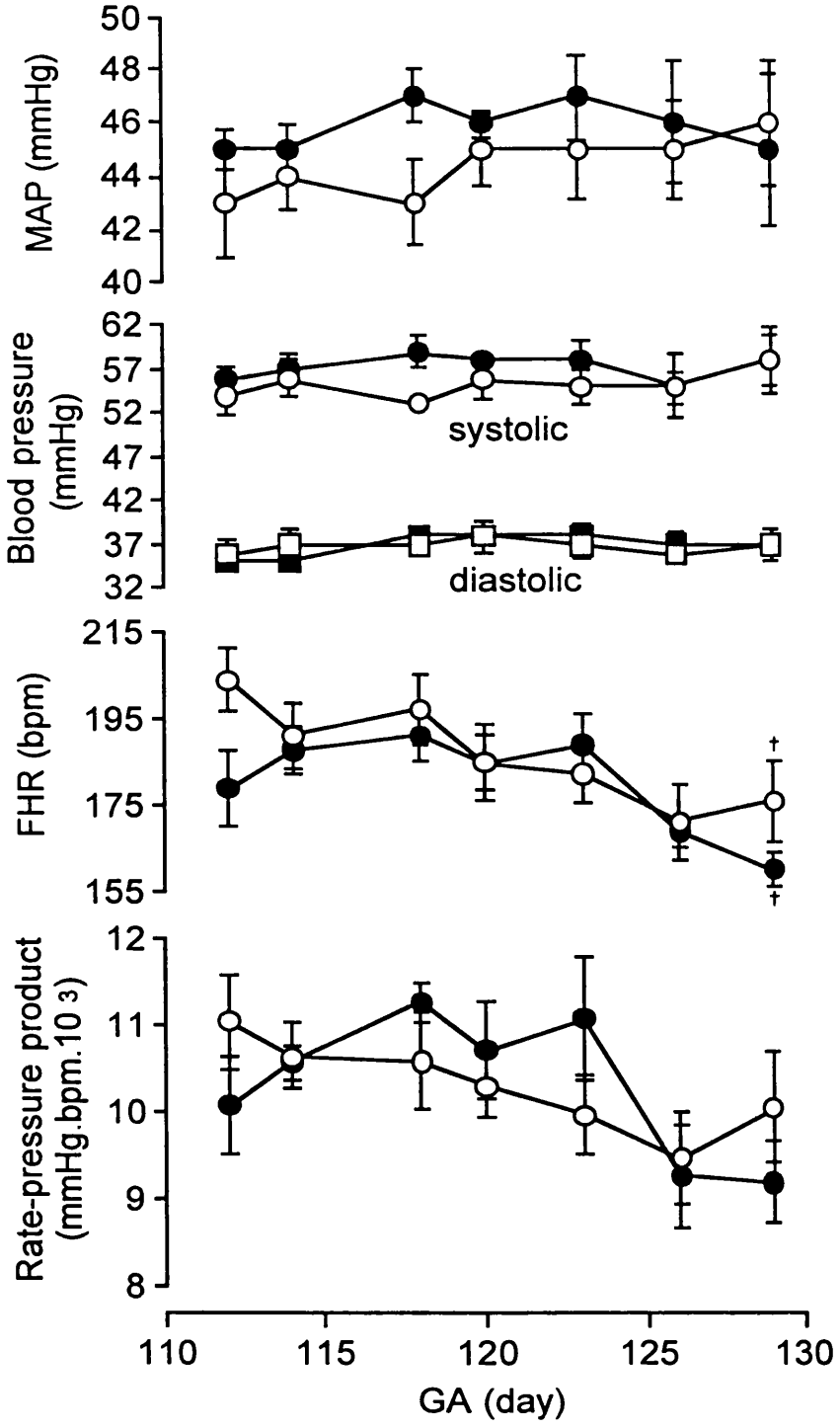

Figure 6.3. MAP, SBP and DBP, FHR and rate-pressure product in HE (o) and LI (•) fetuses over the course of the study. † $P < 0.05$, 112 d vs. 129 d. Values are mean ± S.E.M. (See also Appendix 8).

6.3.2.ii *Cardiovascular reflexes*

Baroreflex

The baroreflex curves for HE and LI fetuses are shown in figure 6.4.

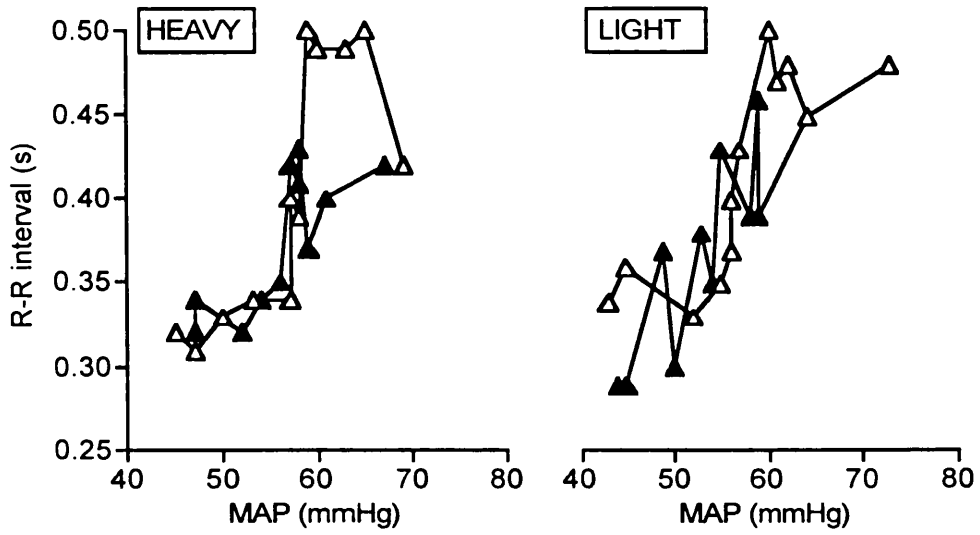

Figure 6.4. Baroreflex curves for HE and LI fetuses at 112 d GA (Δ) and 129 d GA (▲). Values are mean (S.E.M. not included for clarity).

In HE animals, the slope of the steep portion of the baroreflex curve tended ($P = 0.15$) to decrease between 112 d and 129 d GA. But, in LI fetuses there was no such tendency (Fig. 6.5). So, the gain of the reflex decreased in HE fetuses but not in LI fetuses. It is worth noting, also, that the gain of the baroreflex in LI fetuses at both ages was similar to that seen in HE fetuses at 129 d GA (Fig. 6.5).

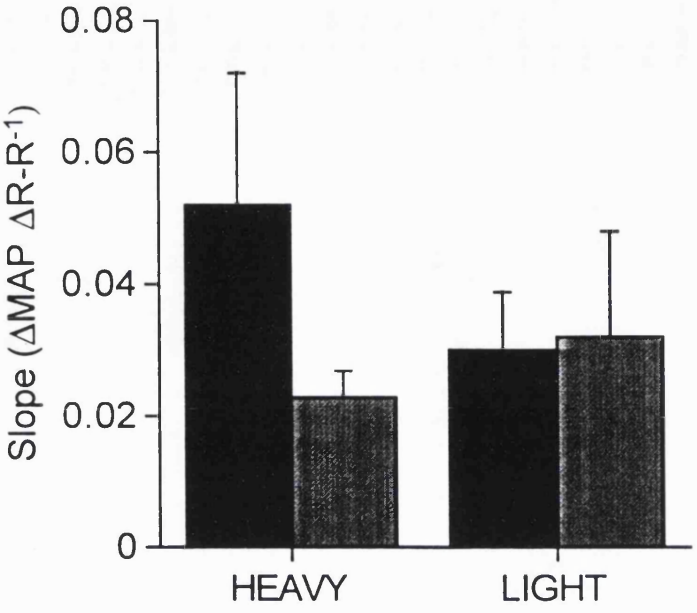

Figure 6.5. Slope of the steep part of the baroreflex curve in HE and LI fetuses at 112 d (■) and 129 d (▩) GA. Values are mean ± S.E.M.

Chemoreflex

There was no significant change in the bradycardic response to hypoxia between 112 d and 129 d GA in HE fetuses, whereas in LI fetuses there was a tendency (P = 0.09) for the response to increase (Fig. 6.6). Although the magnitude of the hypertensive response to hypoxia increased between 112 days and 129 days gestation in both HE and LI fetuses, the increase only reached statistical significance in PD (Fig. 6.6).

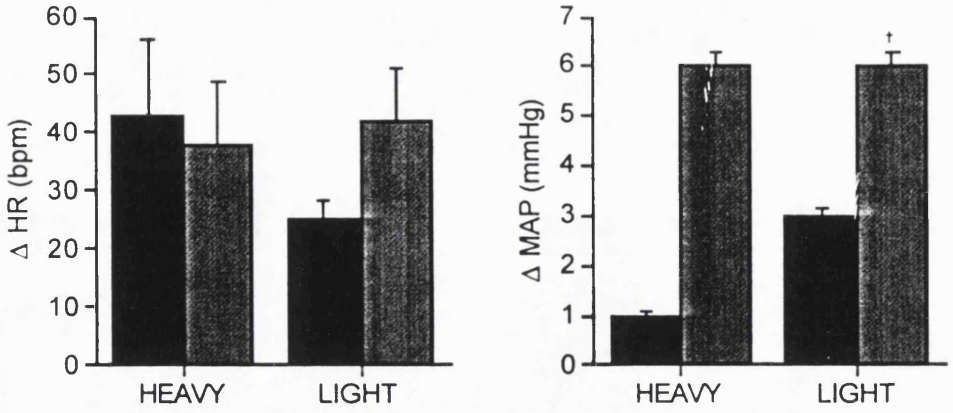

Figure 6.6. The bradycardic (5 min after the onset of hypoxia) and hypertensive (15 min after the onset of hypoxia) responses to hypoxaemia in HE and LI fetuses at 112 d (■) and 129 d (▣) GA. † $P <$ 0.05, 112 d vs. 129 d. Values are mean ± S.E.M.

6.3.2.iii Femoral flow and femoral vascular resistance

Femoral flow rose in both groups of fetuses between 112 d and 129 d GA, but the increase was not significant in either group. The somewhat steeper trajectory in HE fetuses was, however significantly ($P < 0.05$) different to that of LI fetuses (Fig. 6.7). There was no significant change in vascular resistance with increasing gestational age in either group, and nor was there a significant difference between groups (Fig. 6.7).

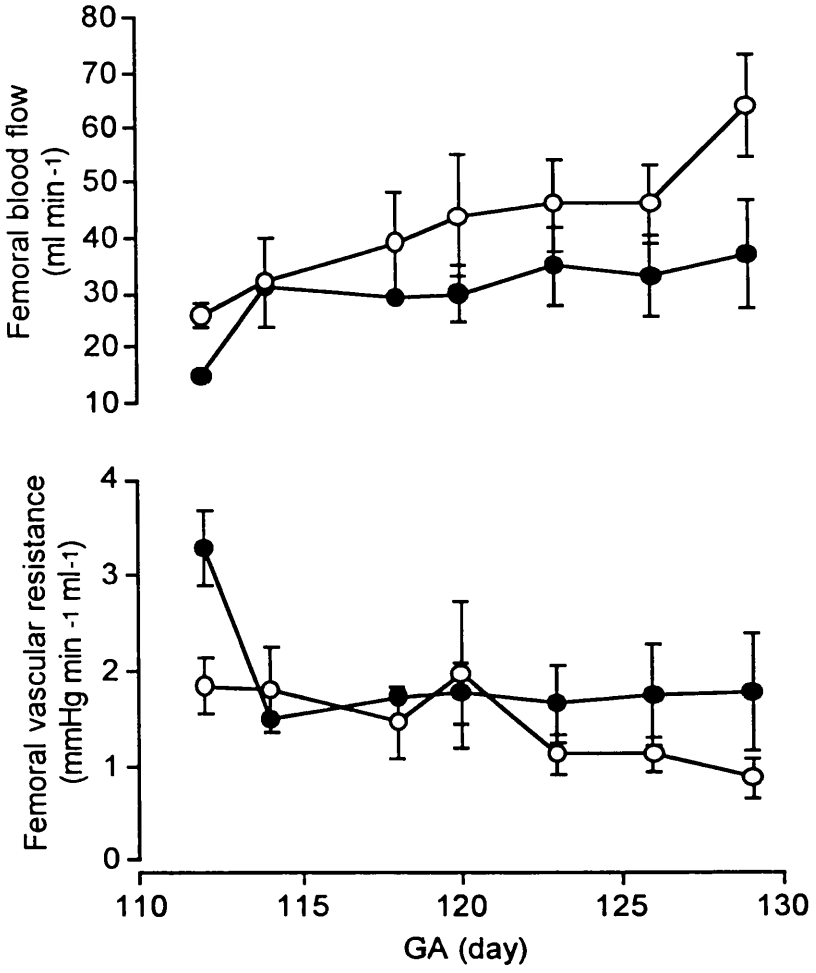

Figure 6.7. Femoral blood flow and femoral vascular resistance in PU (o) (n=3) and PD (●) (n=3) fetuses. Values are mean ± S.E.M.

6.3.3 Endocrine levels

Blood removal for hormone analysis was limited to 3 samples over the course of the study, at 112 d, 118 d and 129 d GA, so as to avoid the fetuses becoming anaemic.

6.3.3.i Catecholamines

The concentration of adrenaline was higher in HE than in LI fetuses at 112 d GA, but there was no difference thereafter. Noradrenaline concentrations were the same in both groups throughout the study (Fig. 6.8). Concentrations did not change significantly between 112 d and 129 d GA in either group.

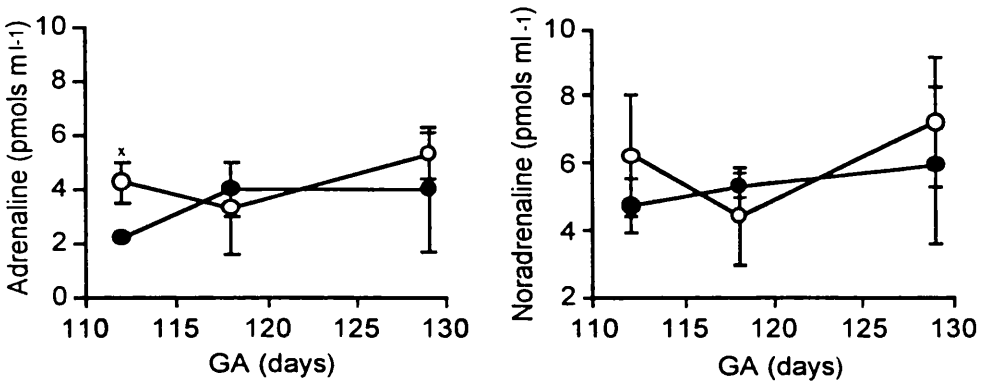

Figure 6.8. Fetal adrenaline and noradrenaline concentrations over the 3 weeks of the study in HE (o) and LI (●) fetuses. ^{x} $P < 0.05$, HE vs. LI. Values are mean ± S.E.M.

6.3.3.ii Cortisol

Cortisol concentrations were the same in LI and HE at 112 d and 118 d GA. At 129 d GA mean cortisol concentration rose dramatically in LI, whereas that of PU rose only very slightly (Fig. 6.9). The increase at 129 d GA was not, however, significant in either group. It is interesting that there was a large degree of variability in cortisol concentrations between LI animals at 129 d GA, which was not the case for HE fetuses.

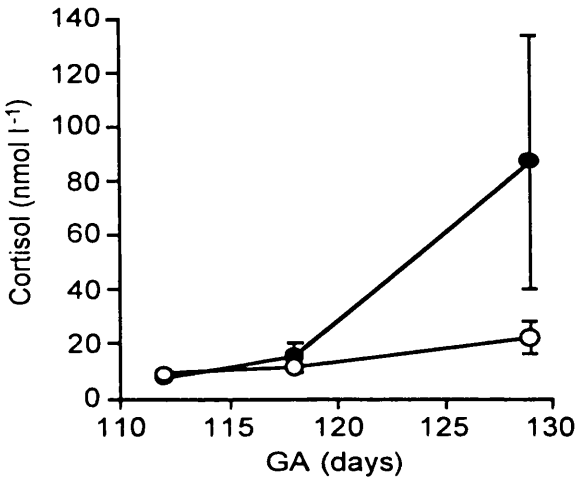

Figure 6.9. Cortisol concentration over the course of the study in HE (o) and LI (•) fetuses. Values are mean ± S.E.M.

6.3.4 Blood gases, haemoglobin, glucose and lactate

PaO_2 appeared to decrease over the course of the study in both HE and LI fetuses, but the decrease was not significant in either group. There was no difference between groups (Fig. 6.10).

Similarly, lactate appeared to increase over the 3 weeks in both groups. The increase was not significant in HE fetuses, but was approaching significance (P = 0.06) in LI fetuses. There was no difference between groups (Fig. 6.10).

$PaCO_2$, haemoglobin, pH, and glucose concentrations did not change significantly with increasing gestational age, and there were no significant differences between groups (Fig. 6.10).

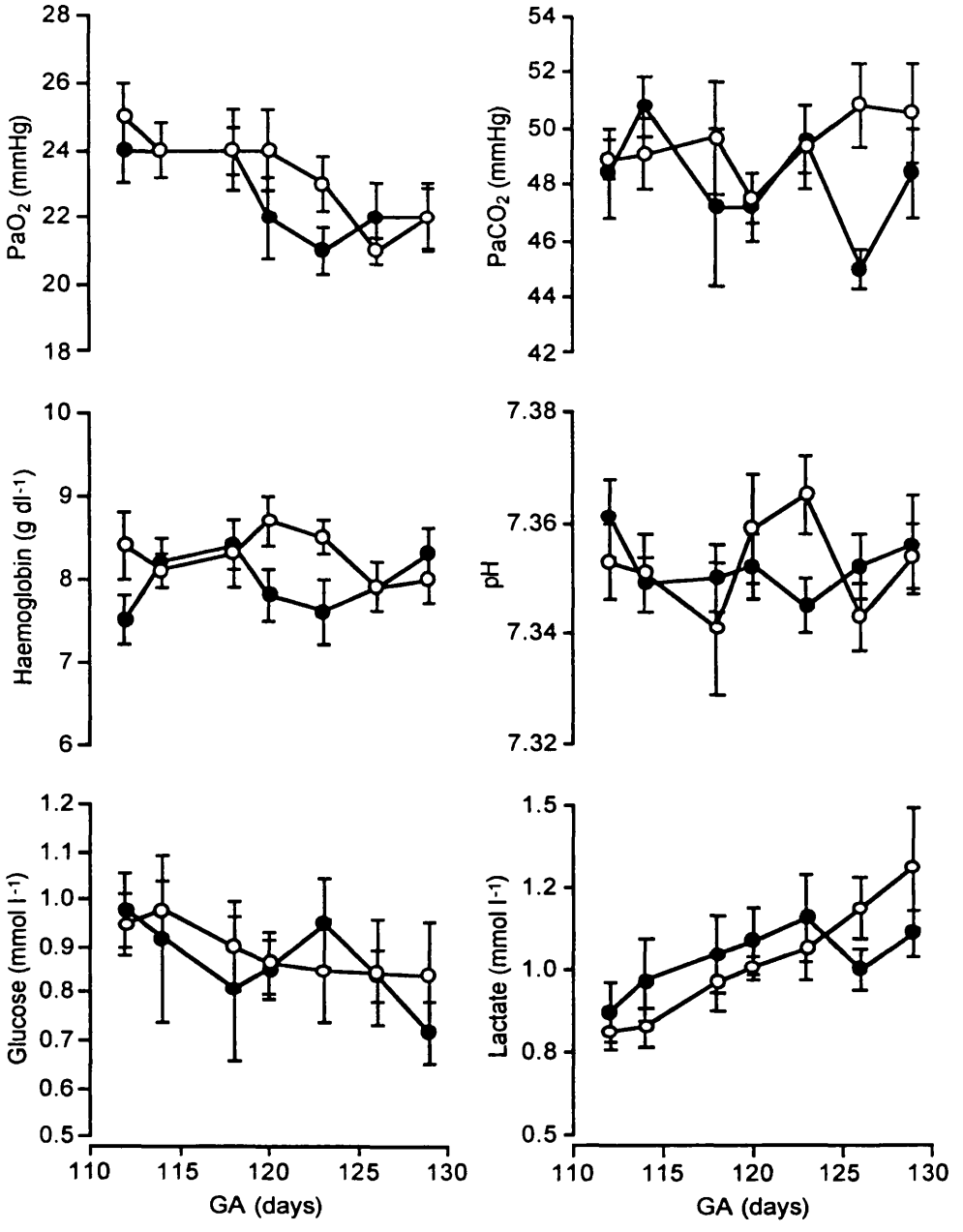

Figure 6.10. PaO_2, $PaCO_2$, pH, haemoglobin, glucose and lactate in HE (o) and LI (●) fetuses over the course of the study. Values are mean ± S.E.M.

6.3.5 Fetal size

6.3.5.i Weights and lengths

LI fetuses were significantly lighter than HE fetuses (Table 6.1) The absolute organ weights *per se* were similar for both groups, except for the kidneys which were significantly lighter in LI fetuses than in HE (Table 6.1). Expressed as a percentage of body weight, so as to take account of the difference in BW between the two groups, it was interesting that not only were the kidneys smaller in LI fetuses, but their brains were larger than those of HE. It may be worth noting also, that, although it was not significant, the livers of LI fetuses tended to be smaller than HE (Table 6.1).

CRL, AC and femur length were not significantly different between LI and HE fetuses at 112 d GA (Fig.6.11), though at 129 d GA AC was significantly greater in HE fetuses, whilst CRL and femur length were similar (Table 6.1, Fig. 6.11).

		HEAVY	LIGHT
Gestational age	GA at start	111 ± 0.2	112 ± 0.4
	GA at end	129 ± 1.2	129 ± 0.7
Absolute weights	Body weight (g)	4117 ± 100	3783 ± 124 x
	Brain weight (g)	44.0 ± 1.1	44.9 ± 0.8
	Heart weight (g)	27.1 ± 0.7	24.9 ± 1.0
	Lung weight (g)	112.8 ± 4.8	98.8 ± 4.8
	Liver weight (g)	126.4 ± 9.7	110.2 ± 5.9
	Kidney weight (g)	14.6 ± 0.9	11.4 ± 0.6 **
	Adrenal weight (g)	0.19 ± 0.01	0.17 ± 0.02
	Perirenal fat weight (g)	19.4 ± 1.1	22.2 ± 6.5
	Pancreas weight (g)	3.2 ± 0.4	3.4 ± 0.6
	Spleen weight (g)	9.7 ± 0.8	7.0 ± 0.6
	Thymus weight (g)	12.3 ± 1.3	9.7 ± 0.8
	Thyroid weight (g)	1.0 ± 0.1	1.0 ± 0.1
Proportional organ weights	Brain % BW	1.08 ± 0.03	1.21 ± 0.05 x
	Heart % BW	0.66 ± 0.01	0.66 ± 0.02
	Lung % BW	2.77 ± 0.15	2.61 ± 0.08
	Liver % BW	3.05 ± 0.20	2.93 ± 0.16
	Kidney % BW	0.36 ± 0.02	0.31 ± 0.02 x
	Adrenal % BW	0.005 ± 0.0001	0.005 ± 0.0001
	Perirenal fat % BW	0.47 ± 0.02	0.58 ± 0.16
	Pancreas % BW	0.08 ± 0.01	0.09 ± 0.01
	Spleen % BW	0.23 ± 0.02	0.24 ± 0.02
	Thymus % BW	0.30 ± 0.03	0.26 ± 0.02
	Thyroid % BW	0.02 ± 0.003	0.03 ± 0.002
Change in length	CRL	31 ± 9	22 ± 4
	AC	65 ± 5	39 ± 7 x
	Femur	57 ± 5	60 ± 1
Placenta	Total placental weight (g)	444 ± 29	380 ± 33
	Placental to FBW ratio	0.11 ± 0.006	0.10 ± 0.008

Table 6.1. Body and organ weights, organ weights as a percentage of body weight (BW), and gestational age (GA) at the start and end of the experimental period in HE and LI fetuses. x $P < 0.05$, ** $P < 0.005$, HE vs. LI. Values are mean ± S.E.M.

6.3.5.ii Growth

The increase in CRL and femur length between 112 d and 129 d GA was comparable between groups, however there was a significantly greater increase of AC in HE than LI fetuses (Table 6.1, Fig. 6.11).

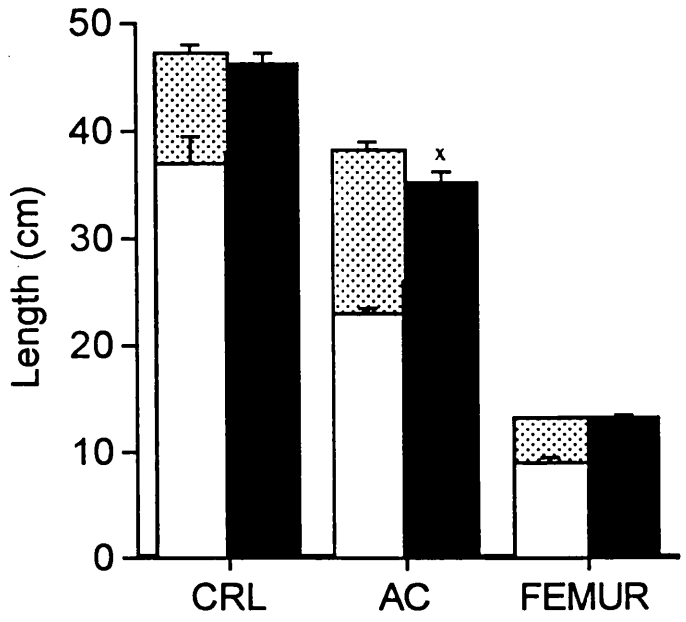

Figure 6.11. CRL, AC and femur length as measured at surgery (107 d GA) (□/■) and at post-mortem (129 d GA) (▨/▩) in HE (lighter coloured bars) and LI (darker coloured bars) fetuses. ^{x} $P < 0.05$, HE vs. LI at post-mortem. Values are mean ± S.E.M.

The decreased girth growth in LI may reflect a decreased liver growth, since, as I have already mentioned, liver weights tended to be less in LI (Table 6.1). In fact, if we look at the correlation of AC as measured at post-mortem to liver weight measured at the same time, there is a positive correlation (Fig. 6.12).

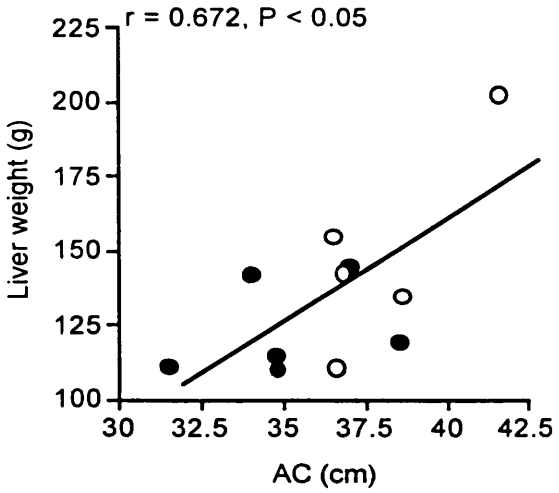

Figure 6.12. Correlation of AC with liver weight at 129 d GA in HE (o) and LI (●) fetuses.

6.3.5.iii Factors relating to FBW

Maternal weight

FBW at 129 d GA correlated positively with maternal BW at mating and at 129 d GA (Fig. 6.13). Change in maternal BW between 0 d and 129 d GA was not, however, correlated to FBW at 129 d GA (Fig. 6.13).

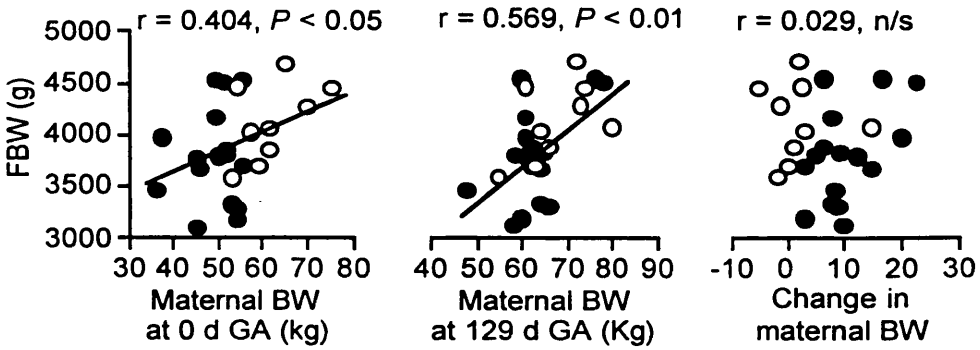

Figure 6.13. Correlation of FBW in HE (o) and LI (●) at 129 d GA to maternal BW at 0 d GA and at 129 d GA, and to the change in maternal BW between 0 d and 129 d GA.

Placental parameters

FBW showed a significant positive correlation with total placental weight, mean placentome weight, and mean placentome diameter; and a significant negative correlation with the total number of placentomes, and the mean diameter to weight ratio of placentomes (which gives an idea of the density of the placentome) (Fig. 6.14). (Unfortunately I did not measure placentome volume, which could have been done quite easily by measuring the displacement of water, so I was unable to calculate the actual densities of placentomes). There was no correlation of FBW with mean placentome thickness or the ratio of mean placentome diameter to placentome thickness (which gives an indication of the shape of the placentome).

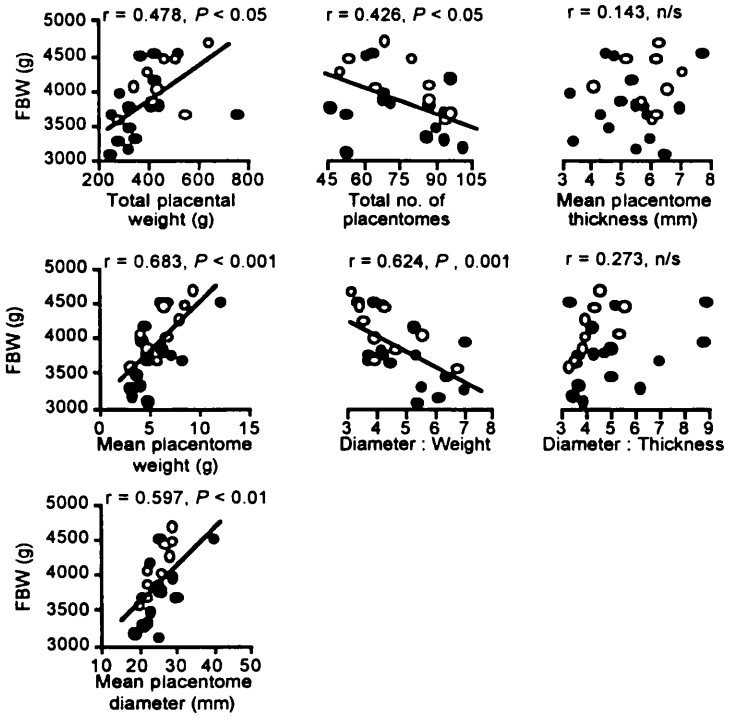

Figure 6.14. Correlation of FBW in HE (o) and LI (•) fetuses to: total placental weight, mean placentome weight, mean placentome diameter (left-hand side), total number of placentomes, mean placentome diameter to weight ratio (middle), mean placentome thickness, and mean placentome diameter to thickness ratio. Values are mean (S.E.M. omitted for clarity).

6.3.6 Placenta

6.3.6.i Weight

Total placental weight was not significantly different between the two groups and nor was placental to fetal body weight ratio (Table 6.1), although LI placentae tended ($P = 0.2$) to be lighter than HE placentae (Table 6.1).

6.3.6.ii Factors relating to placental weight

Placentomes

It was surprising to find that total placental weight did not correlate with the total number of placentomes (Fig. 6.15). There was also no correlation of total placental weight with placentome thickness (Fig. 6.15). There was, however, a significant positive correlation of total placental weight with mean placentome diameter (Fig. 6.15).

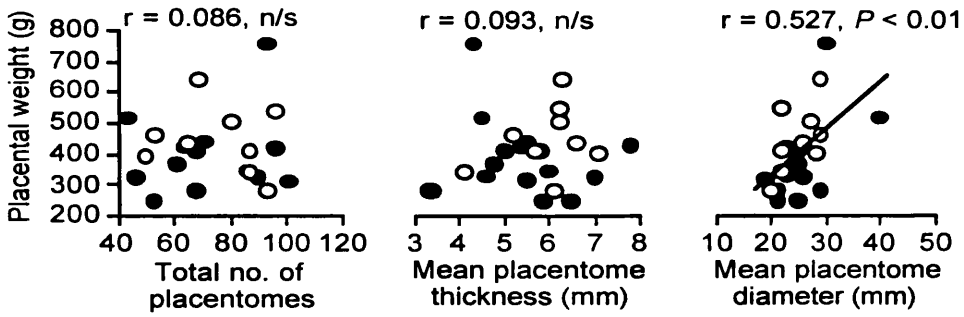

Figure 6.15. Correlation of total placental weight in HE (o) and LI (●) sheep to total number of placentomes, mean placentome thickness, and mean placentome diameter. Values are mean (S.E.M. omitted for clarity).

Maternal weight

There was a significant positive correlation of total placental weight with maternal BW at 0 d GA and a significant negative correlation with change in maternal BW between 0 d and 129 d GA. There was no significant correlation of placental weight with maternal weight at 129 d GA (Fig. 6.16).

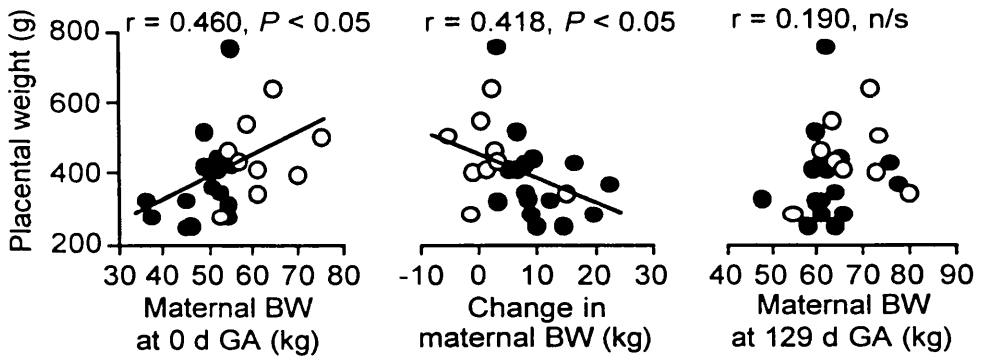

Figure 6.16. Correlation of placental weight in HE (o) and LI (●) sheep to maternal BW at mating and at post-mortem, and to the change in maternal BW between mating and post-mortem.

6.3.6.iii Morphology

In both HE and LI the majority of placentomes were type A and B. There was, however, a shift of distribution to the right in LI fetuses, i.e. an increasing proportion of type D placentomes (Fig. 6.17).

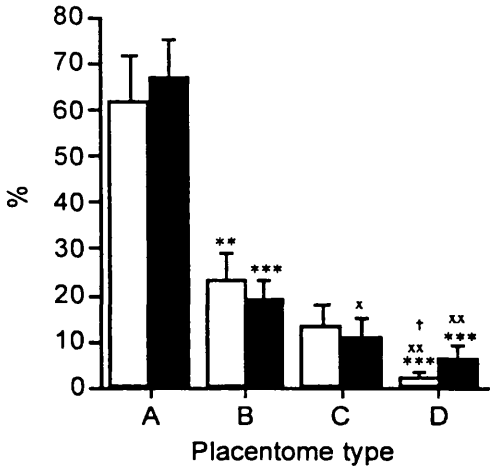

Figure 6.17. Percentage of each type of placentome, A, B, C and D (based on the morphological classification system of Vatnick *et al.*, 1991) in HE (□) and LI (■) placentae. ** $P < 0.005$, *** $P < 0.001$, vs. A; ^{xx} $P < 0.005$, ^{x} $P < 0.05$, vs. B; † $P < 0.05$, vs. C. Values are mean ± S.E.M.

6.3.7 Fetal behaviour

6.3.7.i Sleep states

In LI the duration of LV-ECoG activity was greater than HV-ECoG activity at 120 d GA (Fig. 6.18, upper right graph), and the incidence of LV-ECoG activity was greater than HV-ECoG activity at both 120 and 123 d GA (Fig. 6.18, lower right graph). In contrast, there was no difference of either incidence or duration of LV- and HV-ECoG activity in HE fetuses (Fig. 6.18, upper and lower left graphs).

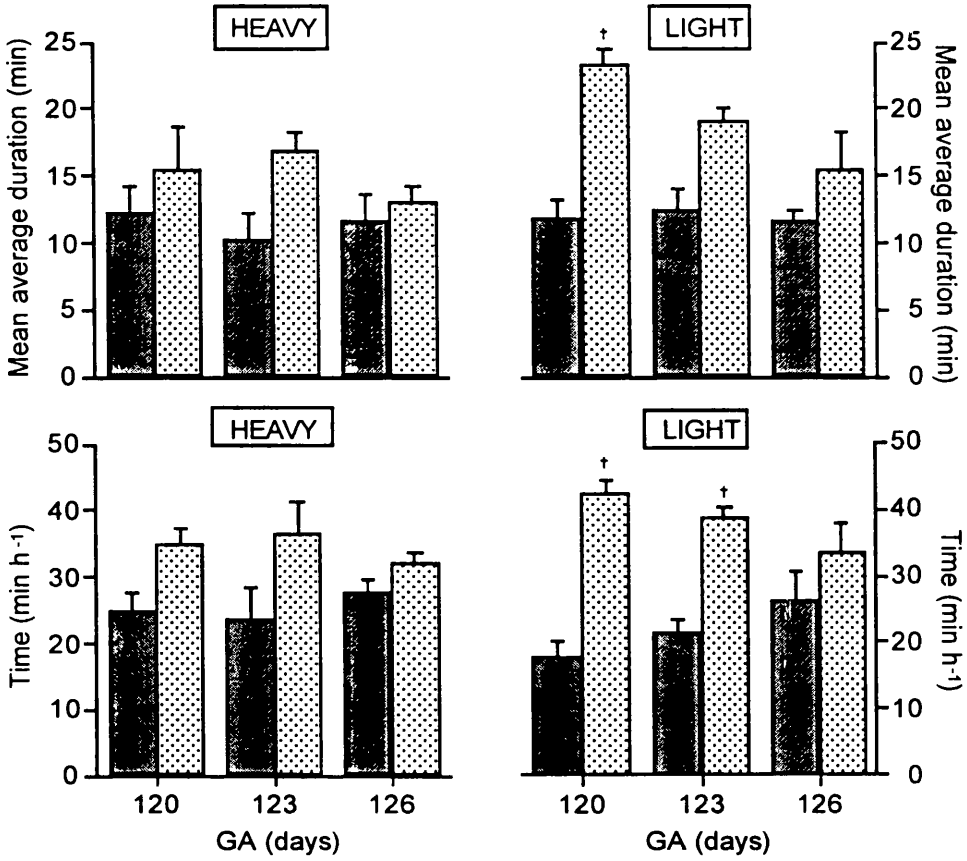

Figure 6.18. Average duration (upper graphs) and incidence (lower graphs) of HV-ECoG activity (■) and LV-ECoG activity (⊡) activity at 120, 123 and 126 d GA in HE (graphs on left) and LI (graphs on right) fetuses. † *P* < HV-ECoG vs. LV-ECoG. Values are mean ± S.E.M.

6.3.7.ii Fetal breathing movements (FBMs)

There was no change in either duration or incidence of FBMs in LI. However, HE showed a decrease in duration between 120 and 126 days gestation, but no change in incidence (Fig. 6.19).

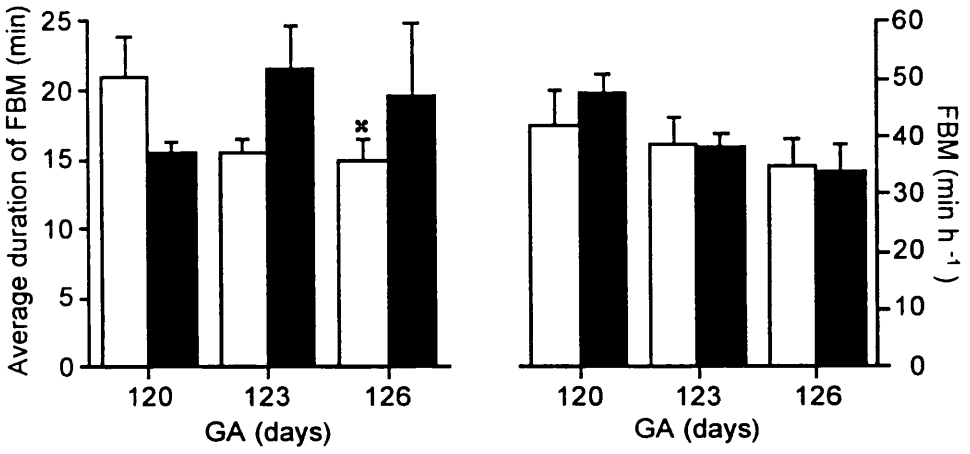

Figure 6.19. Average duration and incidence of FBM in HE (□) and LI (■) fetuses at 120, 123 and 126 days gestation. ⌘ $P < 0.05$, 126 d vs. 120 d GA. Values are mean ± S.E.M.

6.4 DISCUSSION

6.4.1. Cardiovascular development of HE and LI fetuses

6.4.1.i Blood pressure and heart rate

Nutrition and blood pressure development

The finding in this study that blood pressure development was altered in fetuses of ewes that were undernourished during the periconceptual period is interesting in view of observations that have been made in humans. Barker (1992) suggested that undernutrition *in utero* may be an important factor in the predisposition to cardiovascular disease in later life. More recently Godfrey *et al.* (1994) conducted a prospective study looking at the relationship of maternal nutrition to birthweight and blood pressure in offspring at eleven years of age. Maternal nutritional status was determined by measurements of weight, skinfold thickness and haematological status throughout pregnancy. They found that there was a link between poor maternal nutrition and high blood pressure in 10-12 year old children. Campbell *et al.* (1995) found a relationship between blood pressure in men and women aged about 40 years and the balance of maternal intake of animal

proteins and carbohydrate during pregnancy. A somewhat clearer observation of the influence of maternal protein and carbohydrate intake during pregnancy was made by Godfrey *et al.* (1996). They suggested that high carbohydrate intake in early pregnancy combined with a low dairy protein intake in late pregnancy suppresses placental growth, which may have long-term consequences for cardiovascular development of the offspring.

The tendency towards higher pressures in LI (Fig. 6.3) is similar to the findings of Johnston *et al.* (1994), where fetuses whose mothers had been on a low nutritional plane in early pregnancy also tended to have a higher MAP than controls, suggesting that nutrition *in utero* affects the programming of the cardiovascular system. Further evidence to support this idea comes from observations in guinea pigs (Persson & Jansson, 1992) where pups that had severe IUGR, as a result of uterine artery ligation, had elevated blood pressure at 3-4 months of age. Also, rats that were exposed to a reduced protein diet *in utero* and were found to be hypertensive at nine weeks of age (Langley and Jackson, 1994). These findings also compare with observations in my anaemic rat pups that were hypotensive at weaning, then developed hypertension by 40 days of age (Chapter 3). Clearly, maternal nutrition during pregnancy has an effect on fetal MAP in late gestation.

Mechanisms determining the pattern of blood pressure development in LI and HE fetuses

In the light of discussion in the previous chapter regarding the pattern of MAP development, it was notable that in LI fetuses MAP failed to show any increase over the 3 weeks of the study (Fig.6.3). The fact that the SBP of LI fetuses followed a similar pattern of development to MAP, and both were different to the pattern of MAP and SBP development seen in HE suggests that SBP may have influenced MAP development in LI fetuses. DBP development was similar in both LI and HE groups. In both groups, FHR decreased, rate-pressure product did not change significantly (Fig. 6.3), and nor did femoral vascular resistance (Fig. 6.7). Thus, the increase in SBP and MAP in HE fetuses must have been due to increased stroke volume. It was probably also caused by a decrease in aortic compliance, which may occur as a consequence of the rapid accumulation of elastin and collagen that occurs in the aorta during the perinatal period (Bendeck & Langille, 1991). Therefore, the fact that SBP did not increase with time in LI fetuses could have been due to a failure of stroke volume to increase, accompanied perhaps by a fall in

central venous pressure. Heart weight of LI fetuses only tended to be smaller than that of HE, and was the same when expressed as a percentage of body weight (Table 6.1). If there was a significant chronic decrease in stroke volume one would also expect a significant decrease in heart weight. Therefore, decreased central venous pressure is the most likely cause for the pattern of blood pressure seen in LI fetuses. Central venous pressure may have decreased if there was a decrease in placental vascular resistance.

Fetuses that were subjected to either fetal anaemia (Davis & Hohimer, 1991), maternal anaemia (not resulting in fetal anaemia) (Mostello *et al.*, 1991), reduced maternal plasma volume (Daniel *et al.*, 1989), or carunclectomy (Robinson *et al.*, 1983) had lower blood pressure than controls. Lower pressure suggests either a reduced increase or a failure for pressure to increase at all. In fetal anaemia there are changes in fetal vascular resistance that account for the resultant hypotension (anaemia and blood pressure development are discussed in detail in section 3.4.1). Haemoglobin levels in LI fetuses were not significantly different to those of HE fetuses (Fig. 6.10), so anaemia was not an underlying cause for their altered blood pressure development. Mostello *et al.* (1991) suggested that the significantly lower MAP in their fetuses from anaemic ewes may have been due to the smaller size of those fetuses. Certainly this is a possibility as CVO increases ordinarily with increasing fetal weight (Rudolph & Heymann, 1970), which will help to drive pressure up. This raises the possibility that the smaller size of LI fetuses may have been accompanied by a lower CVO than HE fetuses, which could have accounted for the failure for pressure to increase. But, the rate-pressure product was not significantly different between LI and HE fetuses, which implies that CVO may not have been different between them either. Another possible mechanism for the failure of MAP to rise in LI fetuses is hypovolemia, such as was proposed by Daniel *et al.* (1989). Although I did not measure blood volume, there is no indication, e.g. vasoconstriction, that it was less (on a volume per weight basis) in LI compared to HE fetuses. Hypovolemia as a result of haemorrhage has been shown to cause an increase in catecholamines (Meyers *et al.*, 1991) and AII (Iwamoto & Rudolph, 1981), resulting in vasoconstriction. Catecholamine levels in LI fetuses were not elevated (Fig. 6.8) and there was no vasoconstriction (Fig. 6.7), so it seems unlikely that hypovolemia was a mechanism responsible for the failure of MAP to rise in this instance. But, measurement of hormones such as AII, vasopressin and aldosterone would

perhaps have confirmed this view. Robinson *et al.* (1983) found that blood pressure was lower in carunclectomy fetuses compared to controls, presumably due to alterations in placental structure and function. Normally, in the fetal lamb, the proportion of cardiac output to the placenta decreases from about 50% at 60 d GA to about 40% at 145 d GA, indicating a decrease in total fetal body circulatory resistance, or an increase in placental vascular resistance. Decreased fetal body vascular resistance is accounted for by decreased resistance in the lungs, gut, brain and myocardium (Rudolph & Heymann, 1970). But, although the proportion of CVO to the placenta decreases, there is actually an increase in net flow, despite the possible increase in placental vascular resistance suggested by Rudolph & Heymann (1970). Before 115 d GA the increase in net flow is due to a decrease in placental vascular resistance, and after 115 d GA it is pressure driven by the increasing fetal arterial pressure (Carter, 1993). Presumably however, increasing placental vascular resistance also contributes to the rise in pressure during late gestation. It may be, therefore, that the failure for MAP to increase in LI fetuses was due to decreasing placental vascular resistance.

Thus, it seems that, of the mechanisms I have proposed for failure of MAP to increase in LI fetuses, altered placental function resulting in a decrease in placental vascular resistance is the most likely. (I shall discuss the placenta in more detail later on in this chapter, section 6.4.2.iv).

6.4.1.ii Cardiovascular reflexes

The fact that periconceptual maternal nutrition affects cardiovascular development is further emphasised by my finding in this study that there were differences between HE and LI fetuses in the development of their cardiovascular reflex responses.

Baroreflex

The immediately striking finding when looking at baroreflex development in LI and HE fetuses was that the pattern seen in the gain of the reflex (Fig. 6.5) was almost identical to that described in the previous chapter for PU and PD fetuses (see Fig. 5.4 and section 5.5.1.ii). In HE fetuses baroreflex sensitivity tended to decrease (Fig. 6.5), consistent with their rise in MAP, and in the manner expected during this stage of development (Blanco *et al.*, 1988). But, in LI fetuses, gain of the reflex did not change and appeared to already be reset by 112 d GA to a level not seen in HE until 129 d GA (Fig.

6.5). This failure for baroreflex sensitivity to change in LI fetuses is probably not surprising as blood pressure did not rise (Fig. 6.3), but suggests greater baroreflex maturity than HE fetuses.

It is impossible from this study to determine which is 'chicken' and which is 'egg', with reference to baroreflex and MAP development. However, a sustained elevation in arterial pressure would tend to cause a decrease in baroreflex gain, whereas if gain was lower, this would tend to let pressure rise. So, in this study, presumably arterial pressure was cause and baroreflex was effect. We may speculate as to the possible mechanisms underlying the lower sensitivity of the baroreflex in LI fetuses. In section 5.5.1.ii I discussed at some length the different mechanisms for altered baroreflex sensitivity, so I shall not describe them in depth again here. Possibilities are:

1) Flow-related changes in the mechanical properties of the vessel walls of the aorta and carotid artery may have occurred in LI fetuses, resulting in altered vascular compliance which is known to affect baroreceptor resetting (Landgren, 1952; Andresen, 1984). The failure for flow to increase in LI fetuses (Fig. 6.7), presumably as a result of the fact that pressure did not increase, may have had an effect on the vascular structure, as flow is one determinant of vascular remodelling (Langille, 1993; Struijker-Boudier *et al.*, 1995). Possibly the carotid sinus of LI fetuses was less distensible than HE at 112 d GA, as it has been shown (Andresen *et al.*, 1980) that the carotid sinus of the newborn rat is more distensible than that of adults.

2) There may have been altered release of paracrine factors in LI fetuses. It has been demonstrated in the isolated carotid sinus of rabbits that impaired PGI_2 production results in decreased baroreceptor sensitivity (Chen *et al.*, 1990; Chapleau *et al.*, 1991) and it has also been shown in human adults that the fatty acid composition of the diet is associated with changes in PGI_2 synthesis (Sinclair & Mann, 1996). It may be that in LI fetuses the morphological changes seen in their placentae (Fig. 6.17) affected the transport of fatty acids across the placenta, which could have resulted in altered fetal PGI_2 synthesis.

Chemoreflex

The tendency for the bradycardic response to hypoxaemia to increase between 112 d GA and 129 d GA, and the significant increase in the hypertensive response of LI fetuses (Fig. 6.6) bears a striking resemblance to the

chemoreflex response of PD fetuses in the previous chapter (Fig. 5.5, discussed in section 5.5.1.iii). LI fetuses did not have lower blood oxygen levels than HE fetuses, so hypoxaemia is a not a potential mechanism for their altered chemoreflex development, as it was in PD fetuses.

Enhanced chemoreflex activity may have consequences for the fetus later in life, either at birth or after birth when it encounters periods of hypoxia.

6.4.2 Placental growth and morphology

I have already alluded to the idea that changes in placental structure and function may underlie the altered blood pressure development of LI fetuses. So, I shall now go on to discuss the placenta.

6.4.2.i Total placental weight

Total placental weight was not significantly different between LI and HE fetuses, though it showed a tendency to be lower in LI fetuses (Table 6.1). This is similar to findings in women exposed to famine conditions during early pregnancy (Stein & Susser, 1975). Placental weights in women exposed to famine conditions only during early gestation had lighter placentae, though it was found that the effect of late gestation famine exposure had a more profound effect on placental weight. In the sheep, Jacobs *et al.* (1988a) found that hypobaric hypoxaemia tended to cause a reduction in placental weight, and Ahokas *et al.* (1984) that undernutrition in the rat between 5 d GA (0.2 gestation) and 21 d (term) caused a significant decrease in placental weight. Faichney & White (1987), on the other hand, found that undernutrition in the sheep between 50 d GA and 135 d GA caused a significant increase in placental weight, as did DeBarro *et al.* (1992) in response to a periconceptual nutritional challenge. These differences in outcome are probably due to different severities of malnutrition between studies and inconsistencies in time and period of onset. Unfortunately, there have not, to my knowledge, been any dose-control studies performed, which would help to explain these differences. Nonetheless, maternal nutrition does seem to affect placental weight.

6.4.2.ii Placental morphology

Placental morphology was also affected by maternal nutritional plane. Poor periconceptual maternal nutritional status resulted in an increased proportion

of D-type placentomes (Fig. 6.17), a change which is said to reflect compensatory growth of the placenta in order to maintain fetal growth (Alexander *et al.*, 1964; Vatnick *et al.*, 1991). Fetal growth was, however, impaired in LI fetuses (Table 6.1) which suggests that their placental function may not have been adequate for maintaining fetal growth. The possibility arises that D-type placentomes are not, in fact, the most efficient placentome type for placental function. Alternatively, it may be that, had there been more of a shift towards a greater proportion of D-type placentomes in LI fetuses than there were, fetal growth would have been maintained, an idea that is investigated in the next chapter (Chapter 7).

6.4.2.iii Placentome number, thickness and diameter

There was no correlation of either placentome number or thickness with total placental weight, whereas there was a significant positive correlation with placentome diameter (Fig. 6.15).

I was most surprised to find that number seemed to have no influence, particularly as Alexander (1964) reported a significant positive relationship between the number of cotyledons and total cotyledon weight. However, most of the sheep in Alexander's (1964) study had between 0 and 84 caruncles removed, so total placental weight covered a large range, from about 120g to about 800g. This is somewhat greater than the total placental weight range of 245g to 753g in my study. Had he not produced such extremes in placental weight as he did, it is possible that Alexander (1964) may not have found a correlation.

Placentome thickness ranged from about 2 mm to 10 mm, whereas diameter ranged from about 5 mm to 85 mm, so I was not surprised that total placental weight was not influenced by thickness but was strongly associated with diameter.

6.4.2.iv Maternal weight and placental growth

The significant positive correlation of placental weight in late gestation with maternal body weight at mating (Fig. 6.16) shows that maternal nutritional status at conception is important in determining the future growth of the placenta. Both IGF-I and IGF-II are expressed in the maternal caruncles (Stevenson *et al.*, 1994) and circulating levels in fetal plasma show a positive correlation with placental weight (Owens *et al.*, 1994). The correlation of

IGFs and placental weight with placentome number was not shown, which makes the results a little unclear with regard to placental growth. It may have been that a reduced number of placentomes resulted in reduced IGF levels, rather than that reduced IGF levels resulted in decreased placental growth. Also, it would have been nice to know the IGF levels in the placentomes themselves. However, the results of Owens *et al.* (1994) suggest that IGF-I and IGF-II may be regulators of placental growth, and that they may be fetal in origin, as well as placental. Recent work has shown that IGF-II is likely to stimulate proliferative and/or metabolic activity within developing placentomes (Reynolds *et al.*, 1995). The above studies suggest that decreased expression of placental IGFs may occur if maternal nutrition is sub-optimal at conception and during early placental proliferation, so resulting in reduced placental growth.

Vascular endothelial growth factor (VEGF) is an angiogenic growth factor which may also be important for placental growth. Several varieties of VEGF have recently been shown to be produced in the first-trimester human placenta, where it was suggested they may be important for inducing and maintaining placental angiogenesis (Anthony *et al.*, 1994). Hypoxia has been shown to up-regulate VEGF expression, which may reflect a mechanism whereby placental metabolic requirement influences placental vascularisation (Wheeler *et al.*, 1995). It may be that deficiencies of metabolic substrates other than oxygen also influence the expression of VEGFs. Increased VEGF expression is one mechanism whereby placental vascular resistance could be reduced.

6.4.3 Fetal growth

LI fetuses showed an asymmetrical pattern of growth. At 129 d GA they were smaller than HE fetuses, as can be seen by their lower body weight (Table 6.1) and smaller AC (Fig. 6.11), and their brains were larger and their kidneys smaller, as a proportion of body weight (Table 6.1), than those of HE fetuses. Between 107 d GA and 129 d GA CRL and femur length of LI fetuses increased at a similar rate to HE fetuses, but AC growth was significantly reduced (Table 6.1, Fig. 6.11).

6.4.3.i *Maternal determinants of fetal growth*

Size

It is known that maternal body size influences the eventual size of offspring, as was demonstrated in the classic "Shire and Shetland" studies conducted by Walton and Hammond (Yates, 1988). Furthermore, it has been demonstrated in the sheep (Vallet & Christenson, 1993) that if there is a decrease in uterine space, the offspring born are smaller than controls. In a less extreme situation than those cited above, however, Kemp *et al.* (1988) studied the relationship of frame size and nutritional restriction of ewes with size of offspring. They found that there was little effect of ewe frame size on carcass characteristics of lambs slaughtered at 41 kg and 50 kg, but that there was an effect of nutrition. This suggests that normal variations in maternal body size, independent of nutrition, do not have an adverse effect on fetal growth. This seems logical, because there is much variation in the frame size of normal healthy women of reproductive age, and it would be disastrous to survival of the species if small women were destined to produce disadvantaged offspring.

Nutrition

I mentioned above that maternal nutrition has a greater effect on offspring's growth than frame size (Kemp *et al.*, 1988). Certainly evidence from both animal (Mellor & Matheson, 1979; Mellor & Murray, 1981; Faichney & White, 1987; DeBarro *et al.*, 1992; Bauer *et al.*, 1995; Harding & Johnston, 1995; Harding, 1995) and human (Gruenwald, 1967; Stein & Susser, 1975a & b; Godfrey *et al.* 1991, 1994 & 1996; Barker, 1992, 1994 & 1996; Stein *et al.*, 1996) studies confirm that maternal undernutrition at conception and during pregnancy result in impaired fetal growth. The positive correlation of FBW with maternal BW in HE and LI animals (Fig. 6.13) is in agreement with this. Furthermore, this correlation and the fact that there was no correlation of FBW with the change in maternal BW during pregnancy (Fig. 6.13) supports studies carried out in humans (Peckham and Christianson, 1971; Simpson *et al.*, 1975; Brown *et al.*, 1981) where it was observed that women who were underweight at conception had babies of low birth weight, despite similar weight gain to normal-weight women during pregnancy.

Thus, underweight mothers are considered to be a risk factor for fetal growth retardation.

The mechanisms underlying the association between maternal weight and fetal growth are not entirely understood. Of course, if the supply of substrates for metabolism and tissue accretion is inadequate then growth will be impaired. Rosso *et al.* (1992) suggested another mechanism for fetal growth retardation in underweight women. They showed that there is a significant positive correlation between maternal weight and maternal plasma volume, and between maternal plasma volume and both newborn weight and placental weight. They suggested that reduced maternal plasma volume leads to reduced maternal cardiac output, which results in diminished uterine blood flow and thus IUGR. Reduced maternal plasma volume in the pregnant ewe has also been seen to prevent the increase in uterine blood flow that occurs as pregnancy progresses, though impaired fetal growth did not always result (Daniel *et al.*, 1989). In the rat, dams fed 50% of the average daily food intake of controls were found to have preserved maternal hepatic blood flow whilst there was a 30% reduction of flow to the placenta, which was suggested to be the result of increased placental vascular resistance (Ahokas *et al.*, 1984). They found that both fetal and placental weight were lower in the diet-restricted rats. So, maternal undernutrition may impair fetal growth through decreased substrate delivery to the fetus, as a result of decreased uterine blood flow, or as a result of decreased vascular resistance.

However, the mechanisms limiting fetal growth may vary depending on timing of insult. The effect of maternal undernutrition on fetal growth varies depending on when during gestation the period of deprivation occurred (Stein & Susser, 1975a & b; Faichney & White, 1987; Harding & Johnston, 1995; Harding, 1995; Owens *et al.*, 1995).

During the periconceptual period and in early pregnancy reduced substrate supply is generally associated with symmetrical growth retardation (Owens *et al.*, 1995), though outcome does vary from study to study (see Table 1.2 and section 1.7.2). Asymmetrical growth patterns, such as I observed in LI fetuses, are generally observed when nutrient supply is reduced in mid through to late pregnancy, late pregnancy, or for the whole of pregnancy. Uterine artery ligation (Detmer *et al.*, 1991; Jansson *et al.*, 1986) in guinea pigs results in brain sparing, as does undernutrition in late gestation (Stein & Susser, 1975b; Harding & Johnston, 1995; Harding, 1995), placental

embolization during late gestation (Creasy *et al.*, 1972; Murotsuki *et al.*, 1996a), hypoxaemia during late gestation (Jacobs *et al.*, 1988a), acute uterine artery ligation in late gestation (Tanaka *et al.*, 1994), hypoxaemia throughout gestation (Bacon *et al.*, 1984; Jacobs *et al.*, 1988a), and carunclectomy (Owens *et al.*, 1986). All these studies also produced significant alterations in placental weight, except Jacobs *et al.* (1988a) where the decrease in placental weight did not reach significance. This raises the possibility that reduced substrate supply early in gestation may have affected the interaction between fetal and placental growth during late gestation in LI fetuses.

6.4.3.ii Placental size and fetal growth

In my LI and HE fetuses I observed the well established positive correlation of FBW with placental weight (Fig. 6.14). FBW also showed a significant positive correlation with mean placentome weight and mean placentome diameter (Fig. 6.14). This suggests that in the sheep, the optimum placenta for fetal growth is one where the placentomes are large and heavy, though the thickness of the placentome does not influence fetal growth (fig. 6.12). This agrees with the study of Alexander (1964), where he observed an increase in individual placentome weight after carunclectomy. He concluded that up until 100 d GA in the sheep, fetal growth could be sustained with little placental tissue. But, after 100 d GA (0.68 gestation), when there is an increase in fetal growth rate, placental size limits fetal growth. Similarly, in guinea pigs where one uterine artery was ligated (Jansson *et al.*, 1986), prior to 45 d GA (0.66 gestation, term in the guinea pig being about 68 days), there was no effect on fetal growth although placental weight was reduced. But, by 55 d GA (0.8 gestation) fetal growth was impaired. This suggests that in the first half of gestation, prior to 0.66 gestation, growth of the fetal guinea pig was not limited by the size of the placenta. Somewhat differently, DeBarro *et al.* (1992) found in sheep undernourished during the periconceptual period, that fetal growth was reduced by 90 d GA (0.6 gestation) and was associated with increased placental weight. Evidently, both small and large placentae may be indicative of impaired placental function. The possibility arises that the somewhat smaller LI placentae may have limited fetal growth.

I was surprised to find that there was a negative correlation of FBW with number of placentomes (Fig. 6.14), but this is similar to Alexander's (1964) observation that fetal weight correlated better with the weight of functional cotyledons than with their number. There was also a negative correlation of

FBW with placentome diameter/placentome weight ratio (Fig. 6.14). This suggests that less dense placentomes are desirable for optimal fetal growth. It may be that denser placentomes are made up of more stroma and connective tissue than villi, so the surface area for exchange of substances is reduced, which would, of course, not be optimal for fetal growth. If stromal tissue is indeed more dense than villous tissue, this idea is supported by the fact that at 70-90 d GA, the placentomes are made up of a large amount of fetal connective tissue, then as gestation proceeds there is increasing sub-division of the villi at the fetomaternal haemotrophic exchange surface, so by term the villous surface area is greatly increased and there is very little fetal or maternal connective tissue (Wooding & Flint, 1994). There was no correlation of FBW with placentome diameter/placentome thickness ratio, suggesting that placentome shape is not a determinant of placentome functionality. Placental weight and density, therefore, appear to be closely correlated to fetal growth.

6.4.3.iii Placental morphology and fetal growth

It may be that placental morphology is also important for fetal growth, as the different morphological types of placentome (A, B, C, D) may have different functional capacities. Unfortunately, there is not to my knowledge any literature on the comparative histology or function of the different morphological types of placentome, such as they are described here. This is, however, an area of work that we are pursuing in our laboratory.

In LI fetuses there was a shift of distribution to the right, from A-type to D-type placentomes (Fig. 6.17). It is possible that this shift reflects placental compensation to the nutritional challenge. It has previously been suggested (Vatnick *et al.*, 1991) that the shift to the right represents compensatory growth of the fetal placenta so as to maintain fetal growth. It is interesting to consider this notion with reference to comparative placentation of other ruminants. The predominant placentome type in sheep and goats is concave to the fetal side (i.e. A-type), in the antelope placentomes tend to be flat (i.e. C-type), and those of the cow are convex (i.e. D-type) (Wooding & Flint, 1994). What is interesting about this is that the progresssion from A- through to D-type placentomes occurs with increasing species size. The sheep is smaller than most antelope, which in turn are smaller than cattle. Maybe D-type placentomes have a greater fetal to placental exchange surface area. Again, comparative studies between species suggest that this may indeed be so, as the villi in the placentomes of the deer and the cow are more complexly

branched than those of the sheep (Wooding & Flint, 1994). Further support for the idea that D-type placentomes are a compensatory mechanism for optimising fetal growth, is the natural increase in the number of D-type placentomes that occurs during late gestation and the greater number of D-type placentomes seen in twin pregnancies, in sheep.

It would appear, in the sheep, that information on the relative distribution and characteristics of the different placentome types may be a better correlate of fetal growth than information on the placenta as a whole, regardless of morphology. This issue was investigated and is discussed in greater depth in the next chapter (Chapter 7).

6.4.3.iv Fetal metabolism

Although compensatory placental growth may have occurred in LI fetuses, it was not adequate to maintain fetal growth. PaO_2, CaO_2, glucose and lactate concentrations were similar in both HE and LI fetuses (Fig. 6.10) which suggests that LI fetuses slowed down their metabolism, and thus growth, to adapt to the reduced nutrient supply.

The supply of glucose to the fetus is directly related to maternal plasma glucose concentration, uterine glucose uptake, and the arterial plasma concentration gradient between mother and fetus (Aldoretta *et al.*, 1995). After 30 days gestation, maternal food supply was *ad lib* in both LI and HE ewes, so maternal glucose levels would have been normal in both groups. It is possible that the placental morphological alterations seen in LI fetuses were accompanied by altered levels of the transporter proteins GLUT-1 and GLUT-3 that mediate the uptake and transfer of glucose (Hay, 1996), so despite adequate maternal levels of glucose, the amount getting to the fetus may have been reduced. In this connection it is interesting that not all SGA infants exhibit hypoglycaemia (Hawdon *et al.*, 1992). It is also possible that there was endogenous production of glucose from amino acids in the livers of LI fetuses by gluconeogenesis. This could have been mediated by the catabolic effects of cortisol, as cortisol concentrations did tend to be increased in LI fetuses after 118 d GA, though increased levels of cortisol cannot explain the maintenance of blood glucose concentration prior to 118 d GA.

Fetal hypoxaemia is commonly observed in cases of fetal growth failure in both humans (Montemagno & Soothill, 1995) and animals (Owens *et al.*, 1995). Furthermore, the redistribution of blood flow in response to

hypoxaemia in favour of the brain, heart and adrenals at the expense of the kidneys and other peripheral organs is now well documented (Cohn *et al.*, 1974; Itzkovitz *et al.*, 1987). Such redistribution of blood flow results in asymmetrical fetal growth such as I observed in LI fetuses (Table 6.1).This raises the possibility that reduced oxygen supply may also have been responsible for the slower growth and altered growth pattern of LI fetuses. If placental vascularity was affected in LI animals (see section 6.4.2.iv), it may be that the transfer of oxygen across the placenta was not sufficient for normal fetal growth.

6.4.3.v Fetal growth and blood pressure development

The previous 3 chapters have suggested a link between fetal growth and the pattern of blood pressure development. This is an association that once again is borne out in this study. LI fetuses displayed reduced growth rate compared to HE (Table 6.1) and did not show the expected rise in MAP (Boddy *et al.*, 1974; Kitanaka *et al.*, 1989; Daniel *et al.*, 1989; Mostello *et al.*, 1991; Kamitomo *et al.*, 1994; Gagnon *et al.*, 1994; Murotsuki *et al.*, 1996a) that was seen in HE fetuses. It is notable that, despite tending to run at slightly higher pressures than HE (Fig. 6.3), LI fetuses showed the same pattern of MAP development as was seen in PD fetuses in the previous chapter (Fig. 5.2, section 5.5.1.i). This suggests that it is not so much the actual pressure level that is important, but the developmental pattern of MAP.

In the previous chapter (section 5.5.3.iii) I made the suggestion that cortisol may be one of the factors linking fetal blood pressure development and growth. It is interesting, therefore that, although not significant and although the variance was large, in LI fetuses there was a dramatic increase in cortisol concentration between 118 d GA and 129 d GA (Fig. 6.8). The pattern of cortisol secretion seen in LI fetuses was similar to that seen in fetuses where placental embolization was carried out for 21 days (Murotsuki *et al.*, 1996b) and may contribute to the growth restriction that occurred in both. With regard to the variable increases in cortisol levels that I found, it is notable that variability was also greater in the embolization sheep fetuses of Murotsuki *et al.* (1996b) when cortisol levels increased.

However, although cortisol levels increased in LI fetuses, MAP levels did not. This is perhaps not surprising in view of the fact that cortisol does not have a direct effect on blood pressure. Cortisol selectively stimulates the

synthesis of adrenaline by specifically inducing the enzyme phenylethanolamine-N-methyltransferase which converts noradrenaline to adrenaline. Since neither adrenaline nor noradrenaline concentrations were elevated (Fig. 6.7) in LI fetuses, it is probably no surprise that MAP did not rise in LI. However, because of the permissive effect that cortisol has on adrenaline synthesis one would expect adrenaline synthesis to be increased in the presence of elevated cortisol levels. It is possible that tyrosine, the first amino acid in the chain of synthesis that forms adrenaline, was a rate limiting factor. The possible role for cortisol in blood pressure development is discussed in detail in the next chapter (section 7.4.2.ii).

6.4.4 Fetal behaviour

There appeared to be perturbations in the development of fetal behaviour as a consequence of undernutrition in early pregnancy. This was seen as increased incidence and duration of LV-ECoG activity at 120 and 123 d GA in LI fetuses, but not in HE fetuses (Fig. 6.18). Also, the incidence of FBMs decreased from 120 d to 126 d GA in LI but not HE fetuses, whereas there was a decrease in duration of FBM in HE fetuses but not LI (Fig. 6.17). Mirmiran (1995) hypothesised that rapid eye movement (REM) sleep (the same as LV-ECoG activity) in early life is important in influencing neuronal growth, synaptic plasticity, learning and unlearning, and various other neural functions. He suggested this as a reason why the fetus and neonate spend so much time in this state. He also postulated that LV-ECoG activity in early life may serve either as an indicator of the degree of brain maturation or as a predictor of neurological outcome. If it is indicative of the relative maturity of the brain, the decrease of both incidence and duration of LV-ECoG activity in LI fetuses suggests that their neurological development may have been more advanced then HE fetuses. Accelerated maturation of the brainstem (and lungs) is seen in some SGA babies born of pregnancies associated with placental insufficiency (Amiel-Tison & Pettigrew, 1991). Amiel-Tison & Pettigrew (1991) also suggested that elevated glucocorticoid levels may be involved in the mechanisms responsible for advanced maturation, which is interesting in view of the fact that LI fetuses showed a large rise in cortisol (Fig. 6.9).

In the interests of cardiovascular development these findings are very interesting. Brainstem processes are known to regulate the gain of chemo- and baroreflexes in the fetus and neonate (Moore & Hanson, 1994), thus it

seems possible that there may be links between the behavioural changes observed here and those in reflex gain following reduced periconceptual nutritional plane. It remains to be established whether these effects are causally related.

The possible intrauterine origins of cardiovascular disease and diabetes are now well publicised (Barker, 1994 & 1996). But, it may be that other neonatal and adult pathological conditions are also programmed *in utero*, such as SIDS (Mirmiran, 1995) and schizophrenia (Munk-Jorgensen & Mortensen, 1993).

6.5 CONCLUSION

These results show that:

1) Maternal nutrition at conception and in early pregnancy affects fetal blood pressure and cardiovascular reflex development in late gestation.

2) There are interesting trends in relation to fetal growth and blood pressure and reflex development.

3) Placental gross morphology is altered by an early nutritional insult.

Thus, it would appear that reduced placental function results in impaired fetal growth which is related to abnormal cardiovascular development. However, further work investigating placental alterations in response to periconceptual undernutrition is warranted.

6.6 SUMMARY

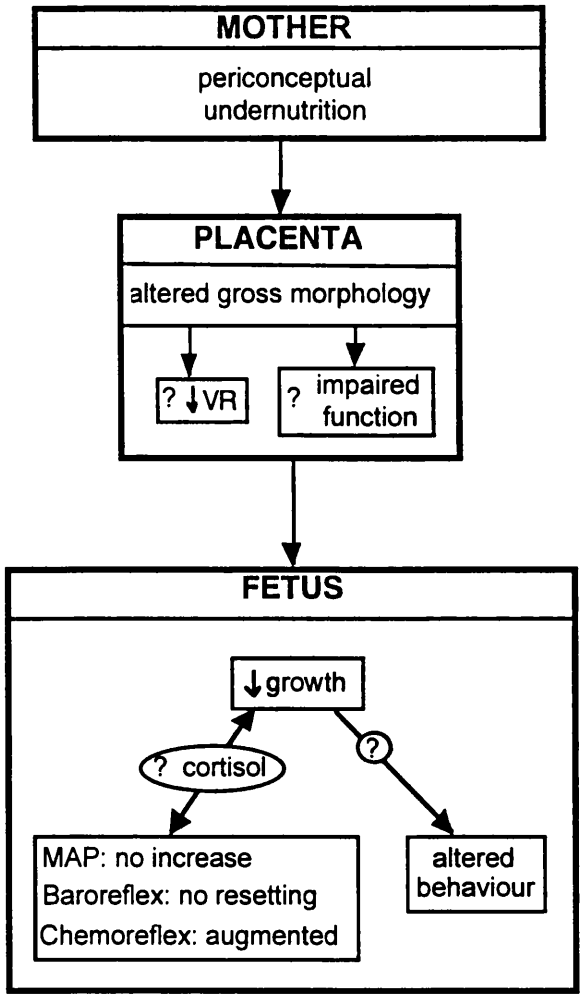

Figure 6.20. Diagram summarising the findings and possible mechanisms involved in fetal cardiovascular development as a result of periconceptual maternal undernutrition.

Chapter 7

FETAL AND PLACENTAL MORPHOMETRY AND PLACENTAL MORPHOLOGY: THE EFFECT OF PERICONCEPTUAL NUTRITION

7.1 INTRODUCTION

Human and animal studies suggest that maternal nutritional status at conception and in early pregnancy affects both fetal (Osofsky, 1975; DeBarro *et al.*, 1992) and placental (DeBarro *et al.*, 1992) growth, and results in perturbations in cardiovascular development (Barker *et al.*, 1993; Stein *et al.*, 1996).

However, the effects of undernutrition on growth are variable. In the placenta, generally, anaemia is associated with hypertrophy (Beisher *et al.*, 1970). Whilst, in some cases, caloric restriction during early pregnancy is associated with a decrease in placental weight (Stein & Susser, 1975a & b), and in others it has been observed to result in an increase in placental weight (DeBarro *et al.*, 1992). Similarly, the effects on fetal growth are variable. In humans, periconceptual and early pregnancy undernutrition has been reported to result in no significant effect (Stein & Susser, 1875a & b), and a definite decrease (Antonov, 1947; Rosso *et al.*, 1992) in birth weight. In the sheep, undernutrition during early pregnancy has been observed to have no significant effect (Harding & Johnston, 1995) and in another study to result in a decrease (DeBarro *et al.*, 1992) in fetal growth. Nonetheless, both placental overgrowth (Beisher *et al.*, 1970, Godfrey *et al.*, 1991) and undergrowth (Bonds *et al.*: 1984, Martyn *et al.*, 1996; Godfrey *et al.*, 1996; Campbell *et al.*, 1996) have been associated with adverse perinatal outcome.

However, placental weight alone may not be a suitable measure of placental adaptation to adverse conditions during pregnancy. For example, in guinea pigs exposed to 12% O_2 throughout gestation, it was found that placental weight was not significantly affected, however there was a significant effect on placental structure and function (Bacon *et al.*, 1984). Similarly, in the sheep, interesting changes in placental morphology have been observed in relation to the number of conceptuses and thus the nutrient demands placed upon the placenta by the fetus (Vatnick *et al.*, 1991).

On the basis of the observations made by various researchers, discussed above, and on the strength of my previous findings (Chapter 6) that there were alterations in placental morphology as a result of maternal periconceptual undernutrition, it was my hypothesis that maternal periconceptual undernutrition would result in altered placental morphology and function, perhaps resulting in impaired fetal growth. So, it was my aim to reproduce

the morphological alterations in the placenta, as a result of periconceptual and early gestation maternal undernutrition, that I had seen previously, and to make a more thorough investigation of the effects of such changes on the placenta.

7.2 EXPERIMENTAL DESIGN

7.2.1 Sheep used

The ewes used in this study were all of the Clun Forest breed and all were mated with a Clun Forest ram. The Clun Forest is a lowland breed and generally has twin or triplet pregnancies.

After mating, the ewes were housed in groups of 4, in pens with raised flooring, until about 125 d GA.

7.2.2 Experimental procedure

14 days prior to mating, ewes were randomly assigned to one of two groups, Light (LI) or Heavy (HE). LI ewes were fed a restricted diet of 85% their recommended nutrient requirement and HE ewes were fed a complete diet *ad lib.* HE ewes continued to receive food unrestricted throughout pregnancy. LI ewes were maintained on the restricted diet after mating until 70 days gestation, thereafter they received the complete diet *ad lib.* Maternal body weights (BW) and body condition score (BCS) were recorded weekly for the duration of the study.

At 130 days gestation 6 twin-bearing ewes from each group were selected, killed and an extensive post-mortem was carried out (as described in section 2.2.9.ii).

7.2.2.i Body condition scoring

All the sheep in this study were body condition scored by Professor David Noakes at the Royal Veterinary College, where they were housed at the time.

Russel (1991) gives a clear description of body condition scoring, and I have drawn from that text to give the following description. BCS is a quantitative measure of fatness. BCS assessment is made by palpating the ewe in the

lumbar region, around the backbone in the region immediately behind the last rib and above the kidneys. Assessment involves:

1) Estimating the prominence (sharpness and roundness) of the spinous processes of the lumbar vertebrae, by the degree of fat cover.

2) Judging the extent of muscular and fatty tissue below the transverse tissues by the ease with which the fingers can be passed under the ends of the transverse processes.

3) Estimating the amount of muscle and fat in the area between the spinous and transverse processes.

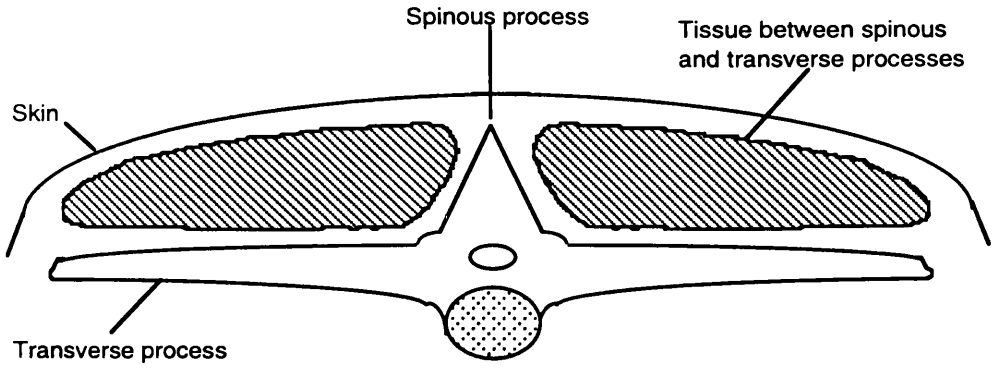

Figure 7.1. The backbone in the lumbar region of the sheep.

Having done this assessment a score on the scale 0-5 is then awarded. 0 is extremely emaciated and 5 is very fat. Generally, in agricultural practice, it is considered that score 3 - 3.5 is desirable for ewes going to the tup.

7.3 RESULTS

7.3.1 Maternal body weight and body condition score

LI ewes began losing weight at the beginning of the study and continued to lose weight until about 63 d GA (Fig. 7.2). Weight loss then decreased, until about 119 d GA at which time they began to put on weight. So, by the end of the study maternal BW of LI and HE ewes was the same (Fig. 7.2). HE ewes

did not show much change in weight until about 70 d GA after which time weight increased (Fig. 7.2). It can be seen clearly from figure 7.2 that the weight profiles of the two groups of ewes were significantly different over the duration of pregnancy.

BCS in HE ewes remained at about score 3 for the whole of the study, whereas that of LI ewes dropped progressively throughout pregnancy from about score 3 to about score 1.5 (Fig. 7.2).

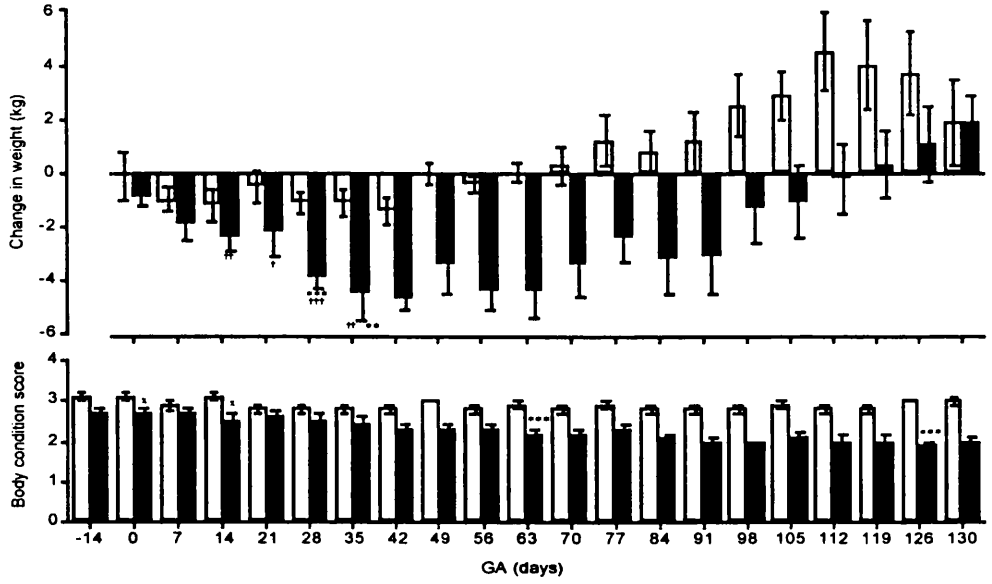

Figure 7.2. Change in maternal BW and BCS in HE (□) and LI (■) ewes during pregnancy. ^{x} $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$, HE vs. LI; † $P < 0.05$, ††† $P < 0.001$, vs. 0 d GA. Values are mean ± S.E.M.

There was no correlation between maternal BW and BCS. For each BCS there were a range of BWs (Fig. 7.3).

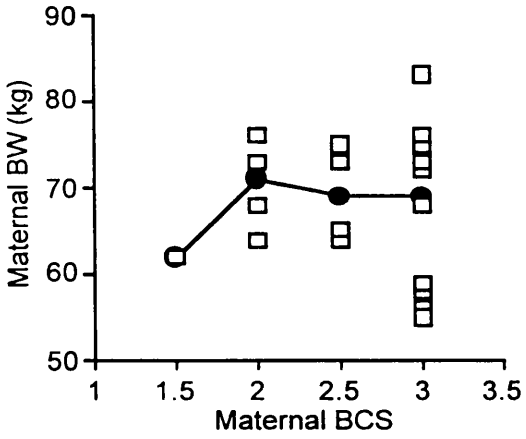

Figure 7.3. Relationship of maternal BW with BCS. The filled in circles (●) show the mean weight at each BCS.

7.3.2 Placenta

With the exception of looking at overall placental morphology (Fig. 7.4), I have looked at only A- and D-type placentomes. I decided to do this because A and D placentomes are easily classified, whereas it was not always so easy to be definitive about the classification of B- and C-type placentomes. Therefore, it is possible that some Bs were classified as C's and vice versa. Thus, by choosing to look at only As and Ds, I hoped to minimise any observer error.

7.3.2.i Morphology

There was an interesting difference between the distribution of A, B, C and D-type placentomes in LI and HE placentae. In HE placentae there were significantly more A than D-type placentomes, whereas in LI placentae there was no difference in the number of A and D-type placentomes, i.e. there was a shift to the right in LI placentae (Fig. 7.4). This difference in placentome distribution between the two groups resulted in a striking and significant difference between the proportional placentome distribution in LI and HE animals (Fig. 7.4).

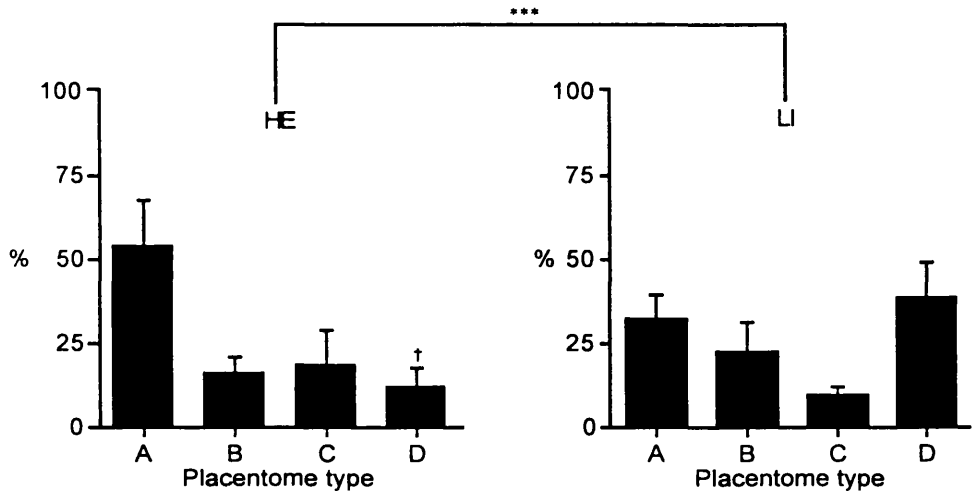

Figure 7.4. Distribution of A-, B-, C- and D-type placentomes (morphological classification of Vatnick *et al.*, 1991). *** $P < 0.001$ HE vs. LI; † $P < 0.05$, A vs. D. Values are mean ± S.E.M.

There was no significant difference between LI and HE placentae in the total number of A-type placentomes. However, total weight, thickness and diameter of A-type placentomes were significantly greater in HE compared to LI placentae (Fig. 7.5) and no significant differences were seen between groups in total number, weight, thickness and diameter of D-type placentomes, although all were consistently greater in LI placentae (Fig. 7.5).

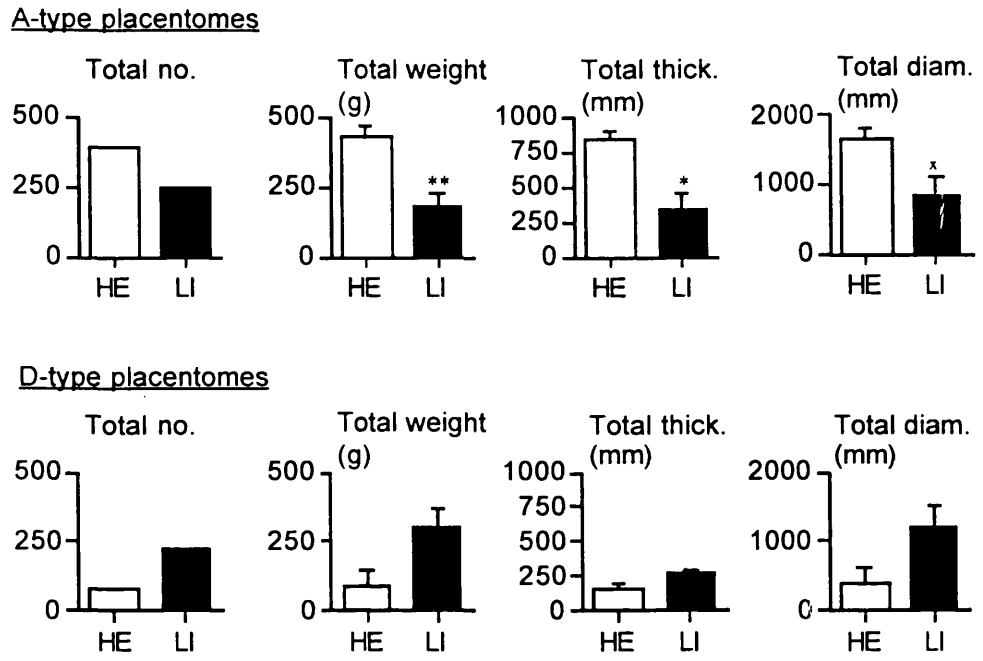

Figure 7.5. Total number, weight, thickness and diameter of A- and D-type placentomes. ^{x} $P < 0.05$, * $P < 0.01$, ** $P < 0.005$. **Values are mean** ± S.E.M.

There was no significant difference in the mean number, weight, thickness and diameter of A-type placentomes between LI and HE placentae (Fig. 7.6). The mean number of D-type placentomes tended ($P = 0.07$) to be greater in LI placentae, but there was no significant difference between groups in mean weight, thickness and diameter of D-type placentomes (Fig. 7.6). It may be worth noting that, as with the total values (Fig. 7.5), mean placentome number, weight, thickness and diameter of D-type placentomes were also consistently greater in LI fetuses.

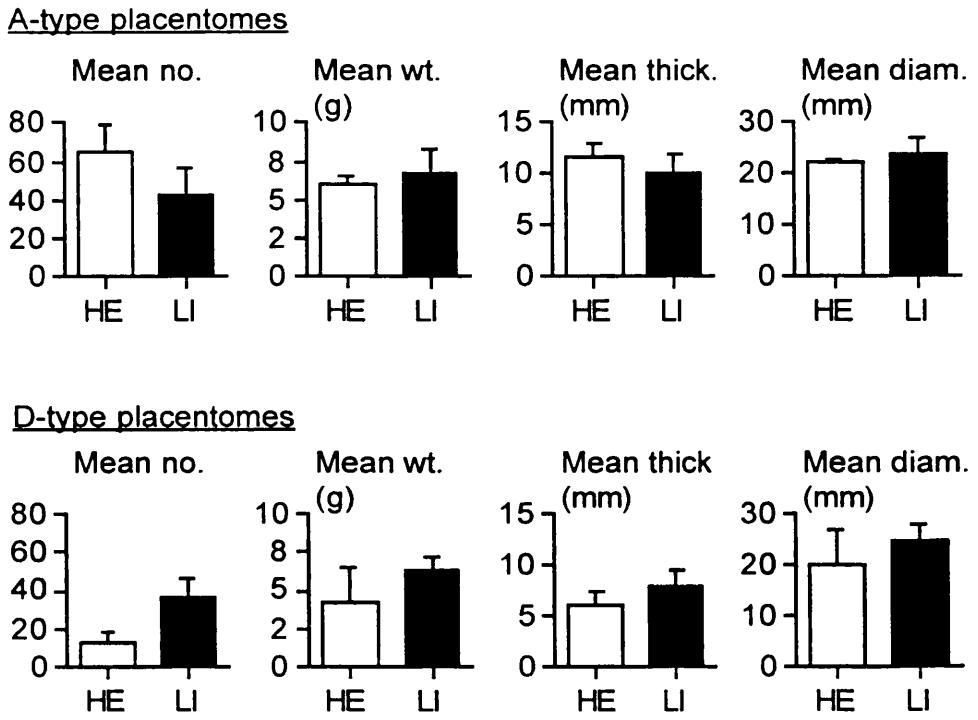

Figure 7.6. Mean number, weight, thickness and diameter of A- and D-type placentomes in HE and LI animals. Values are mean ± S.E.M.

It was interesting to find that total weight of A- and D-type placentomes showed a positive correlation with each other, and there were similar weak correlations for thickness ($P = 0.2$) and diameter ($P = 0.1$) (Fig. 7.7). So, as the total weight of A increased, that of D decreased.

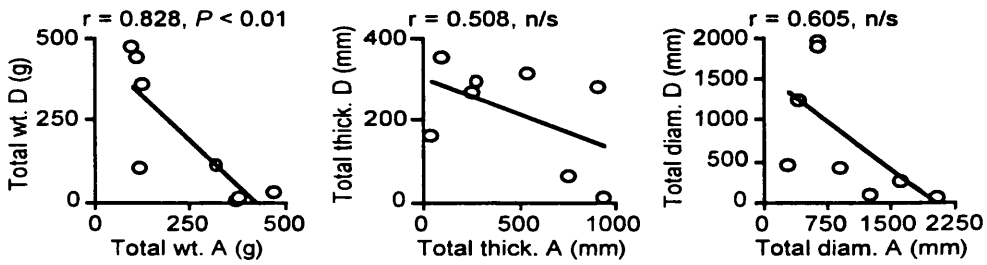

Fig. 7.7. Correlation of total weight, thickness and diameter of A- and D-type placentomes. Values are mean (S.E.M. Omitted for clarity).

7.3.2.ii Weight

Total placental weight was not significantly different between the two groups (Table 7.1).

Maternal influences on placental weight

Placental weight showed a significant positive correlation with maternal body weight at 0 d GA, but no correlation with the change in body weight between 0 d and 130 d GA, or with maternal body weight at 130 d GA (Fig. 7.8). There was no correlation of placental weight with BCS at 0 d or 130 d GA, or with the change in BCS (Fig. 7.8).

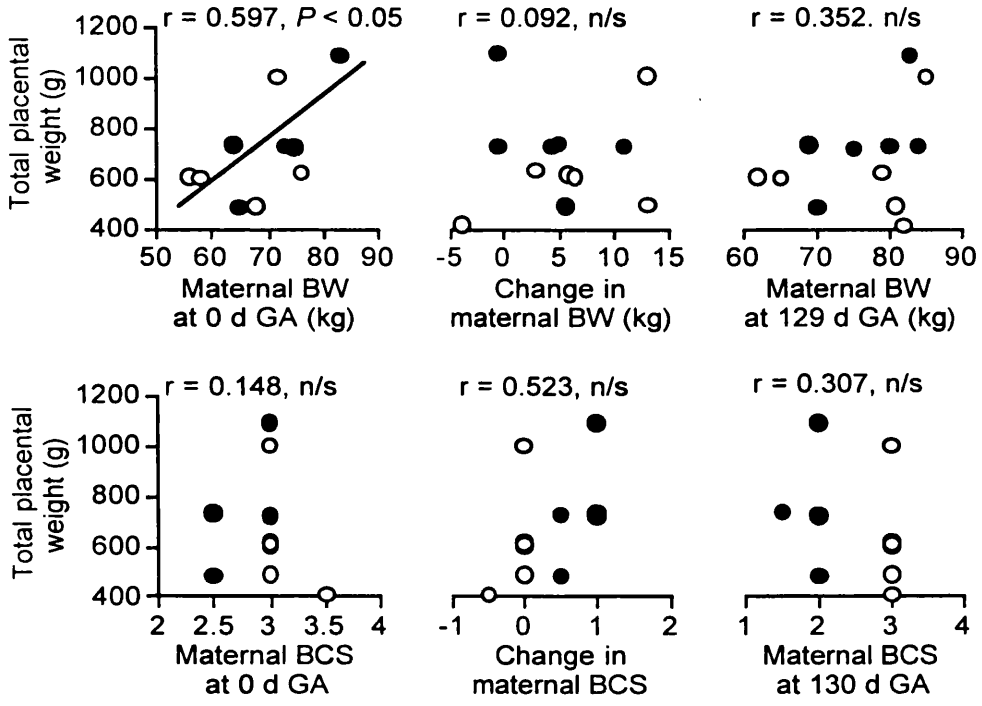

Figure 7.8. Correlation, in HE (o) and LI (●) sheep, of placental weight with maternal BW and maternal BCS at 0 d GA, 130 d GA, and with the change in maternal BW and BCS between 0 and 130 d GA. Values are mean (S.E.M. omitted for clarity).

Influence of placentome size and number on total placental weight

Placental weight did not correlate with the total number of placentomes, total placentome thickness, mean placentome thickness, or mean placentome diameter. It did, however, show a significant correlation with total placentome diameter (Fig. 7.9).

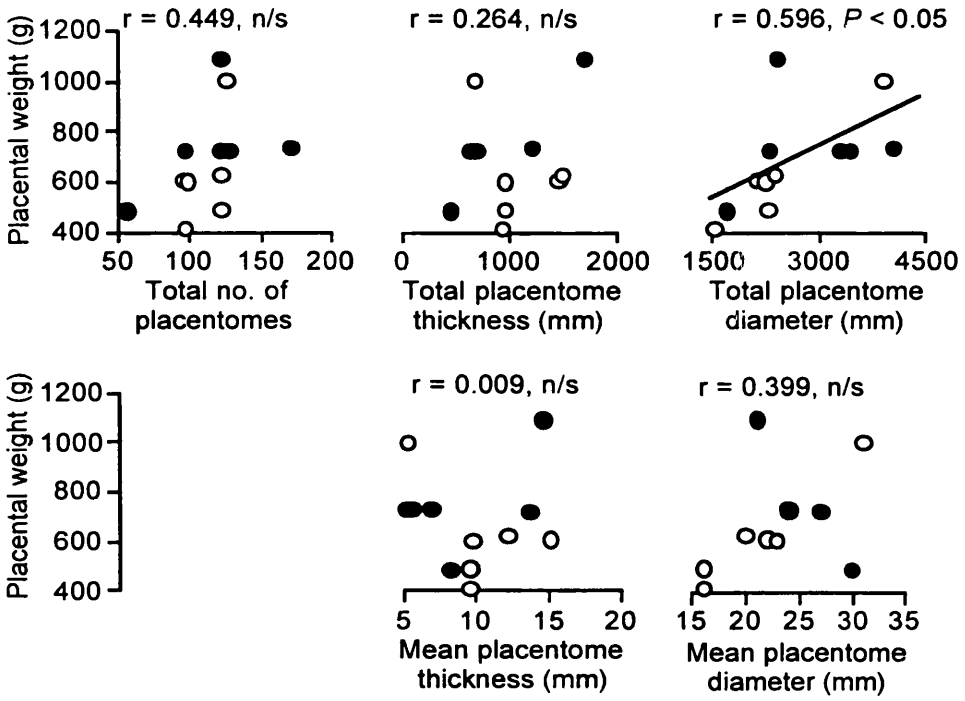

Fig. 7.9. Correlation of total placental weight in HE (o) and LI (●) placentae with total number, thickness and diameter of placentomes, and with mean placentome thickness and diameter. Values are mean (S.E.M. omitted for clarity).

Influence of A- and D-type placentomes on placental weight

Neither weight, thickness, nor diameter of either A- or D-type placentomes correlated with total placental weight (Fig. 7.10).

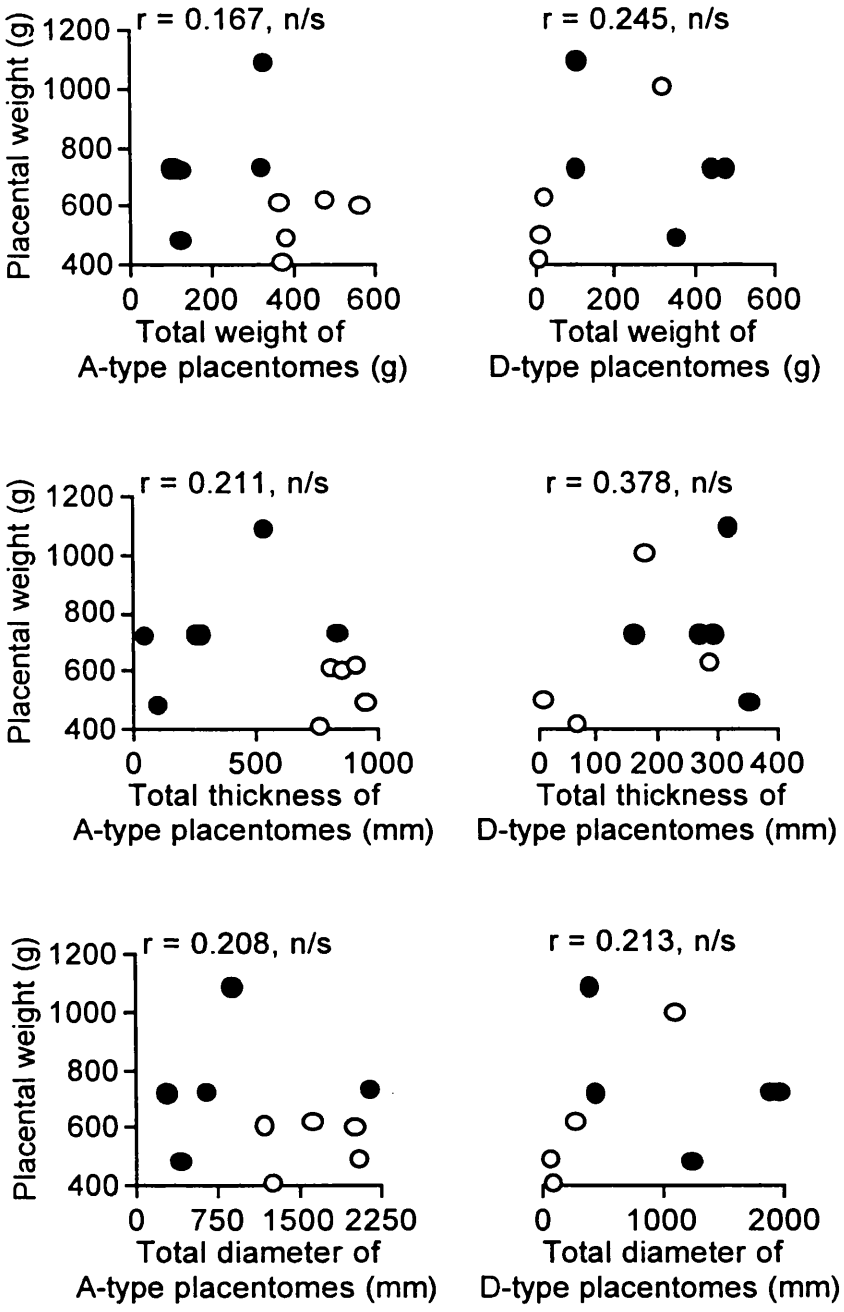

Figure 7.10. Correlation of total weight, thickness and diameter of A- and D-type placentae, in HE (o) and LI (●) sheep, with total placental weight. Values are mean (S.E.M. omitted for clarity).

7.3.3 Fetal size

7.3.3.i Weights and lengths

Body weights were similar for both groups, as were brain, heart, lung, liver, kidney, perirenal fat, pancreas, spleen, thymus and thyroid weights. The adrenals, however, were significantly larger in LI fetuses compared to HE (Table 7.1). Preservation of fetal growth was further evident by the fact that CRL, AC and femur length were not significantly different between the two groups of fetuses (Table 7.1).

	HE	LI
GA (days)	130 ± 0.9	129 ± 0.9
Fetal weights (g)		
Fetal body	3632 ± 170	3521 ± 117
Brain	45.0 ± 0.85	45.3 ± 0.73
Heart	24.3 ± 1.05	24.0 ± 0.88
Lung	97.8 ± 7.70	102.3 ± 4.03
Liver	90.2 ± 5.30	99.6 ± 4.05
Kidney	10.0 ± 0.49	10.0 ± 0.23
Adrenal	0.20 ± 0.02	0.24 ± 0.02 ^{x}
Perirenal fat	11.2 ± 1.04	12.3 ± 0.90
Pancreas	2.64 ± 0.27	3.08 ± 0.18
Spleen	4.67 ± 0.29	4.97 ± 0.22
Thymus	15.23 ± 2.16	16.26 ± 1.01
Thyroid	0.85 ± 0.10	0.93 ± 0.07
Lengths (cm)		
CRL	47 ± 0.9	49 ± 0.8
AC	35 ± 0.9	34 ± 1.1
Femur	10 ± 0.3	10 ± 0.3
Placental weight (g)	623 ± 83	746 ± 80

Table 7.1. GA, body and organ weights, and placental weight at 130 days gestation in HE (n=12) and LI (n=12) fetuses. ^{x} $P < 0.05$, HE vs. LI. Values are mean ± S.E.M.

7.3.3.ii *Factors relating to FBW*

Maternal weight

FBW at 130 d GA correlated positively with the change in maternal BW between 0 d and 130 d GA (Fig. 7.11). There was no correlation of FBW at 130 d GA with maternal BW or maternal BCS at either 0 d or 130 d GA, or with the change in BCS between 0 d and 130 d GA (Fig. 7.11).

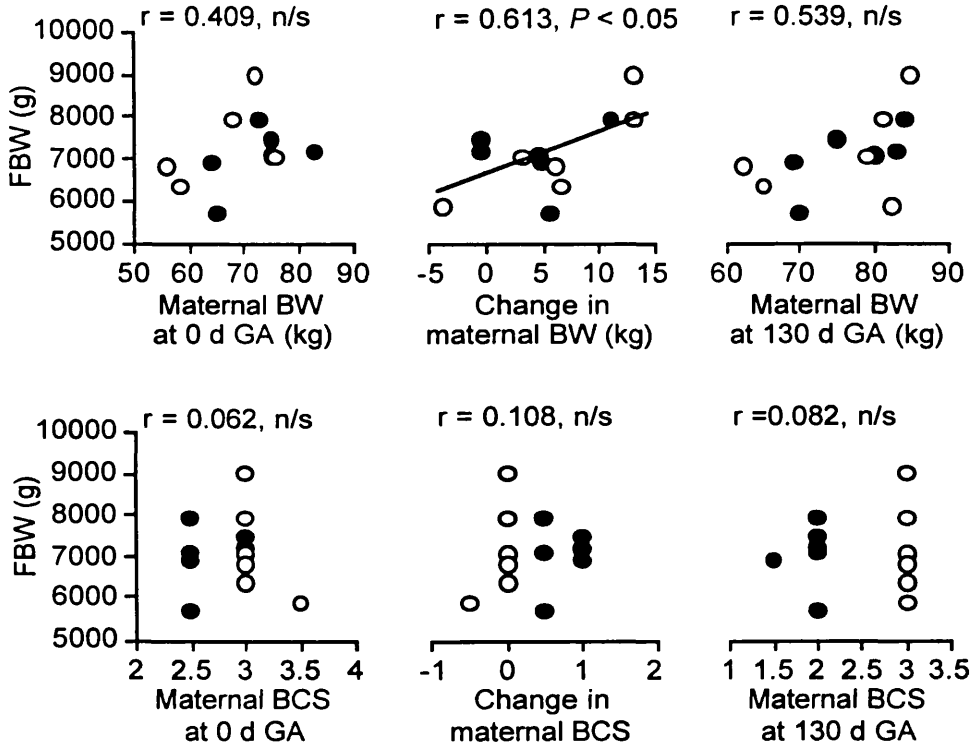

Figure 7.11. Correlation of FBW of HE (o) and LI (●) fetuses at 130 d GA with maternal BW and BCS at 0 d and 130 d GA, and with their change between 0 d and 130 d GA. Values are mean (S.E.M. omitted for clarity).

Placental weight, thickness and diameter

Both total placental weight and total placentome diameter showed a significant positive correlation with FBW at 130 d GA (Fig. 7.12). There was no

correlation of FBW at 130 d GA with total placentome thickness or with mean placentome weight, thickness and diameter (Fig. 7.12).

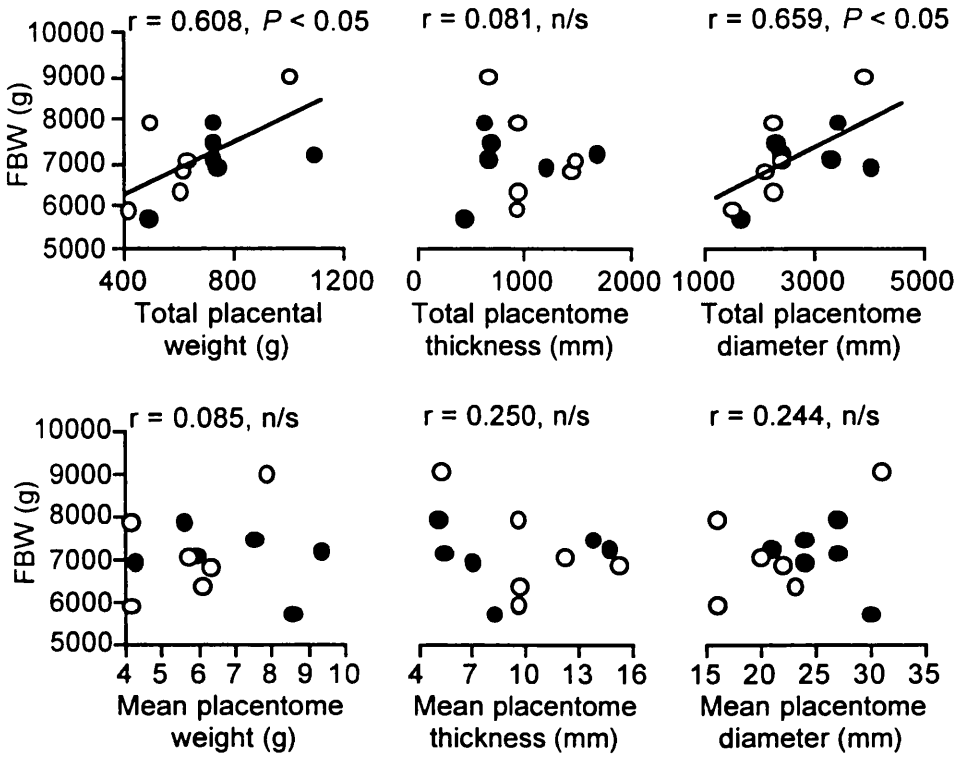

Figure 7.12. Correlation in FBW of HE (o) and LI (●) fetuses at 130 d GA with total and mean placentome weight, thickness and diameter at 130 d GA. Values are mean (S.E.M. omitted for clarity).

Placental morphology

FBW at 130 d GA showed no correlation with total number, weight, thickness and diameter of either A- or D-type placentomes (Fig. 7.13).

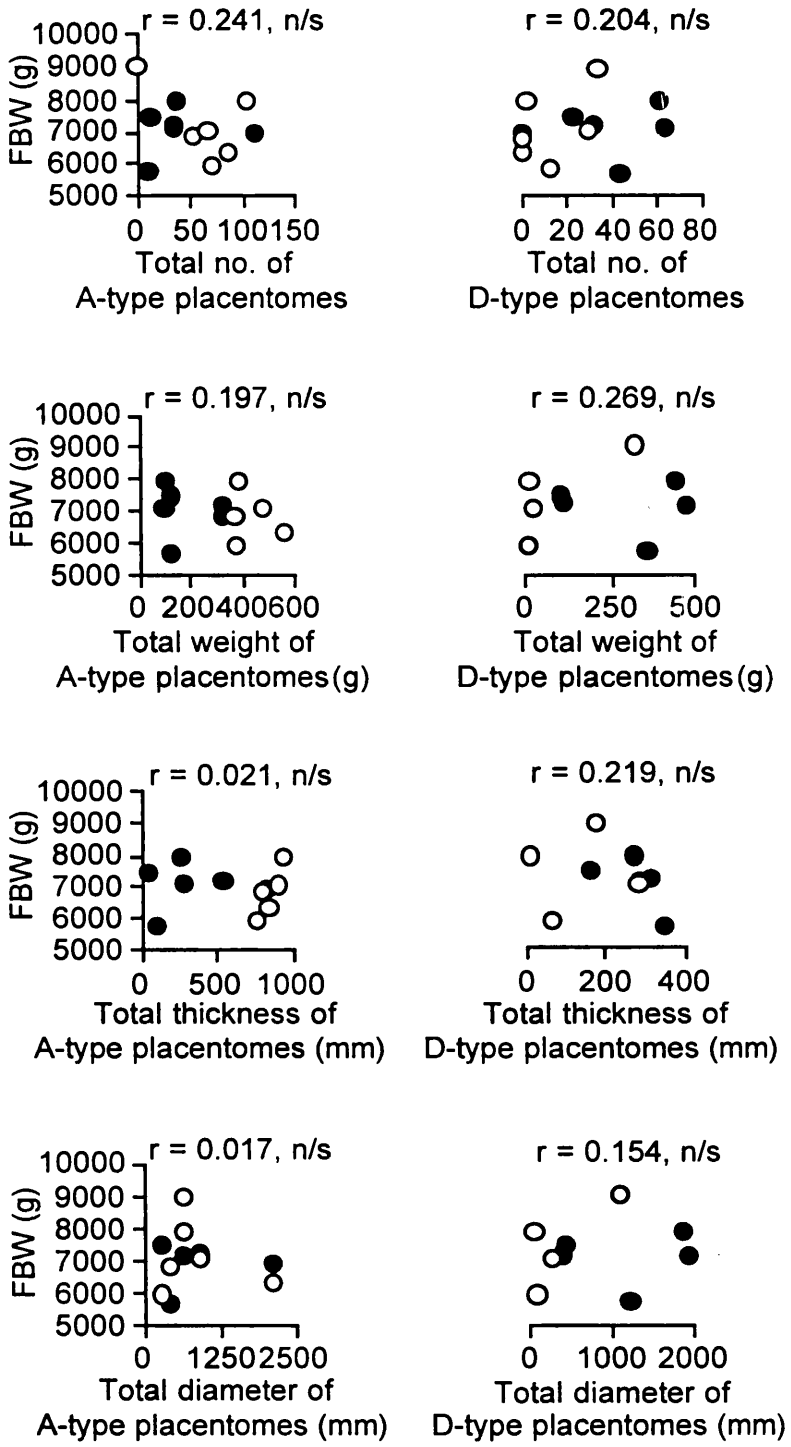

Fig. 7.13. Correlation of FBW at 130 d GA in HE (o) and LI (●) sheep with total number, weight, thickness and diameter of both A- and D-type placentomes. Values are mean (S.E.M. omitted for clarity).

Similarly, there was no correlation of FBW at 130 d GA with mean weight, thickness and diameter of A- and D-type placentomes (Fig. 7.14).

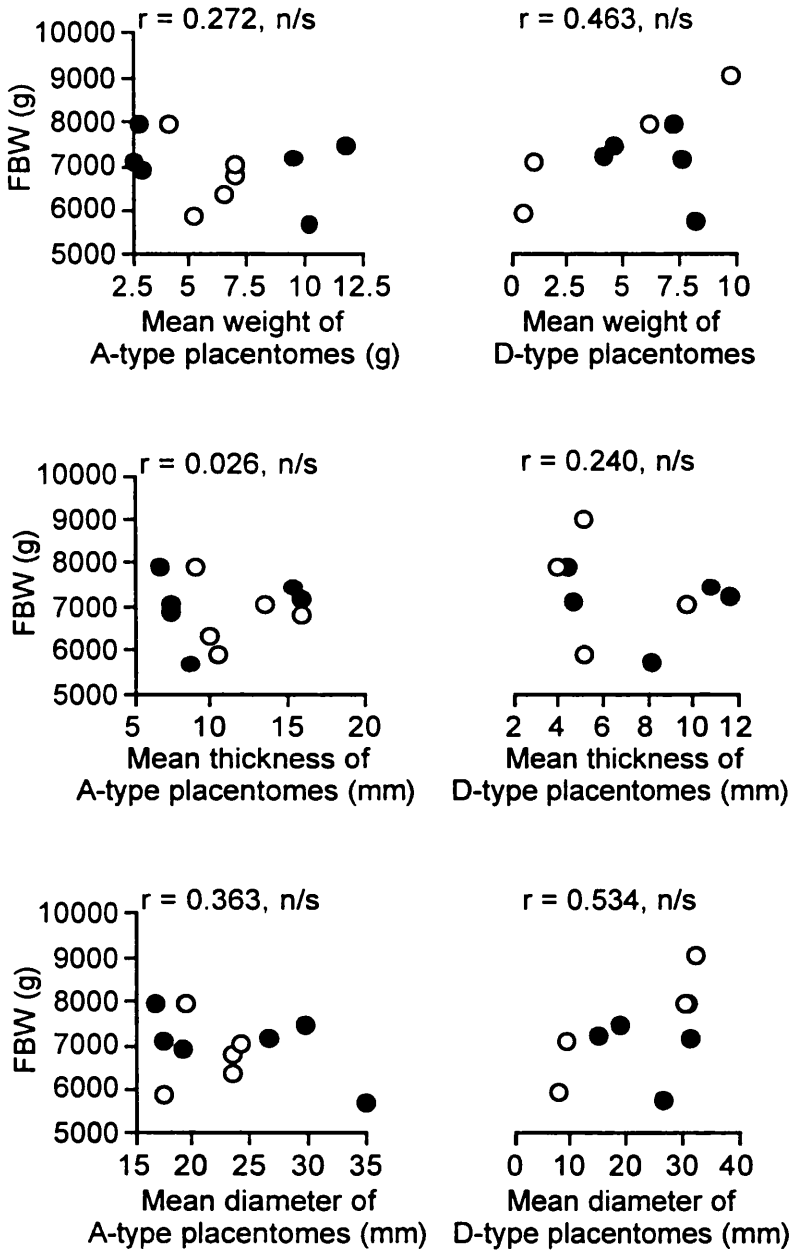

Figure 7.14. Correlation of FBW of HE (o) and LI (●) fetuses at 130 d GA with mean weight, thickness and diameter of A- and D-type placentomes. Values are mean (S.E.M. omitted for clarity).

7.4. DISCUSSION

7.4.1 Placenta

7.4.1.ii Morphology

Whole placenta

There was an extremely pronounced difference in the distribution of A, B, C, and D placentomes between HE and LI placentae (Fig. 7.4). Similar to my findings in the previous study (Fig. 6.17), there was a shift in distribution to the right i.e. towards more D-type placentomes in LI placentae. As I discussed in the last chapter (section 6.4.2.ii), this may reflect compensatory growth of the placenta, such as was suggested by Alexander (1964) and Vatnick *et al.* (1991), in response to the periconceptual nutritional challenge.

The effect of undernutrition on A- and D-type placentomes

Undernutrition early in pregnancy produced significant differences between HE and LI placentae in terms of various characteristics of A-type placentomes, but no significant effect on D-type placentomes (Fig. 7.5). The total number of A-type placentomes was not significantly different between LI and HE animals, but total weight, thickness and diameter were decreased in LI placentae. The suggestion is, therefore, that A-type placentomes are more susceptible than D-type placentomes to undernutrition of a moderate degree at conception and during early pregnancy. However, the placenta is an extremely plastic organ, as is demonstrated by the inverse relationship between size of A- and D-type placentomes (Fig. 7.7). Thus, it seems that a decrease in size of A-type placentomes results in an increase in size of D-type placentomes. So, overall placental weight does not change, but morphology, and perhaps function, does. This would explain why total placental weight was not significantly different between the two groups.

7.4.1.ii Total weight

Influence of maternal BW and BCS

LI and HE placentae were not significantly different from each other in terms of weight (Table 7.1), despite the significant loss of weight and BCS in LI ewes during early pregnancy (Fig. 7.2). This may be because the nutritional challenge, which caused the loss of weight in LI ewes, was not severe

enough to affect placental growth and metabolism. Alternatively, it may be that the nutritional challenge did have an affect on placental growth and function, that was not reflected in overall placental weight. Indeed, differences in morphology (discussed below) suggest that this was the case.

Placental weight showed a significant positive correlation with maternal BW at 0 d GA (Fig. 7.8), consistent with my findings in the previous chapter (Fig. 6.16). Previously (Fig. 6.16) I found that there was an inverse relationship between placental weight and the change in maternal BW over the course of pregnancy. But, in the study described here, I found no correlation at all. The changes in maternal BW observed in this study varied between a 4 kg loss and 13 kg gain (Fig. 7.8), whereas in the previous study maternal BW varied between a 5 kg loss and a 23 kg gain in weight (Fig. 6.16). It may be that only substantially large increases or decreases in maternal weight influence placental weight. Maternal body weight at 130 d GA did not correlate with placental weight, which is the same as my finding in the previous study (Fig. 6.16). Placental growth and differentiation are most rapid early on in gestation (Owens *et al.*, 1995), and, in the sheep, the definitive placenta is formed by 70-90 d GA (Wooding & Flint, 1994). Therefore, consistent with my findings, one would not expect events after about 70 d GA to have as great an effect on placental weight as events occurring before that time. Placental weight showed no correlation with maternal BCS, which is perhaps surprising, as BCS is an assessment of fatness and thus body reserves. However, that said, only maternal BW at mating correlated with placental weight, so perhaps one would expect only BCS at mating (and not the change in BCS and that at 129 d GA) to correlate wth placental weight. All the ewes used in this study were the same breed and were of similar frame size, so one would expect that as weight increased so too would BCS. However, this was not found to be the case (Fig. 7.3). In this study, therefore, BCS may not have been as sensitive a measure of fatness and body reserves as maternal BW. Therefore, it is perhaps not surprising that BCS did not correlate with placental weight at all.

Influence of placentome characteristics

Number and thickness of placentomes did not influence total placental weight, but there was a positive correlation with total placentome diameter (Fig. 7.9), similar to my findings in the previous study (Fig. 6.15). Presumably, therefore, increasing placentome size, as a result of increased diameter,

represents growth of the densest placental tissue. Whereas, increased growth due to increased placentome thickness, is the result of growth of less dense tissue. I am not aware of any studies that have differentiated the densities of the various tissues within the placenta, therefore it is not possible to say which type of tissue grows most with an increase in diameter and which with an increase in thickness.

Influence of morphology

Above I mentioned the plasticity of the placenta, and showed that increasing size of D-type placentomes is accompanied by decreasing size of A-type placentomes (Fig. 7.7).

This finding suggests a preservation of total placental weight, despite a shift in the balance of A- and D-type placentomes. This is borne out by the finding that total placental weight does not correlate with weight, thickness or diameter of A- or D-type placentomes (Fig. 7.10). Other investigators (Alexander, 1964; Jansson *et al.*, 1986) have shown that size of the placenta is a limiting factor for fetal growth, particularly in late gestation. It makes sense, therefore for the placenta to adapt to stress by maintaining capacity but improving function, which is what may have occurred in my LI sheep, if D-type placentomes are more efficient than A-type placentomes at transfer of nutrients to the fetus. There is, to my knowledge, no literature on the comparative function of the different morphological types of ovine placentome. However, we are at present in our laboratory investigating the differences in villi density between A- and D-type placentomes.

7.4.2 Fetus

7.4.2.i Growth

Fetal body weight at post-mortem was not significantly different between groups at 130 d GA (Table 7.1). Likewise, CRL, AC and femur length were similar for both groups. and organ weights were also similar, except for the adrenals which were significantly larger in LI fetuses. Therefore, despite a periconceptual nutritional challenge, it is clear that fetal growth was preserved in LI fetuses. This preservation of growth may have been the result of successful adaptation by the placenta to the nutritional challenge, as suggested above.

Interaction with maternal nutrition

FBW correlated with the change in maternal BW between 0 d and 130 d GA, but there was no relationship with maternal weight at 0 d GA or 130 d GA (Fig. 7.11), contrary to my previous findings (Fig. 6.13) and those of Rosso *et al.* (1992) in the human. This difference may be influenced by the fact that only twin-bearing ewes were used in this study, whereas my previous study and that of Rosso *et al.* (1992) studied only singleton pregnancies. There is evidence to suggest that uterine space affects growth *in utero* (Vallet & Christenson, 1993). A crowded uterine environment was seen to result in reduced fetal weight by as early as 25 d GA in pigs (term = 115 d). Therefore, it is possible that, in the twin pregnancies the presence of another conceptus was of greater influence on fetal growth than maternal body reserves during early gestation, particularly as the maternal nutritional insult was only moderate. This is supported by the review of Cogswell & Yip (1995) on fetal and maternal factors that influence the distribution of birthweight. It can be seen in that report that low pre-pregnancy BMI has a less profound effect on birthweight than increasing plurality. It is therefore perhaps not surprising that in this study with twins, FBW was influenced less by maternal BW at mating than in my study with singletons (previous chapter). The association that I found of FBW with the change in maternal BW during pregnancy (Fig. 7.11) was most probably due, in part at least, to increasing weight due to the increasing weight of the fetus itself. This effect will obviously be greater in twin pregnancies than singleton. Thus the observed association in this study, but not in the one previous to this. In the literature, the association of maternal weight gain during pregnancy with birthweight seems to be a matter of disagreement. Cogswell & Yip (1995) maintain that there is an association, but Rosso's (1992) findings do not support this view. The results of other studies (Prentice *et al.*, 1983; Abrams & Laros; 1986; Cogswell *et al.*, 1995) would seem to indicate that significant maternal weight gain during pregnancy improves birthweight in underweight women, but that there is no consistent beneficial effect in women who are not underweight when they conceive.

Interaction with placental growth

As discussed in section 7.4.1.ii, there was an association of maternal weight at conception with placental weight (Fig. 7.8). It is interesting, therefore, that I also found that total placental weight correlated positively with FBW (Fig.

7.12). Thus, whilst maternal nutritional status at mating had no direct effect on fetal growth, there is obviously an association between maternal weight at conception and fetal growth that is mediated by the placenta. In agreement with my previous study (Fig. 6.14), placental diameter as well as weight, but not thickness, showed a positive correlation with FBW (Fig. 7.12).

The fact that placental morphology was significantly altered in response to the nutritional challenge (Fig. 7.4) and the fact that fetal growth was preserved (Table 7.1), suggests that the altered placental morphology may have been a mechanism responsible for the preservation of fetal growth. But, there was not any correlation of number, weight, thickness or diameter of either A- or D-type placentomes with FBW (Figs. 7.13 & 7.14). Thus, there must have been structural and/or functional alterations in the placentomes that allowed for normal growth of LI fetuses.

It has been reported (Cheung & Brace, 1996) that ovine placental VEGF mRNA levels increase with increasing gestational age. In normal ovine pregnancies there is also an increase in D-type placentomes in late gestation. It is therefore possible that the increased VEGF expression correlates with number and/or mass of D-type placentomes. If this is the case, VEGF expression may have been increased in LI placentae, thus resulting in increased angiogenesis (Wheeler *et al.*, 1995; Cheung & Brace, 1996) and permeability (Cheung & Brace, 1996), so maintaining nutrient delivery to the fetus despite maternal undernutrition.

It is possible that there is differential expression between A- and D-type placentomes in the release of other substances such as the IGFs, growth hormone variant (GHV), corticotrophin releasing hormone (CRH), lactogen hCG and NO, all of which influence fetal growth.

7.4.2.ii Cardiovascular development

Unfortunately I did not have any measurements relating to the cardiovascular function of the fetuses in this study. However, the adrenals of LI fetuses were larger than those of HE (Table 7.1) which may reflect increased catecholamine and/or cortisol release. Gagnon *et al.* (1994) observed larger adrenals in their fetuses that also had increased levels of plasma noradrenaline and cortisol. This raises the possibility that vascular tone in LI fetuses may have been elevated, which would have resulted in altered blood pressure development.

Endogenous production of cortisol and catecholamines

CRH and AVP released from the hypothalamus stimulate the pituitary corticotrophs to produce ACTH. Within the corticotrophs in the pars distalis of the pituitary, CRH and AVP stimulate the synthesis of POMC mRNA which is translated into POMC. POMC is then cleaved to produce ACTH and a number of other biologically active peptides (Matthews & Challis, 1995). ACTH stimulates the adrenal cortex to produce cortisol. Hypoxaemia (Jackson *et al.*, 1989; Giussani *et al.*, 1994b), decreased blood volume (Rose *et al.*, 1978), and 'stress' cause an increase in the endogenous secretion of cortisol. The fact that fetal growth was preserved in LI fetuses, suggests that they were not hypoxaemic. If they were hypovolemic one may expect that they would also have been lighter, which they were not. Therefore, it is unlikely that there was any elevation of cortisol levels in LI fetuses due to hypoxaemia or hypovolemia. It has also been suggested, however, that afferent chemo- and baroreceptor activity may reflexly control the release of cortisol (Giussani *et al.*, 1994b). It may be that if the chemo- and baroreflexes of LI fetuses were altered, such as was the case in the LI fetuses in chapter 6, basal cortisol levels may also have been affected.

Noradrenaline is synthesised in the adrenal medulla from dopamine in a reaction catalysed by dopamine β-hydroxylase. The enzyme phenylethanolamine-N-methyltransferase, which is specifically induced by cortisol, then converts noradrenaline to adrenaline. Fetal adrenomedullary cells are stimulated directly by hypoxaemia to release catecholamines (Cheung, 1989) and there is also an increase in plasma noradrenaline levels in response to hypotension (Rawashdeh *et al.*, 1988). It is possible, in light of my previous findings presented in this thesis, that cardiovascular development of LI fetuses was not normal and they could have been hypotensive. If this was the case it could have resulted in increased catecholamine output.

Exogenous cortisol

Recently the idea has been put forward that dysfunction of the placental glucocorticoid barrier leads to increased exposure of the fetus to exogenous glucocorticoids, which may lead to low birth weight and subsequent hypertension in the offspring (Edwards *et al.*, 1993). Edwards *et al.* (1993) hypothesised that reduced placental 11β-HSD activity would result in such

changes. The same group demonstrated in rats that 11β-HSD activity showed a positive correlation with fetal weight and a negative correlation with placental weight, i.e. rats with a high placental /birth weight ratio had lower placental 11β-HSD activity (Benediktsson *et al.*, 1993). They suggested that these rats may be predisposed to hypertension in adulthood, since Barker *et al.* (1990) had shown an association of hypertension in later life with high placental /birth weight ratio. More recently in support of this idea they have shown that blood pressure was significantly higher in offspring whose mothers were treated with carbenoxolone, a potent inhibitor of 11β -HSD 2 (Lindsay *et al.*, 1996). In my study, although total placental weight was not different between LI and HE fetuses (Table 7.1) there was a significant decrease in the size of A-type placentomes in LI placentae (Fig. 7.5). It may be that this decrease in A-type placentomes resulted in a decrease in 11β-HSD activity. If so, we may speculate that LI fetuses were exposed to higher concentrations of maternal cortisol than HE fetuses, which may have altered their cardiovascular development and function.

It has been shown *in vitro* that cytotrophoblasts incubated for 4 hours show no detectable 11β-HSD activity (Shepherd, *et al.*, 1996). However, when allowed to incubate for up to 3 days, by which time the cytotrophoblasts had formed syncytia, 11β-HSD activity was observed (Shepherd & McGarrigle, personal communication). This suggests that 11β-HSD activity is confined to the syncytiotrophoblasts, which indeed seems to be the case in the human placenta (Krozowski *et al.*, 1995). Thus, a decrease in syncytiotrophoblast area may result in decreased 11β-HSD activity. The site of 11β-HSD activity has not been localised in the ovine placenta but, on the basis of the human data, it may be that it is situated in the trophectoderm. If this were the case, decreased area of trophectoderm could result in decreased 11β-HSD activity. Maybe the reduced size of A-type placentomes resulted in a decrease in trophectoderm area.

Mechanisms of blood pressure programming by glucocorticoids

There are several mechanisms whereby increased glucocorticoids in the fetus may result in altered blood pressure development. These include altered HPA axis function, changes in the pattern of α and β adrenoceptor expression in various organs, and apoptosis.

1) *Altered HPA function.* Cortisol has a negative feedback effect on ACTH secretion and is also associated with a decrease in POMC mRNA levels (Matthews *et al.*, 1995). During late gestation there is a rise in cortisol concentration towards term (Rose *et al.*, 1978; Tangalakis *et al.*, 1992; Matthews *et al.*, 1995), despite the negative feedback effects of cortisol on ACTH. This suggests that maturation of the axis may involve increased hypothalamic drive to the pituitary so that negative feedback control is overcome, altered to a new set point, or no longer active (Matthews *et al.*, 1995). Thus, chronically elevated cortisol levels, whether endogenous or exogenous in origin, may cause resetting of the axis, similar to that observed in late gestation, which could result in perturbed blood pressure development. However, there is also an increase in corticosteroid-binding globulin (CBG) during late gestation (Ballard *et al.*, 1982) which, it has been suggested, may decrease the levels of free cortisol in the circulation, thus modifying the feedback of cortisol to the pituitary (Berdusco *et al.*, 1995).

2) *Apoptosis.* Oka *et al.* (1993) proposed a mechanism whereby hypertension may result. They suggested that elevated fetal glucocorticoid levels may cause apoptosis of cells in the hippocampus. Mineralocorticoid and glucocorticoid receptors are expressed in hippocampal cells and are involved in glucocorticoid feedback inhibition of the HPA axis. Thus, as well as having effects on mood, behaviour, memory and other cognitive functions, glucocorticoids in the hippocampus also modulate neuroendocrine function (Yau *et al.*, 1995). Receptor loss, as a consequence of apoptosis, as proposed by Oka *et al.* (1993), would cause desensitisation to cortisol feedback resulting in cortisol hypersecretion and hypertension. Old rats with failure of negative feedback control are often found to have elevated glucocorticoid levels (Yau *et al.*, 1995), and increased apoptosis has been observed in the cortex, striatum, hippocampus and thalamus of hypertensive mice (Hamet *et al.*, 1995).

3) *Vascular tone.* If cortisol levels were elevated in LI fetuses, vascular tone may have increased, which would of course have implications for blood pressure development. In their review on the role of corticosteroids in vascular tone, Walker & Williams (1992) suggested various potential biochemical signals in the vascular wall that may be affected by glucocorticoids. These included angiotensin-converting enzyme (ACE), phospholipase A_2, α- and β-adrenoceptor agonists, PGE_1, adenylate cyclase,

ANP, guanylate cyclase, inducible NO synthase, and cell surface receptors. It is interesting, therefore, that increased cortisol levels in the immature (103-120 d GA) ovine fetus cause an increase in blood pressure, which, it was suggested is due to enhanced vascular sensitivity to AII (Tangalakis *et al.*, 1992). Also, low-dose dexamethasone in prenatal rats promotes the ability of β-adrenoceptors to stimulate adenylate cyclase (Bian *et al.*, 1992), which could result in altered vascular responsivity to vasoconstrictors. The development of cholinergic projections in the developing neonatal rat brain is also enhanced by fetal (17-19 d GA, term =21d) dexamethasone treatment (Zahalka *et al.*, 1993).

7.5 CONCLUSIONS

1) Consistent with the results of Chapter 6, maternal undernutrition at conception and during early pregnancy produces a significant change in placental morphology in the sheep.

2) The change in placental morphology is not accompanied by a significant increase in placental weight, but there is a significant decrease in the size of A-type placentomes and a consistent tendency for D-type placentomes to be larger.

3) These alterations in placental morphology are successful in maintaining fetal growth, through mechanisms that are unknown.

4) In the light of previous findings, it is possible that, although fetal growth is preserved, cardiovascular development may not be normal.

Chapter 8

FINAL DISCUSSION

8.1 OVERVIEW OF THESIS

The work described in this thesis had two main aims. The first was to determine whether the findings described by Barker (1994) could be reproduced in an animal model. The second was to investigate the mechanisms involved in the nutritional effects on the development of blood pressure in the fetus, so as to understand how hypertension in adult life may result from events *in utero.*

The main points to emerge from the work described are as follows:

Chapter 3

Severe maternal nutritional iron-deficiency anaemia results in hypertension of the offspring. However, adult hypertension is preceded by hypotension during neonatal life, which highlights the importance of investigating the development of blood pressure rather than just taking point measurements. There is an association between growth and blood pressure development in the neonate, but the mechanisms linking the two are obscure. Cardiovascular development during fetal life is probably important for future outcome, and fetal hypoxaemia is proposed as an important factor influencing fetal growth and blood pressure development. Thus, the results of this study suggested that fetal hypoxaemia may be an important factor in the aetiology of adult hypertension.

Chapter 4

Despite the apparent involvement of hypoxaemia in blood pressure development, repeated acute isocapnic hypoxaemia of a moderate degree during late gestation does not affect blood pressure development in the fetal sheep.

Chapter 5

Blood pressure development of individual fetuses during late gestation may follow different trajectories. Not all fetuses show the expected increase in MAP during late gestation. As in the neonate, the pattern of blood pressure development and growth are also associated in the fetus. Abnormal blood pressure development is accompanied by perturbed cardiovascular reflex

development. Fetal hypoxaemia again appears to be a possible candidate for the cause of disturbed blood pressure development. The reason for the hypoxaemia is unknown, however it is suggested that it may be the result of placental insufficiency.

Chapter 6

Periconceptual maternal undernutrition has a significant effect on placental morphology, but not on placental weight, and may also result in reduced fetal growth. Blood pressure and cardiovascular reflex development are also affected during late gestation, but there is no associated hypoxaemia. It therefore appears that fetal growth and blood pressure development in late gestation are affected by the development of the placenta.

Chapter 7

In the sheep, the placental morphological alterations that occur as a result of periconceptual undernutrition are a decrease in size and number of A-type placentomes and an increase in size and number of D-type placentomes. This change in placental morphology seems to be an adaptation to periconceptual undernutrition that is successful in preserving fetal body growth. However, blood pressure development may be disturbed, possibly as a result.

Summary

Thus, in summary, maternal nutritional anaemia during pregnancy is associated with reduced fetal and neonatal growth, and hypertension in later life. This may relate to blood pressure development during fetal life, thus it is interesting that MAP development in individual fetuses may follow distinct trajectories during late gestation. Also, abnormal blood pressure development is accompanied by alterations in cardiovascular reflex development. Chronic hypoxaemia during fetal life may be an important factor in the aetiology of adult hypertension, however, repeated acute hypoxaemia for 2 weeks during late gestation does not affect fetal cardiovascular development and periconceptual undernutrition does not result in hypoxaemia during late gestation. Periconceptual undernutrition does, however, perturb fetal cardiovascular development during late gestation, an effect that seems to be the result of alterations in placental function.

These results demonstrate that maternal nutritional status at conception and during early pregnancy has important implications for the future cardiovascular development of the conceptus.

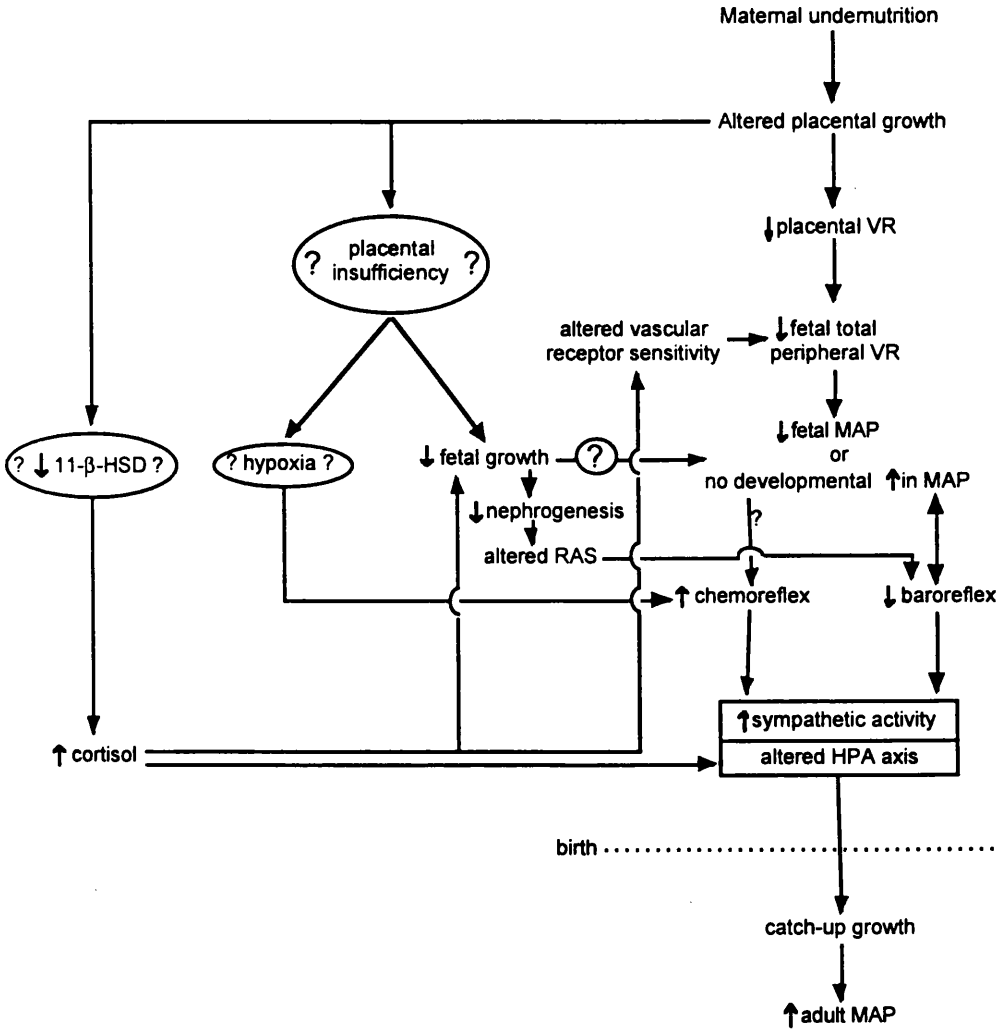

Figure 8.1. Flow diagram to show the possible mechanisms involved in the development of hypertension in adult life as a result of undernutrition during the periconceptual period.

8.2 QUESTIONS

Inevitably, there are a number of questions that arise as a result of the findings described in this thesis.

Is hypoxaemia a cause of altered cardiovascular development in response to maternal undernutrition or not?

My initial findings (Chapters 3 and 5) suggested that hypoxaemia may be involved in abnormal blood pressure development. However, my subsequent findings (Chapter 6) did not implicate hypoxaemia as a cause for perturbed cardiovascular development. Therefore, in cases of maternal undernutrition during early pregnancy, the role of hypoxaemia in blood pressure development during late gestation is questionable.

What is the mechanism for the altered chemoreflex development?

Chemoreflex development was perturbed as a result of periconceptual undernutrition, however it was not accompanied by hypoxaemia, hypercapnia or acidosis. Thus, if resetting occurred at the level of the peripheral chemoreceptors, the mechanism remains to be elucidated. It is possible that there was also resetting of central chemoreceptors, perhaps as a result of altered cerebral metabolism and blood flows. Increased cerebral blood flow would result in CO_2 washout. But, one would expect to see a degree of brain sparing if cerebral blood had increased, therefore CO_2 washout is an unlikely mechanism. Alternatively, blood flow may have decreased resulting in a build up of CO_2. Again, this seems unlikely to have occurred in this instance, as there was no resultant decrease in brain growth. The only other factor that could affect chemoreflex activity is blood pressure. The possibility arises, therefore, that abnormal blood pressure development was responsible for the altered chemoreflex development. Nonetheless, whatever the mechanism, an increase in chemoreflex gain will result in an increase in arterial pressure during hypoxaemia, e.g. at birth.

Baroreflex and blood pressure development. Cause and effect.

The results presented in this thesis do not distinguish between cause and effect with respect to blood pressure and baroreflex development. Was the

failure for baroreflex resetting to occur caused by the failure for blood pressure to increase, or did abnormal baroreflex development result in the abnormal blood pressure development that was observed? The gain of the baroreflex was low in those fetuses that did not show normal blood pressure development, despite the fact that their blood pressures failed to increase with advancing gestational age. This suggests that, during late gestation, reduced baroreceptor sensitivity was the 'cause', and the development of pressure the 'effect'. Such a decrease in baroreflex gain will fail to protect against any increase in arterial pressure.

An intriguing question is, what happened earlier in gestation? There is the possibility that there was a rise in blood pressure early on in undernourished animals, resulting in baroreceptor resetting, i.e. undernourished animals may have had accelerated blood pressure development.

Is there a mechanistic link between growth and blood pressure development?

Certainly blood pressure and growth are linked. However, whether they are causally linked or not is not clear from the results presented in this thesis. The observation that growth and blood pressure are linked is not a new one, however the link between them is not understood.

Histologically and histochemically, how does maternal undernutrition affect the placenta?

I have shown that periconceptual undernutrition results in gross morphological changes of the placenta. However, the question remains as to what these morphological changes mean functionally. Is there an increase in the surface area for exchange of nutrients between mother and fetus, e.g. increased villous growth? Or, perhaps the capillary walls within the terminal villi become thinner to allow more efficient exchange of substances. Alternatively, or perhaps, as well, it is possible that there are alterations in the expression of various growth factors and their receptors, e.g. the IGFs, VEGF. Maybe levels of 11-β-HSD are altered, which, as discussed in Chapter 7, would lead to altered levels of cortisol transfer between mother and fetus.

8.3 FUTURE WORK

As a result of the questions posed above there are various areas of further investigation that need to be pursued.

Longitudinal study

It has became increasingly clear that it is necessary to carry out a longitudinal study investigating the effects of a periconceptual nutritional challenge, by looking at not only fetal cardiovascular development, but cardiovascular development during the neonatal period and through into adult life. It is clear from the results presented in this thesis that, in order for us to understand the mechanisms underlying the development of hypertension in adult life, we have to follow the development of an individual. Measurements taken at discrete times during development do not give us enough information to understand the possible factors involved.

Effect of added stress

Further work also needs to be done to investigate whether or not nutritionally deprived fetuses represent a high-risk group that are more susceptible to stresses encountered later in life. Fetal hypoxaemia that occurs during labour may prove a life-threatening event to fetuses that have abnormal chemoreflex development. This may be investigated by imposing a hypoxaemic insult on fetuses during late gestation by methods such as uterine artery occlusion or placental embolization.

The adolescent growth spurt may also be regarded as a stress. It is possible that adolescents who were undernourished *in utero* have an exaggerated increase in blood pressure during this period of development. Because the mechanisms involved linking growth and blood pressure are not understood, it is difficult to know exactly how to investigate the stress of growth. But, perhaps it could be studied by infusing with growth hormone (GH) to cause a growth spurt.

Vascular responses

The suggestion that peripheral resistance is altered warrants investigation of vessel responses to various agonists, firstly to establish whether vascular reactivity is actually altered, and secondly to identify the vasoconstrictors

and/or vasodilators that may be involved. Such investigations can be carried out *in vitro* using a small vessel myograph.

Placenta

More in-depth studies of the placenta are necessary. Histological analysis needs to be carried out to investigate the pattern and degree of villous and capillary growth in response to periconceptual undernutrition. The expression of IGFs, IGF-Rs and 11-β-HSD in the placentae of undernourished ewes also needs to be investigated, so that we have a better idea as to what the morphological changes that we see mean from a functional point of view.

HPA axis

Finally, because cortisol is strongly implicated in the perturbations of both growth and blood pressure that result from periconceptual undernutrition, it is necessary to study the development of the HPA axis. In particular, we need to investigate whether changes in fetal plasma cortisol alter the programming of the development of the HPA axis, including the sensitivity of its feedback control mechanisms.

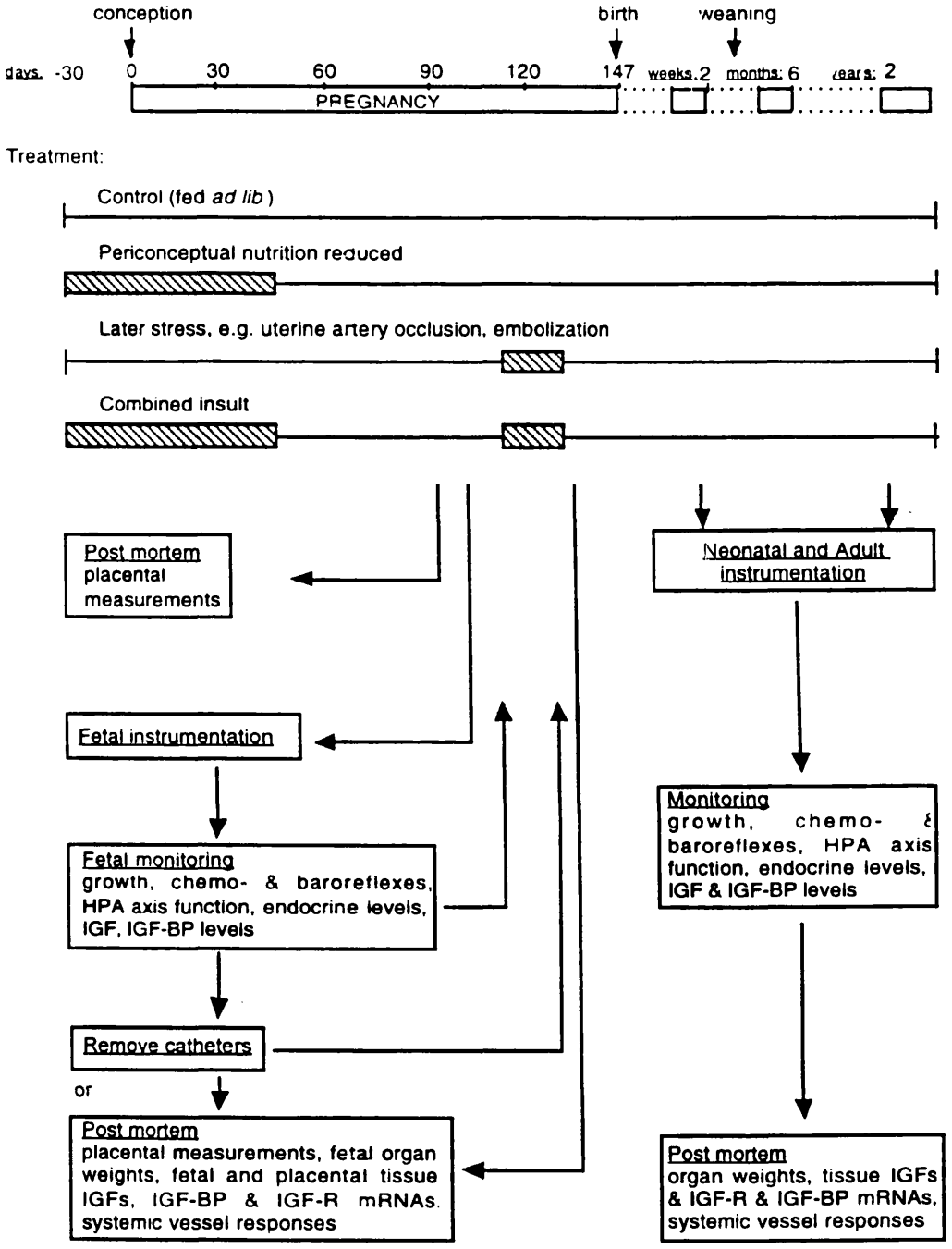

Figure 8.2. Illustration of the experimental design whereby the above mentioned work may be carried out. (This represents the plan of future work in our laboratory).

8.4 HOW HAS THE WORK PRESENTED IN THIS THESIS CONTRIBUTED TO THE FIELD OF FETAL PHYSIOLOGY?

The contributions of the work described in this thesis are as follows:

1) It adds further support to the idea that events during fetal life affect cardiovascular development later in life.

2) It has established that the sheep is a good model for studying the effects of maternal undernutrition on cardiovascular development.

3) It has led to further questions regarding the mechanisms involved, and has thus provided a number of avenues of further research.

REFERENCES

ABRAMS, B.F. & LAROS, Jr., R.K. (1986). Prepregnancy weight, weight gain, and birth weight. *American Journal of Obstetrics & Gynecology* **154**, 503-509.

AHOKAS, R.A., REYNOLDS, S.L., ANDERSON, G.D. & LIPSHITZ, J. (1984). Maternal organ distribution of cardiac output in diet-restricted pregnant rats. *Journal of Nutrition* **114**, 2262-2268.

AKAGI, K., BERDUSCO, E.T.M. & CHALLIS, J.R.G. (1990). Cortisol inhibits ACTH but not the AVP response to hypoxaemia in fetal lambs at days 123-128 of gestation. *Journal of Developmental Physiology* **14**, 319-324.

ALDORETTA, P.W. & HAY Jr., W. (1995) Metabolic substrates for fetal energy metabolism and growth. *Clinics in Perinatology* **22(1)**, 15-36.

ALEXANDER, G. (1964). Studies on the placenta of the sheep (*Ovis aries L.*). Effect of surgical reduction in the number of caruncles. *Journal of Reproduction and Fertility* **7**, 307-322.

ALLEN, W.R. (1975). Endocrine functions of the placenta. In: *Comparative Placentation. Essays in Structure and Function*, ed. Steven, D.H. pp. 214-267. Academic Press.

AMIEL-TISON, C. & PETTIGREW, A.G. (1991). Adaptive changes in the developing brain during intrauterine stress. *Brain Development* **13**, 67-76.

ANAND, I.S & CHANDRASHEKHAR, Y. (1993a). Reduced inhibition of endothelial-derived relaxing factor causes the hyperdynamic circulation in chronic severe anemia. *Medical Hypotheses* **41**, 225-228.

ANAND, I.S., CHANDRASHEKHAR, Y., FERRARI, R., POOLE-WILSON, P.A. & HARRIS, P.C. (1993). Pathogenesis of oedema in chronic severe anaemia: studies of body water and sodium, renal function, haemodynamic variables, and plasma hormones. *British Heart Journal* **70**, 357-362.

ANAND, I.S., CHANDRASHEKHAR, Y., WANDER, G.S. & CHAWLA, L.S. (1995). Endothelium-derived relaxing factor is important in mediating the high output state in chronic severe anaemia. *Journal of the American College of Cardiology* **25**, 1402-1407.

ANDERSON, D.F., PARKS, C.M. & FABER, J.J. (1986). Fetal O_2 consumption in sheep during controlled long-term reductions in umbilical blood flow. *American Journal of Physiology* **250(6)**, H1037-H1042.

ANDRESEN, M.C. (1984). Short- and long-term determinants of baroreceptor function in aged normotensive and spontaneously hypertensive rats. *Circulation Research* **54(6)**, 750-759.

ANDRESEN, M.C., KURAOKA, S. & BROWN, A.M. (1980). Baroreceptor function and changes in strain sensitivity in normotensive and spontaneously hypertensive rats. *Circulation Research* **47**, 821-828.

ANTHONY, F.W., WHEELER, T., ELCOCK, C.L. PICKETT, M. & THOMAS, E.J. (1994). Short report: identification of a specific pattern of vascular endothelial growth factor mRNA expression in human placenta and cultured placental fibroblasts. *Placenta* **15**, 557-561.

ANTONOV, A.N. (1947). Children born during the siege of Leningrad in 1942. *Journal of Pediatrics* **30**, 250-259.

BAAN, Jr., J., BOEKKOOI, P.F., TEITEL, D.F. & RUDOLPH, A.M. (1993). Heart rate fall during acute hypoxemia: a measure of chemoreceptor response in fetal sheep. *Journal of Developmental Physiology* **19**, 105-111.

BACON, B.J., GILBERT, R.D., KAUFMANN, P., SMITH, A.D., TREVINO, F.T. & LOMGO, L.D. (1984). Placental anatomy and diffusing capacity in guinea pigs following long-term maternal hypoxia. *Placenta* **5**, 475-488.

BALL, R.H., ESPINOZA, M.I., PARER, J.T., ALON, E., VERTOMMEN, J. & JOHNSON, J. (1994). Regional blood flow in asphyxiated fetuses with seizures. *American Journal of Obstetrics & Gynecology* **170(1)**, 156-161.

BALLARD, P.L., KITTERMAN, J.A., BLAND, R.D., CLYMAN, R.I., GLUCKMAN, P.D., PLATZKER, A.C.G., KAPLAN, S. & GRUMBACH, M.M. (1982). Ontogeny and regulation of corticosteroid binding globulin capacity in plasma of fetal and newborn lambs. *Endocrinology* **110**, 359-366.

BARKER, D.J.P. & OSMOND, C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *The Lancet* **1**, 1077-1081.

BARKER, D.J.P. & OSMOND, C. (1987). Death rates from stroke in England and Wales predicted from past maternal mortality. *British Medical Journal* **295**, 83-86.

BARKER, D.J.P. & SULTAN, H.Y. (1995). Fetal programming of human disease. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 3: Growth* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 255-274. Cambridge University Press.

BARKER, D.J.P. (1990). The intrauterine origins of adult hypertension. In: *Fetal autonomy and adaptation*. Ed. Dawes, G.S., Borruto, F., Zacutti, A. & Zacutti, A. Jr., pp. 29-37. John Wiley & Sons.

BARKER, D.J.P. (1991). The intrauterine environment and adult cardiovascular disease. *Ciba Foundation Symposium* **156**, 3-16.

BARKER, D.J.P. (1992a). Symposium on 'impact of diet on critical events in development'. The effect of nutrition of the fetus and neonate on cardiovascular disease in adult life. *Proceedings of the Nutrition Society* **51**, 135-144.

BARKER, D.J.P. (1994). *Mothers, babies, and Disease in Later Life.* BMJ Publishing Group.

BARKER, D.J.P. (1996). Fetal undernutrition and disease in later life. In: *Reviews of Reproduction.* (In press).

BARKER, D.J.P., BULL, A.R., OSMOND, C. & SIMMONDS, S.J. (1990). Fetal and placental size and risk of hypertension in adult life. *British Medical Journal* **301**, 259-262.

BARKER, D.J.P., GODFREY, K.M., OSMOND, C. & BULL, A. (1992b). The relation of fetal length, ponderal index and head circumference to blood pressure and the risk of hypertension in adult life. *Pediatric and Perinatal Epidemiology* **6**, 35-44.

BARKER, D.J.P., MARTYN, C.N., OSMOND, C. & WIELD, G.A. (1995). Abnormal liver growth in utero and death from coronary heart disease. *British Medical Journal* **310**, 703-704.

BARKER, D.J.P., MEADE, T.W., FALL, C.H.D., LEE, A., OSMOND, C., PHIPPS, K. & STIRLING, Y. (1992c). Relation of fetal and infant growth to plasma fibrinogen and factor VII concentrations in adult life. *British Medical Journal* **304**, 147-152.

BARKER, D.J.P., OSMOND, C. & LAW, C.M. (1989a). The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. *Journal of Epidemiology and Community Health* **43**, 237-240.

BARKER, D.J.P., OSMOND, C., GOLDING, J., KUH, D. & WADSWORTH, M.E.J. (1989c). Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *British Medical Journal* **298**, 564-567.

BARKER, D.J.P., OSMOND, C., SIMMONDS, S.J. & WIELD, G.A. (1993a). The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *British Medical Journal* **306**, 422-426.

BARKER, D.J.P., WINTER, P.D., OSMOND, C., MARGETTS, B. & SIMMONDS, S.J. (1989b). Weight in infancy and death from ischaemic heart disease. *The Lancet* **2(8663)**, 577-580.

BASSETT, J.M. (1995). Glucose and fetal growth derangement. In: *Fetus and Neonate. Physiology and cinical applications. Volume 3. Growth,* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 223-254. Cambridge University Press.

BATTAGLIA, F.C. (1992). New concepts in fetal and placental amino acid metabolism. *Journal of Animal Science* **70**, 3258-3263.

BATTAGLIA, F.C., MESCHIA, G. (1988). Fetal nutrition. *Annual Reviews in Nutrition* **8**, 43-61.

BATTAGLIA, F.G., BEHRMAN, R.E., BRUNS, P.D., HELLEGERS, A., MESCHIA, G., SCHRUEFER, J.J., SEEDS, A.E. & BARRON, D.H. (1965). Gestational changes in arteriovenous differences of oxygen across the uterus of the rhesus monkey. *American Journal of Obstetrics & Gynecology* **93(1)**, 32-36.

BATTAGLIA, F.G., BOWES, W., McGAUGHEY, H.R., MAKOWSKI, E.L. & MESCHIA, G. (1969). The effect of fetal exchange transfusions with adult blood upon fetal oxygenation. *Pediatric Research* **3**, 60-65.

BAUER, M.K., BREIER, B.H., HARDING, J.E., VELDHUIS, J.D. & GLUCKMAN, P.D. (1995). The fetal somatotropic axis during long term maternal undernutrition in sheep: evidence for nutritional regulation in utero. *Endocrinology* **136**, 1250-1257.

BEISCHER, N.A., SIVASAMBOO, R., VOHRA, S., SILPISORNKOSAL, S. & REID, S. (1970). Placental hypertrophy in severe pregnancy anaemia. *The Journal of Obstetrics and Gynaecology of the British Commonwealth* **77**, 398-409.

BENDECK, M.P. & LANGILLE, B.L. (1991). Rapid accumulation of elastin and collagen in the aortas of sheep in the immediate perinatal period. *Circulation Research* **69**, 1165-1169.

BENDECK, M.P. & LANGILLE, B.L. (1992). Changes in blood flow distribution during the perinatal period in fetal sheep and lambs. *Canadian Journal of Physiology & Pharmacology* **70**, 1576-1582.

BENEDIKTSSON, R., LINDSAY, R.S., NOBLE, J., SECKL, J.R. & EDWARDS, C.R.W. (1993). Glucocorticoid exposure in utero: new model for adult hypertension. *The Lancet* **341**, 339-341.

BENNET, L. & HANSON, M.A. (1994). Intrauterine compromise: physiological consequences. In: *Early Fetal Growth and Development*, eds. Ward, R.H.T., Smith, S.K. & Donnai, D. pp. 363-370. RCOG Press.

BERDUSCO, E.T.M., YANG, K., HAMMOND, G.L. & CHALLIS, J.R.G. (1995). Corticosteroid-binding globulin (CBG) production by hepatic and extra-hepatic sites in the ovine fetus; effects of CBG on glucocorticoid negative feedback in pituitary cells *in vitro*. *Journal of Endocrinology* **146**, 121-130.

BERKEY, C.S., LAIRD, N.M., VALADIAN, I. & GARDNER, J. (1991). Modelling adolescent blood pressure patterns and their prediction of adult pressures. *Biometrics* **47**, 1005-1018.

BERNSTEIN, D., TEITEL, D.F. & RUDOLPH, A.M. (1988). Chronic anaemia in the newborn lamb: cardiovascular adaptations and comparison to chronic hypoxaemia. *Pediatric Research* **23**, 621-627.

BERRY, C.L., GOSLING, R.G., LAOGUN, A.A. & BRYAN, E. (1976). Anomalous iliac compliance in children with a single umbilical artery. *British Heart Journal* **38**, 510-515.

BIAN, X-P., SEIDLER, F.J. & SLOTKIN, T.A. (1992). Promotional role for glucocorticoids in the development of intracellular signalling: enhanced cardiac and renal adenylate cyclase reactivity to β-adrenergic and non-adrenergic stimuli after low-dose fetal dexamethasone exposure. *Journal of Developmental Physiology* **17**, 289-297.

BISCOE, T.J. & DUCHEN, M.R. (1990). Monitoring PO_2 by the carotid chemoreceptor. *News in Physiological Sciences* **5**, 229-233.

BLANCO, C.E. (1994). Peripheral control of breathing. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 2: Breathing* eds. Hanson, M.A., Spencer, J.A.D, Rodeck, C.H. & Walters, D.V. pp. 85-108. Cambridge University Press.

BLANCO, C.E., DAWES, G.S., HANSON, M.A. & McCOOKE, H.B. (1984). The response to hypoxia of arterial chemoreceptors in fetal sheep and new-born lambs. *Journal of Physiology* **351**, 25-37.

BLANCO, C.E., DAWES, G.S., HANSON, M.A. & McCOOKE, H.B. (1988). Carotid baroreceptors in fetal and newborn sheep. *Pediatric Research* **24(3)**, 342-346.

BLOCK, B.S., SCHLAFER, D.H., WENTWORTH, R.A. KREITZER, L.A. & NATHANIELSZ, P.W. (1990). Regional blood flow distribution in fetal sheep with intrauterine growth retardation produced by decreased umbilical placental perfusion. *Journal of Developmental Physiology* **13**, 81-85.

BLOCK, B.S.B., LLANOS, A.J. & CREASY, R.K. (1984). Responses of the growth-retarded fetus to acute hypoxemia. *American Journal of Obstetrics & Gynaecology* **148**, 878-885.

BLUM, W.F. & GLUCKMAN, P.D. (1996). Insulin-like growth factors. In: *Pediatrics & Perinataology. The Scientific Basis,* eds. Gluckman, P.D. & Heymann, M.A. pp. 314-323. Arnold.

BOCKING, A.D., GAGNON, R., WHITE, S.E., HOMAN, J., MILNE, K.M. & RICHARDSON, B.S. (1988). Circulatory responses to prolonged hypoxemia in fetal sheep. *American Journal of Obstetrics & Gynecology* **159**, 1418-1424.

BOCKING, A.D., HARDING, R. & WICKHAM, J.D. (1986). Effects of reduced uterine blood flow on accelerations and decelerations in heart rate of fetal sheep. *American Journal of Obstetrics & Gynecology* **154**, 329-335.

BOCKING, A.D., WHITE, S.E., HOMAN, J. & RICHARDSON, B.S. (1992). Oxygen consumption is maintained in fetal sheep during prolonged hypoxaemia. *Journal of Developmental Physiology* **17**, 169-174.

BODDY, K., ROBINSON, J.S. & RATCLIFFE, J.G. (1975). Pituitary and adrenal function in foetal sheep during the latter half of gestation. *Journal of Endocrinology* **67(2)**, 26P.

BODDY, K; DAWES, G.S., FISHER, R., PINTER, S. & ROBINSON, J.S. (1974). Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. *Journal of Physiology* **243**, 599-618.

BODE-BÖGER, S.M., BÖGER, R.H., KUHN, M., RADERMACHER, J. & FRÖLICH, J.C. (1992). Endothelin release and shift in prostaglandin balance are involved in the modulation of vascular tone by recombinant erythropoietin. *Journal of Cardiovascular Pharmacology* **20(Suppl. 12)**, S25-S28.

BOEKKOOI, P.F., BAAN Jr., J., TEITEL, D. & RUDOLPH, A.M. (1992). Chemoreceptor responsiveness in fetal sheep. *American Journal of Physiology* **263**, H162-H167.

BONDS, D.R., GABBE, S.G., KUMAR, S. & TAYLOR, T. (1984). Fetal weight / placental weight ratio and perinatal outcome. *American Journal of Obstetrics & Gynecology* **149**, 195-200.

BOONE JR., J.B. & MCMILLEN, D. (1994). Proenkephalin gene expression is altered in the brain of spontaneously hypertensive rats during the development of hypertension. *Molecular Brain Research* **24**, 320-326.

BRACE, R.A. (1993). Regulation of blood volume in utero. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 1: The circulation.* eds. Hanson, M.A., Spencer, J.A.D, Rodeck, C.H. & Walters, D.V. pp. 75-99. Cambridge University Press.

BRACE, R.A. (1994). Fetal fluid balance. In: *Textbook of Fetal Physiology*, eds. Thorburn, G.D. & Harding, R. pp. 205-218. Oxford University Press.

BRACE, R.A. (1996). Fetal and amniotic fluid balance. In: *Pediatrics & Perinataology. The Scientific Basis,* eds. Gluckman, P.D. & Heymann, M.A. pp. 233-242. Arnold.

BRISTOW, J.D. (1996). Cardiac and myocardial structure and myocardial cellular and molecular function. In: *Pediatrics and Perinatology. The Scientific Basis,* eds: Gluckman, P.D. & Heymann, M.A. pp. 710-724. Arnold.

BROUGHTON-PIPKIN, F., LUMBERS, E.R. & MOTT, J.C. (1974). Factors influencing plasma renin and angiotensin II in the conscious pregnant ewe and its foetus. *Journal of Physiology* **243**, 619-636.

BROWN, A.M. (1980). Receptors under pressure. An update on baroreceptors. *Circulation Research* **46(1)**, 1-10.

BROWN, J.E., JACOBSON, H.N., ASKUE, L.H. & PEICK, M.G. (1981). Influence of pregnancy weight gain on the size ofinfants born to underweight women. *Obstetrics & Gynecology* **57**, 13-17.

BRUCE, N.W. & CABRAL, D.A. (1975). Effects of maternal blood loss on embryonic and placental development in the rat. *Journal of Reproduction and Fertility* **45**, 349-356.

BURD, L.I., JONES Jr., M.D., SIMMONS, M.A., MAKOWSKI, E.L., MESCHIA, G. & BATTAGLIA, F.C. (1975). Placental production and foetal utilisation of lactate and pyruvate. *Nature* **254**, 710-711.

BURTON, P.J., SMITH, R.E., KROZOWSKI, Z.S. ¶ WADDELL, B.J. (1996). Zonal distribution of 11β-hydroxysteroid dehydrogenase types 1 and 2 messenger ribonucleic acid expression in the rat placenta and decidua during late pregnancy. *Biology of Reproduction* **55**, 1023-1028.

BUSSE, R., MÜLSCH, A., FLEMING, I. & HECKER, M. (1993). Mechanisms of Nitric oxide release from the vascular endothelium. *Circulation* **87** (suppl. V), V18-25.

CALKINS, L.A. (1937). Placental variation. An analytical determination of its clinical importance. *American Journal of Obstetrics & Gynecology* **33**, 280-291.

CALLAN, N.A. & WITTER, F.R. (1990). Intrauterine growth retardation: characteristics, risk factors and gestational age. *International Journal of Gynaecology and Obstetrics* **33(3)**, 215-220.

CAMPAGNOLE-SANTOS, M., HERINGER, S.B., BATISTA, E.N. KHOSLA, M.C. & SANTOS, R.A.S. (1992). Differential baroreceptor reflex modulation by centrally infused angiotensin peptides. *American Journal of Physiology* **263**, R89- R94.

CAMPBELL, D.M., HALL, M.H., BARKER, D.J.P., CROSS, J., SHIELL, A.W. & GODFREY, K.M. (1996). Diet in pregnancy and the offspring's blood pressure 40 years later. *British Journal of Obstetrics & Gynaecology* **103**, 273-280.

CARTER, A.M. (1989). Factors affecting gas transfer across the placenta and the oxygen supply to the fetus. *Journal of Developmental Physiology* **12**, 305-322.

CARTER, A.M., CHALLIS, J.R.G. & SVENDSEN, P. (1996). Regional adrenal blood flow responses to adrenocorticotropic hormone after chronic embolization of the fetal placental circulation in sheep. *Journal of Endocrinology* **148**, 517-522.

CHALLIS, J., HAN, V.K.M., RAMIREZ, M., McDONALD, T.J., FRASER, M., YANG, K., GIBB, W., MATTHEWS, S.G., MUROTSUKI, J. & GAGNON, R. (1995). Fetal placental interaction: who talks to whom. *Proceedings of the 4th. International Conference on Veterinary Perinatology, Cambridge, U.K.*

CHAPLEAU, M.W., HAJDUCZOK, G. & ABBOUD, F.M. (1991). Paracrine modulation of baroreceptor activity by vascular endothelium. *News in Physiological Science* **6**, 210-214.

CHAPLEAU, M.W., HAJDUCZOK, G. & ABBOUD, F.M. (1992). Suppression of baroreceptor discharge be endothelin at high carotid sinus pressure. *American Journal of Physiology* **263**, R103-R108.

CHAPLEAU, M.W., HAJDUCZOK, G. 7 ABBOUD, F.M. (1988). Mechanisms of resetting or arterial baroreceptors: an overview. *American Journal of Medical Sciences* **295(4)**, 327-334.

CHARD, T. (1994). Insulin-like growth factors and their binding proteins in the control of human fetal growth. In: *Early Fetal Growth and Development*, , eds. Ward, R.H.T., Smith, S.K. & Donnai, D. pp. 293-316. RCOG Press.

CHATFIELD, B.A., McMURTRY, I.F., HALL, S.L. & ABMAN, S.H. (1991). Hemodynamic effects of endothelin-1 on ovine fetal pulmonary circulation. *American Journal of Physiology* **261**, R182-R187.

CHEN, H-G. & WOOD, C.E. (1992). Reflex control of fetal arterial pressure and hormonal responses to slow hemorrhage. *American Journal of Physiology* **262**, H225-H233.

CHEN, H.I., CHAPLEAU, M.W., McDOWELL, T.S. & ABOUD, F.M. (1990). Prostaglandins contribute to activation of baroreceptors in rabbits. Possible paracrine influence of endothelium. *Circulation Research* **67**, 1394-1404.

CHEUNG, C.Y. & BRACE, R.A. (1988). Fetal hypoxia elevates plasma atrial natriuretic factor concentration. *American Journal of Obstetrics & Gynaecology* **159**, 1263-1268.

CHEUNG, C.Y. & BRACE, R.A. (1996). Vascular endothelial growth factor/vascular permeability factor expression in the ovine fetus and placenta: possible role in angiogenesis and permeability. *Fetal & Neonatal Physiological Society* **23rd. Annual Meeting**, 9.

CHEUNG, C.Y. (1989). Direct adrenal medullary catecholamine response to hypoxia in fetal sheep. *Journal of Neurochemistry* **52**, 148-153.

CHEUNG, C.Y. (1991). Role of endogenous atrial natriuretic factor in the regulation of fetal cardiovascular and renal function. *American Journal of Obstetrics & Gynecology* **165**, 1558-1567).

CHEUNG, C.Y. (1992). Autonomic and arginine vasopresssin modulation of the hypoxia-induced atrial natriuretic factor release in immature and mature ovine fetuses. *American Journal of Obstetrics & Gynecology* **167**, 144301453.

CHEUNG, C.Y. (1994). Regulation of atrial natriuretic factor release by endothelin in ovine fetuses. *American Journal of Physiology* **267**, R380-R386.

CHEUNG, C.Y., GIBBS, D.M. & BRACE, R.A. (1987). Atrial natriuretic factor in maternal and fetal sheep. *American Journal of Physiology* **252**, E279-E282.

CHIU, A.T., HERBLIN, W.F., McCALL, D.E., ARDECKY, R.J., CARINI, D.J., DUNCIA, J.V., PEASE, L.J., WONG, P.C., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1989). Identification of angiotensin II receptor subtypes. *Biochemical and Biophysical Research Communications* **165(1)**, 196-203.

COGSWELL, M.E. & YIP, R. (1995). The influence of fetal and maternal factors on the distribution of birthweight. *Seminars in Perinatology* **19(3)**, 222-240.

COGSWELL, M.E., SERDULA, M.K., HUNGERFORD, D.W. & YIP, R. (1985). Gestational weight gain among average-weight and overweight women - what is excessive? *American Journal of Obstetrics & Gynecology* **172(2)**, 705-712.

COHN, H.E., SACKS, E.J., HEYMANN, M.A. & RUDOLPH, A.M. (1974). Cardiovascular responses to hypoxemia and acidemia in fetal lambs. *American Journal of Obstetrics & Gynecology* **120**, 817-824.

COMLINE, R.S. & SILVER, M. (1975). Placental transfer of blood gases. *British Medical Bulletin* **31(1)**, 25-31.

COMLINE, R.S., SILVER, I.A. & SILVER, M. (1965). Factors responsible for the stimulation of the adrenal medulla during asphyxia in the foetal lamb. *Journal of Physiology* **178**, 211-238.

CONLEY, A.J. & MASON, J.I. (1994). Endocrine function of the placenta. In: *Textbook of Fetal Physiology*, eds. Thorburn, G.D. & Harding, R. pp.16-29. Oxford University Press.

COURT, D.J., PARER, J.T. BLOCK, B.S.B. & LLANOS, A.J. (1984). Effects of beta-adrenergic blockade on blood flow distribution during hypoxaemia in fetal sheep. *Journal of Developmental Physiology* **6**, 349-358.

COUSTAN, D.R., HANING, R.V. & SINGER, D.B. (1995). *Human Reproduction: Growth and Development.* Little Brown and Company.

CREASY, R.K., BARRETT, C.T., DESWIET, M., KAHANPÄÄ, K.V. & RUDOLPH, A.M. (1972). Experimental intrauterine growth retardation in the sheep. *American Journal of Obstetrics & Gynecology* **112(4)**, 566-573.

CUNNINGHAM, F.G., McDONALD, P.C., GANT, N.F., LEVENO, K.J., & GILSTRAP, L.C. (1993). *Williams Obstetrics* **19th. edition**, Prentice-Hall International Inc.

CURRIE, I.S. & BROOKS, A.N. (1992). Corticotrophin-releasing factors in the hypothalamus of the developing fetal sheep. *Journal of Developmental Physiology* **17**, 241-246.

DALINGHAUS, M., KNOESTER, H., GRATAMA, J.W.C., VAN DER MEER, J., ZIJLSTRA, W.G. & KUIPERS, J.R.G. (1994). Effect of increased whole blood viscosity on regional blood flows in chronically hypoxemic lambs. *American Journal of Physiology* **267**, H471-H476.

DANIEL, S.S., JAMES, L.S., STARK, R.I. & TROPPER, P.J. (1989). Prevention of the normal expansion of maternal plasma volume: a model for chronic fetal hypoxaemia. *Journal of Developmental Physiology* **11**, 225-233.

DANIELIAN, P.J., ALLMAN, A.C.J. & STEER, P.J. (1992). Is obstetric and neonatal outcome worse in fetuses who fail to reach their own growth potential? *British Journal of Obstetrics & Gynaecology* **99**, 452-454.

DAVIS, L.E. & HOHIMER, A.R. (1991). Hemodynamics and organ blood flow in fetal sheep subjected to chronic anemia. *American Journal of Physiology* **261**, R1542-R1548.

DAWES, G.S., LEWIS, B.V., MILLIGAN, J.E., ROACH, M.R. & TALNER, N.S. (1968). Vasomotor responses in the hind limbs of foetal and new-born lambs to asphyxia and aortic chemoreceptor stimulation. *Journal of Physiology* **195**, 55-81.

DAWSON, J.M. & HUDLICKA, O. (1993). Can changes in microcirculation explain capillary growth in skeletal muscle? *International Journal of Experimental Pathology* **74**, 65-71.

DeBARRO, T.M., OWENS, J.A., EARL, C.R. & ROBINSON, J.S. (1992). Mating weight influences the effect of mid-pregnancy nutrition on placental growth in the sheep. *Proceedings of the Australian Nutrition Society,* P 7403

DELPAPA, E.H., EDELSTONE, D.I., MILLEY, J.R. & BALSAN,M. (1992). Effects of chronic maternal anemia on systemic and uteroplacental oxygenation in near-term pregnant sheep. *American Journal of Obstetrics & Gynecology* **166**, 1007-1012.

DEPARTMENT OF HEALTH (1996). *Health and Personal Social Services Statistics for England*, eds: Barker, M., Ainsley, D., Marcelle, M. & Allen, B. pp. 126. Her Majesty's Stationery Office.

DETMER, A., GU, W. & CARTER, A.M. (1991). The blood supply to the heart and brain in the growth retarded guinea pig fetus. *Journal of Developmental Physiology* **15**, 153-160.

DOUGHTY, I.M. & SIBLEY, C.P. (1995). Placental transfer. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 3: Growth,* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 1-29. Cambridge University Press.

DOUGLAS, RENTON, J.P. & WATTS, C. (1971). Placental transfer of iron in the rabbit. *British Journal of Haemotology* **20**, 185-194.

DRABKIN, D.L. & AUSTIN, J.H. (1932). Spectrophotometric studies. I. Spectrophotometric constants for common hemoglobin derivatives in human, dog, and rabbit blood. *Journal of Biological Chemistry* **98**, 719-733.

DWYER, C.M., MADGWICK, A.J.A., CROOK, A.R. & STICKLAND, N.C. (1992). The effect of maternal undernutrition on the growth and development of the guinea pig placenta. *Journal of Developmental Physiology* **18**, 295-302.

DZAU, V.J. (1984). Vascular renin-angiotensin: a possible autocrine or paracrine system in control of vascular function. *Journal of Cardiovascular Pharmacology* **6**, S377-S382.

ECONOMIDES, D.L., NICOLAIDES, K.H., GAHL, W.A., BERNARDINI, I. & EVANS, M.I. (1989). Plasma amino acids in appropriate- and small-for-gestational-age fetuses. *American Journal of Obstetrics and Gynaecology* **161**, 1219-1227.

EDELSTONE, D.I., PAULONE, M.E., MALJOVEC, J.J. & HAGBERG, M. (1987). Effects of maternal anaemia on cardiac output, systemic oxygen consumption, and regional blood flow in pregnant sheep. *American Journal of Obstetrics & Gynecology* **156**, 740-748.

EDWARDS, C.R.W., BENEDIKTSSON, R., LINDSAY, R.S. & SECKL, J.R. (1993). Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? *The Lancet* **341**, 355-357.

EGGENA, P., WILLSEY, P., JAMGOTCHIAN, N., TRUCKENBROD, L., HU, M.S., BARRETT, J.D., EGGENA, M.P., CLEGG, K., NAKHOUL, F. & LEE, D.B.N. (1991). Influence of recombinant human erythropoietin on blood pressure and tissue renin-angiotensin systems. *American Journal of Physiology* **261**, E642-E646.

EKBOM, A., HSIEH, C-C. LIPWORTH, L., WOLK, A., PONTÉN, J., ADAMI, H-O. & TRICHOPOULOS, D. (1996). Perinatal characteristics in relation to incidence of and mortality from prostate cancer. *British Medical Journal* **313**, 337-341.

EKBOM, A., TRICHOPOULOS, D., ADAMI, H-O., HSIEH, C-C. & LAN, S-J. (1992). Evidence of prenatal influences on breast cancer risk. *The Lancet* **340**, 1015-1018.

ELPHICK, M.C., HULL, D. & BROUGHTON PIPKIN, F. (1979). The transfer of fatty acids across the sheep placenta. *Journal of Developmental Physiology* **1**, 31-45.

ENGSTRÖM, W. & HEATH, J.K. (1988). Growth factors in early embryonic development. In: *Fetal and Neonatal Growth,* ed: Cockburn, F. pp. 11-32. John Wiley & Sons.

ERVIN, M.G., ROSS, M.G., LEAKE, R.D. & FISHER, D.A. (1992). V_1- and V_2-receptor contributions to ovine fetal renal and cardiovascular responses to vasopressin. *American Journal of Physiology* **262**, R636-R643.

EVERITT, G.C. (1964). Maternal undernutrition and retarded foetal development in Merino sheep. *Nature* **201 (4926)**, 1341-1342.

FAICHNEY, G.J. & WHITE, G.A. (1987). Effects of maternal nutritional status on fetal and placental growth and on fetal urea synthesis in sheep. *Australian Journal of Biological Sciences* **40**, 365-377.

FAY, R.A. & ELLWOOD, D.A. (1993). Categories of intrauterine growth retardation. *Fetal and Maternal Medicine Review* **5**, 203-212.

FISHER, D.J. (1989). β-adrenergic influence on increased myocardial oxygen consumption during hypoxemia in awake newborn lambs. *Pediatric Research* **25(6)**, 585-590.

FLETCHER, E.C. (1995). The relationship between systemic hypertension and obstructive sleep apnea: facts and theory. *The American Journal of Medicine* **98**, 118-128.

FLETCHER, E.C., BAO, G., & MILLER III, C.C. (1995). Effect of recurrent episodic hypocapnic, eucapnic, and hypercapnic hypoxia on systemic blood pressure. *Journal of Applied Physiology* **78(4)**, 1516-1521.

FLETCHER, E.C., LESSKE, J., BEHM, R., MILLER III, C.C., STAUSS, H. & UNGER, T. (1992). Carotid chemoreceptors, systemic blood pressure, and chronic episodic hypoxia mimicking sleep apnea. *Journal of Applied Physiology* **72(5)**, 1978-1984.

FLETCHER, E.C., LESSKE, J., QIAN, W., MILLER III, C.C. & UNGER, T. (1992). Repetitive, episodic hypoxia causes diurnal elevation of blood pressure in rats. *Hypertension* **19**, 555-561.

FORRESTER, T.E., WILKS, R.J., BENNETT, F.I., SIMEON, D., OSMOND, C., ALLEN, M., CHUNG, A.P. & SCOTT, P. (1996). Fetal growth and cardiovascular risk factors in Jamaican schoolchildren. *British Medical Journal* **312**, 156-160.

FOWDEN, A.L. & SILVER, M. (1995). The effects of thyroid hormones on oxygen and glucose metabolism in the sheep fetus during late gestation. *Journal of Physiology* **482(1)**, 203-213.

FOWDEN, A.L. (1994). Fetal metabolism and energy balance. In: *Textbook of Fetal Physiology*, eds. Thorburn, G.D. & Harding, R. pp. 70-82. Oxford University Press.

FOWDEN, A.L. (1995). Endocrine regulation of fetal growth. *Reproduction, Fertility & Development* **7**, 351-363.

FOWDEN, A.L. (1995). Nutrient requirements for normal fetal growth and metabolism. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 3: Growth,* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 31-56. Cambridge University Press.

FOWDEN, A.L., APATU, R.S.K. & SILVER, M. (1995). The glucogenic capacity of the fetal pig: developmental regulation by cortisol. *Experimental Physiology* **80**, 457-467.

FOWDEN, A.L., COULSON, R.L. & SILVER, M. (1990). Endocrinr regulation of tissue glucose-6-phosphotase activity in the fetal sheep during late gestation. *Endocrinology* **126**, 2823-2830.

FOWDEN, A.L., MIJOVIC, J. & SILVER, M. (1992). The effects of cortisol on hepatic and renal gluconeogenic enzyme activities in the sheep fetus during late gestation. *Journal of Endocrinology* **137**, 213-222.

FOWLER, N.O. & HOLMES, J.C. (1975). Blood viscosity and cardiac output in acute experimental anemia. *Journal of Applied Physiology* **39(3)**, 453-456.

FRANCE, J.T. (1996). Placental endocrine function. In: *Pediatrics & Perinataology. The Scientific Basis,* eds. Gluckman, P.D. & Heymann, M.A. pp. 206-213. Arnold.

FRIESS, U., RASCHER, W., RITZ, E. & GROSS, P. (1995). Failure of arginine-vasopressin and other pressor hormones to increase in severe recurrent dialysis hypotension. *Nephrology Dialysis Transplantation* **10**, 1421-1427.

FUJIMORA, K., ENDO, C., KIN, S., FUNATA, Y., ARAKI, T., SATO, A. & MURATA, Y. (1994). Endocrinologic and biophysical responses to prolonged (24 hour) hypoxemia in fetal goats. *American Journal of Obstetrics & Gynecology* **171**, 470-477.

GAGNON, R., CHALLIS, J., JOHNSTON, L. & FRAHER, L. (1994). Fetal endocrine responses to chronic placental embolization in the late-gestation ovine fetus. *American Journal of Obstetrics & Gynecology* **170**, 929-938.

GAGNON, R., RUNDLE, H., JOHNSTON, L. & HAN, V.K.M. (1995). Alterations in fetal and placental deoxy ribonucleic acid synthesis rates after chronic fetal placental embolization. *American Journal of Obstetrics & Gynecology* **172**, 1451-1458.

GAR,. S.M., RIDELLA, S.A., PETZOLD, A.S. & FALKNER, F. (1981). Maternal hematological levels and pregnancy outcome. *Seminars in Perinatology* **5(2)**, 155-162.

GARCIA, R. (1993). Atrial natriuretic factor in experimental and human hypertension. *News in Physiological Sciences* **8**, 161-164.

GARDNER, D.S., LANGLEY-EVANS, S.C. & JACKSON, A.A. (1995). Glucocorticoid activity in brain regions of rats exposed to a maternal low protein diet. *Proceedings of the 4th. International Conference on Veterinary Perinatology, Cambridge, U.K.*

GIUSSANI, D.A., McGARRIGLE, H.H.G., MOORE, P.J., BENNET, L., SPENCER, J.A.D. & HANSON, M.A. (1994b). Carotid sinus nerve section and the increase in plasma cortisol during acute hypoxia in fetal sheep. *Journal of Physiology* **477(1)**, 75-80.

GIUSSANI, D.A., McGARRIGLE, H.H.G., SPENCER, J.A.D., MOORE, P.J., BENNET, L. & HANSON, M.A. (1994c). Effect of carotid denervation on plasma vasopressin levels during acute hypoxia in the late-gestation sheep fetus. *Journal of Physiology* **477(1)**, 81-87.

GIUSSANI, D.A., RIQUELME, R.A., MORAGA, F.A., McGARRIGLE, H.H.G., GAETE, C.R., SANHUEZA, E.M., HANSON, M.A. & LLANOS, A.J. (1996b). Chemoreflex and endocrine components of cardiovascular responses to acute hypoxemia in the llama fetus. *American Journal of Physiology* **271**, R73-R83.

GIUSSANI, D.A., SPENCER, J.A.D. & HANSON, M.A. (1994a). Fetal cardiovascular reflex responses to hypoxaemia. *Fetal and Maternal Medicine Review* **6**, 17-37.

GIUSSANI, D.A., UNNO, N., MAN, W.K., HING, A., JENKINS, S.L., WENTWORTH, R.A., DERKS, J.B., COLLINS, J.H. & NATHANIELSZ, P.W. (1996c). Vasopressin (AVP) response to repeated, partial umbilical cord occlusion (UCO) in late-gestation unanaesthetized fetal sheep. *Fetal & Neonatal Physiological Society* **23rd. Annual Meeting**, 3.

GIUSSANI, D.A., UNNO, N., MAN, W.K.H., HING, A., JENKINS, S.L., WENTWORTH, R.A., DERKS, J.B., COLLINS, J.H. & NATHANIELSZ, P.W. (1996a). Cardiovascular responses to repeated, partial umbilical cord occlusions (UCO) in the chronically-instrumented sheep fetus. *Journal of the Society for Gynecologic Investigation* **3(2),** 402.

GLUCKMAN, P.D. & HARDING, J.E. (1994). Nutritional and hormonal regulation of fetal growth - evolving concepts. *Acta Paediatrica Supplements* **399**, 60-63.

GLUCKMAN, P.D. (1995). Insulin-like growth factors and their binding proteins. In: *Fetus and Neonate. Physiology and clinical applications. Volume 3. Growth,* eds. Hanson, M.A., Spencer, J.A.D., Rodeck, C.H. pp. 97-115. Cambridge University Press.

GODFREY, K., ROBINSON, S., BARKER, D.J.P., OSMOND, C. & COX, V. (1996). Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *British Medical Journal* **312**, 410-414.

GODFREY, K.M, FORRESTER, T., BARKER, D.J.P., JACKSON, A.A., LANDMAN, J.P. HALL, J.ST.E., COX, V. & OSMOND, C. (1994). Maternal nutritional status in pregnancy and blood pressure in childhood. *British Journal of Obstetrics and Gynaecology* **101**, 398-403.

GODFREY, K.M., REDMAN, C.W.G., BARKER, D.J.P. & OSMOND, C. (1991). The effect of maternal anaemia and iron deficiency on the ratio of fetal weight to placental weight. *British Journal of Obstetrics and Gynaecology* **98**, 886-891.

GREEN, L.R., BENNET, L. & HANSON, M.A. (1996). The role of nitric oxide synthesis in cardiovascular responses to acute hypoxia in the late gestation sheep fetus. *Journal of Physiology* **497(1)**, 271-277.

GRUENWALD, P. (1967). Influence of environmental factors on foetal growth in man. *The Lancet* **XX**, 1026-1028.

GUILLERY, E.N. & ROBILLARD, J.E. (1993). The renin-angiotensin system and blood pressure regulation during infancy and childhood. *Pediatric Clinics of North America* **40(1)**, 61-79.

HALL, J.E. (1994). Renal and cardiovascular mechanisms of hypertension in obesity. *Hypertension* **23**, 381-394.

HAMET, P., RICHARD, L., DAM, T-V., TEIGER, E., ORLOV, S.N., GABOURY, L., GOSSARD, F. & TREMBLAY, J. (1995). Apoptosis in target organs of hypertension. *Hypertension* **26**, 642-648.

HAMILL, O.P. & McBRIDE Jr., D.W. (1994). Molecular mechanisms of mechanoreceptor adaptation. *News in Physiological Sciences* **9**, 53-59.

HAN, V.K.M. & FOWDEN, A.L. (1994). Paracrine regulation of fetal growth. In: *Early Fetal Growth and Development*, eds. Ward, R.H.T., Smith, S.K. & Donnai, D. pp. 275-291. RCOG Press.

HAN, V.K.M. & HILL, D.J. (1994). Growth factors in fetal growth. In: *Textbook of Fetal Physiology*, eds. Thorburn, G.D. & Harding, R. pp. 48-69. Oxford University Press.

HANSON, M.A. (1988). The importance of baro- and chemoreflexes in the control of the fetal cardiovascular system. *Journal of Developmental Physiology* **10**, 491-511.

HANSON, M.A. (1993). The control of heart rate and blood pressure in the fetus: theoretical considerations. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 1: The Circulation,* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 1-22. Cambridge University Press.

HARDING, J.E., JONES, C.T. & ROBINSON, J.S. (1985). Studies on experimental growth retardation in sheep. The effects of a small placenta in restricting transport to and growth of the fetus. *Journal of Developmental Physiology* **7**, 427-442.

HARDING, J.E., JONES, C.T. & ROBINSON, J.S. (1985). Studies on experimental growth retardation in sheep. The effects of a small placenta in restricting transport to and growth of the placenta. *Journal of Developmental Physiology* **7**, 427-442.

HARDING, J.E. & JOHNSTON, B.M. (1995). Nutrition and fetal growth. *Reproduction, Fertility & Development* **7**, 539-547.

HARDING, J.E. (1995). The role of nutrition and pre-natal growth development. *Proceedings of the 4th International Conference on Veterinary Perinatology*, **Cambridge, U.K.**

HARDING, R. (1994). Development of the respiratory system. In: *Textbook of Fetal Physiology*, eds. Thorburn, G.D. & Harding, R. pp. 140-167. Oxford Universitty Press.

HARIK, S.I., HRITZ, M.A. & LAMANNA, J.C. (1995). Hypoxia-induced brain angiogenesis in the adult rat. *Journal of Physiology* **485**, 525-530

HAWDON, J.M., WARD PLATT, M.P., McPHAIL, S., CAMERON, H. & WALKINSHAW, S.A. (1992). Prediction of impaired metabolic adaptation by antenatal Doppler studies in small for gestational age fetuses. *Archives of Disease in Childhood* **67**, 789-792.

HAY, W.W. Placental-fetal interaction: regulation of fetal glucose and amino acid metabolism. (1996). *Proceedings of Frontiers in Maternal, Fetal and Neonatal Health. Programming for a life-time of good health.* **Cornell University, Ithaca, New York, U.S.A..**

HEBBEL, R.P., BERGER, E.M. & EATON J.W. (1980). Effect of increased maternal haemoglobin oxygen affinity on fetal growth in the rat. *Blood* **55(6)**, 969-974.

HEDNER, J.A., WILCOX, I., LAKS, L., GRUNSTEIN, R.R. & SULLIVAN, C.E. (1992). A specific and potent pressor effect of hypoxia in patients with sleep apnea. *American Reviews of Respiratory Disease* **146**, 1240-1245.

HERNANDEZ, J., ZARNEGER, R. & MICHALOPOULOS, G.K. (1992). Characterization of the effects of human placental HGF on rat hepatocytes. *Journal of Cellular Physiology* **150**, 116-121.

HILL, D.J. & HAN, V.K.M. (1996). Role of growth factors in tissue development. In: *Pediatrics and Perinatology. The Scientific Basis,* eds; Gluckman, P.D. & Heymann, M.A. pp.279-283. Arnold.

HINCHLIFFE, S.A., HOWARD, C.V., LYNCH, M.R.J., SARGENT, P.H., JUDD, B.A. & VAN VELZEN, D. (1993). Renal developmental arrest in Sudden Infant Death Syndrome. *Pediatric Pathology* **13**, 333-343.

HINCHLIFFE, S.A., LYNCH, M.R.J., SARGENT, P.H., HOWARD, C.V. & VAN VELZEN, D. (1992). The effect of intrauterine growth retardation on the development of renal nephrons. *British Journal of Obstetrics and Gynaecology* **99**, 296-301.

HOWE, D., WHEELER, T. & PERRING, S. (1994). Measurement of placental volume with real-time ultrasound in mid-pregnancy. *Journal of Clinical Ultrasound* **22**, 77-83.

HOWE, D.T., WHEELER, T. & OSMOND, C. (1995). The influence of maternal haemoglobin and ferritin on mid-pregnancy placental volume. *British Journal of Obstetrics & Gynaecology* **102**, 213-219.

HU, N. & CLARK, E.B. (1989). Hemodynamics of the stage 12 to stage 29 chick embryo. *Circulation Research* **65**, 1665-1670.

HUCH, R. & HUCH, A. (1994). Erythropoietin in obstetrics. *Hemotology/Oncology Clinics of North America* **8(5)**, 1021-1040.

HUDLICKA, O. (1991). What makes blood vessels grow? *Journal of Physiology* **444**, 1-24.

ISMAY, M.J.A., LUMBERS, E.R. & STEVENS, A.D. (1979). The action of angiotensin II on the baroreflex response of the conscious ewe and the conscious fetus. *Journal of Physiology* **288**, 467-479.

ITSKOVITZ, J., LaGAMMA, E.F., BRISTOW, J. & RUDOLPH, A.M. (1991). Cardiovascular responses to hypoxemia in sinoaortic-denervated fetal sheep. *Pediatric Research* **30**, 381-385.

IWAMOTO, H.S. & RUDOLPH, A.M. (1981). Effects of angiotensin II on the blood flow and its distribution in fetal lambs. *Circulation Research* **48**, 183-189.

IWAMOTO, H.S. & RUDOLPH, A.M. (1981a). Effects of angiotensin II on the blood flow and its distribution in fetal lambs. *Circulation Research* **48**, 183-189.

IWAMOTO, H.S. & RUDOLPH, A.M. (1981b). Role of renin-angiotensin system in response to hemorrhage in fetal sheep. *American Journal of Physiology* **240**, H848-H854.

IWAMOTO, H.S., KAUFMAN, T., KEIL, L.C. & RUDOLPH, A.M. (1989). Responses to acute hypoxemia in fetal sheep at 0.6-0.7 gestation. *American Journal of Physiology* **256**, H613-H620.

IWAMOTO, H.S., RUDOLPH, A.M., KEIL, L.C. & HEYMANN, M.A. (1979). Hemodynamic responses of the sheep fetus to vasopressin infusion. *Circulation Research* **44**, 430-436.

IWAMOTO, J., YOSHINAGA, M., YANG, S-P., KRASNEY, E. & KRASNEY, J. (1992). Methylene blue inhibits hypoxic cerebral vasodilation in awake sheep. *Journal of Applied Physiology* **73(6)**, 2226-2232.

JACKSON, B.T., MORRISON, S.H., COHN, H.E. & PIASECKI, G.J. (1989). Adrenal secretion of glucocorticoids during hypoxaemia in fetal sheep. *Endocrinology* **125(5)**, 2751-2757.

JACKSON, M.R., MAYHEW, T.M. & HAAS, J.D. (1987). The volumetric composition of human term placentae: altitudinal, ethnic and sex differences in Bolivia. *Journal of Anatomy* **152**, 173-187.

JACOBS, R., OWENS, J.A., FALCONER, J., WEBSTER, M.E.D. & ROBINSON, J.S. (1988 b). Changes in metabolite concentration in fetal sheep subjected to prolonged hypobaric hypoxia. *Journal of Developmental Physiology* **10**, 113-121.

JACOBS, R., ROBINSON, R.S., OWENS, J.A., FALCONER, J. & WEBSTER, M.E.D. (1988 a). The effect of prolonged hypobaric hypoxia on growth of fetal sheep. *Journal of Developmental Physiology* **10**, 97-112.

JANSSON, T. & PERSSON, E. (1991). Intrauterine growth retarded guinea pigs with more than 45% reduction in birth weight have elevated blood pressure at 3-4 months of age. *Society for Gynecologic Investigation*, Abstract 327.

JANSSON, T., THORDSTEIN, M. & KJELLMER, I. (1986). Placental blood flow and fetal weight following uterine artery ligation. Temporal aspects of intrauterine growth retardation in the guinea pig. *Biology of the Neonate* **49**, 172-180.

JENKIN, G. & NATHANIELSZ, P.W. (1994). Myometrial activity during pregnancy and parturition. In: *Textbook of Fetal Physiology*, eds.Thorburn, G.D. & Harding, R. pp. 405-414. Oxford University Press.

JENSEN, A. & BERGER, R. (1993). Regional distribution of cardiac output. *Fetus and Neonate. Physiology and Clinical applications. Volume 1. The Circulation*, eds. Hanson, M.A., Spencer, J.A.D., Rodeck, C.H. pp.23-74. Cambridge University Press.

JENSEN, A. & HANSON, M.A. (1993). Circulatory responses to acute asphyxia in intact and chemodenervated fetal sheep near term. *Reproduction, Fertility & Development* **7**, 1351-1359.

JENSEN, A. & LANG, U. (1992). Foetal circulatory responses to arrest of uterine blood flow in sheep: effects of chemical sympathectomy. *Journal of Developmental Physiology* **17**, 75-86.

JENSEN, A., KÜNZEL, W. & KASTENDIECK, E. (1985). Repetitive reduction of uterine blood flows and its influence on fetal transcutaneous PO_2 and cardiovascular variables. *Journal of Developmental Physiology* **7**, 75-87.

JENSEN, A., KÜNZEL, W. & KASTENDIECK, E. (1987). Fetal sympathetic activity, transcutaneous PO_2, and skin blood flow during repeated acute asphyxia in sheep. *Journal of Developmental Physiology* **9**, 337-346.

JIA, L., BONAVENTURA, C., BONAVENTURA, J. & STAMLER, J.S. (1996). S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. *Nature* **380**, 221-226.

JIN, L., PANG, Y-Y.S, JOSEPH, A., LI, Y., ROSEN, E.M., BHARGAVA, M.M. & GOLDBERG, I.D. (1993). Rat placental hepatocyte growth factor/ scatter factor: purification, characterization, and developmental regulation. *Proceedings of the Society for Experimental Biology and Medicine* **204**, 75-80.

JOHNSON, M.H. & EVERITT, B.J. (1988). Implantation and the establishment of the placenta. In: *Essential Reproduction*, by Johnson, M.H. & Everitt, B.J. pp. 224-251. Blackwell Scientific Publications.

JOHNSTON, B.M., HARDING, J.E. & GLUCKMAN, P.D. (1994). Development of fetal hypertension after maternal recovery from acute severe undernutrition. *Thorburn Symposium*, Abstract A3.

JONES, C.T. & ROBINSON, R.O. (1975). Plasma catecholamines in foetal and adult sheep. *Journal of Physiology* **248**, 15-33.

JONES, C.T., ROEBUCK, M.M., WALKER, D.W., LAGERCRANTZ, H. & JOHNSTON, B.M. (1987). Cardiovascular, metabolic and endocrine effects of chemical sympathectomy and of adrenal demedullation in fetal sheep. *Journal of Developmental Physiology* **9**, 347-367.

JORGENSEN, C.R., WANG, K., WANG,Y., GOBEL, F.L., NELSON, R.R. & TAYLOR, H. (1973). Effect of propanolol on myocardial oxygen consumption and its hemodynamic correlated during upright exercise. *Circulation* **48**, 1173-1182.

KALES, A., BIXLER, E.O., CADIEUX, R.J., SCHNECK, D.W., SHAW, L.C., LOCKE, T.W., VELA-BUENO, A. & SOLDATOS, C.R. (1984). Sleep apnoea in a hypertensive population. *The Lancet* **2(8410)**, 1005-1008.

KAMITOMO, M., ALONSO, J.G., OKAI, T., LONGO, L.D. & GILBERT, R.D. (1993). Effects of long-term, high-altitude hypoxaemia on ovine fetal cardiac output and blood flow distribution. *American Journal of Obstetrics & Gynaecology* **169**, 701-707.

KAMITOMO, M., LONGO, L.D. & GILBERT, R.D. (1992). Right and left ventricular function in fetal sheep exposed to long-term high-altitude hypoxemia. *American Journal of Physiology* **262**, H399-H405.

KAMITOMO, M., LONGO, L.D. & GILBERT, R.D. (1994). Cardiac function in fetal sheep during two weeks of hypoxemia. *American Journal of Physiology* **266**, R1778-R1785.

KELLY, R.W., NEWNHAM, J.P., JOHNSON, T. & SPEIJERS, E.J. (1987). An ultrasound technique to measure placental growth in ewes. *Australian Journal of Agricultural Research* **38**, 757-764.

KEMP, J.D., VIMINI, R.J. & ELY, D.G. (1988). Influence of maternal frame size and nutritional restriction on growth and development of the postnatal lamb. *Journal of Animal Science* **66**, 3073-3085.

KIM, E-K., WOOD, C.E. & KELLER-WOOD, M. (1995). Characterization of 11β-hydroxysteroid dehydrogenase avtivity in fetal and adult ovine tissue. *Reproduction, Fertility & Development* **7**, 377-383.

KINGDOM, J.C., McQUEEN, J., CONNELL, J.M.C. & WHITTLE, M.J. (1993). Fetal angiotensin II levels and vascular (type I) angiotensin receptors in pregnancies complicated by intrauterine growth retardation. *British Journal of Obstetrics & Gynaecology* **100**, 476-482.

KIRCHHEIM, H.R. (1976). Systemic arterial baroreceptor reflexes. *Physiological Reviews* **56(1)**, 100-176.

KITAMURA, K., JORGENSEN, C.R., GOBEL, F.L., TAYLOR, H.L. & WANG, Y. (1972). Hemodynamic correlates of myocardial oxygen conumption during upright exercise. *Journal of Applied Physiology* **32(4)**, 516-522.

KITANAKA, T., ALONSO, J.G., GILBERT, R.D., SIU, B.L., CLEMONS, G.K. & LONGO, L.D. (1989). Fetal responses to long-term hypoxemia in sheep. *American Journal of Physiology* **256**, R1348-R1354.

KONJE, J.C., TAYLOR, D.J. & RENNIE, M.J. (1996). Application of ultrasonic transit time flowmetry to the measurement of umbilical vein blood flow at caesarean section. *British Journal of Obstetrics & Gynaecology* **103**, 1004-1008.

KOOS, B.J., MASON, B.A. & ERVIN, G. (1994). Adenosine mediates hypoxic release of arginine vasopressin in fetal sheep. *American Journal of Physiology* **266**, R215-R220.

KOWALL, J., CLARK, G., NINO-MURCIA, G. & POWELL, N. (1989). Precipitation of obstructive sleep apnea during pregnancy. *Obstetrics & Gynecology* **74(3 Pt 2)**, 453-455.

KROZOWSKI, Z., MAGUIRE, J.A., STEIN-OAKLEY, A.N., DOWLING, J., SMITH, R.E. & ANDREWS, R.K. (1995). Immunohistochemical localization of the 11β-hydroxysteroid dehydrogenase type II enzyme in human kidney and placenta. *Journal of Clinical Endocrinology & Metabolism* **80(7)**, 1995.

KRÜGER, H. & ARIAS-STELLA, J. (1970) The placenta and the newborn infant at high altitudes. *American Journal of Obstetrics and Gynecology* **106(4)**, 586-591.

LAFEBER, H.N., ROLPH, T.P. & JONES, C.T. (1984). Studies on the growth of the fetal guinea pig. The effects of ligation of the uterine artery on organ growth and development. *Journal of Developmental Physiology* **6**, 441-459.

LANGILLE, B.L. (1993). Remodeling of developing and mature arteries: endothelium, smooth muscle, and matrix. *Journal of Cardiovascular Pharmacology* **21** (suppl. 1), S11-S17.

LANGLEY, S.C. & JACKSON, A.A. (1994) Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clinical Science* **86**, 217-222.

LAW, C.M., DE SWIET, M., OSMOND, C., FAYERS, P.M., BARKER, D.J.P., CRUDDAS, A.M. & FALL, C.H.D. (1993b). Initiation of hypertension in utero and its amplification throughout life. *British Medical Journal* **306**, 24-27.

LAWRENCE, D.L., SKATRUD, J.B. & SHENKER, Y. (1990). Effect of hypoxia on atrial natriuretic factor and aldosterone regulation in humans. *American Journal of Physiology* **258**, E243-E248.

LEON, D.A., KOUPILOVA, I., LITHELL, H.O., BERGLUND, L., MOHSEN, R., VÅGERO, D., LITHELL, U-B. & McKEIGUE, P.M. (1996). Failure to realise growth potential in utero and adult obesity in relation to blood pressure in 50 year old Swedish men. *British Medical Journal* **312**, 401-406.

LEW, R.A. & BAERTSCHI, A.J. (1988). Mechanisms of hypoxia-induced atrial natriuretic factor release from rat hearts. *American Journal of Physiology* **257**, H147-H156.

LINDSAY, R.S., LINDSAY, R.M., EDWARDS, C.R.W. & SECKL, J.R. (1996). Inhibition of 11b-hydroxysteroid dehydrogenase in pregnant rats and the programming of blood pressure in the offspring. *Hypertension* **27**, 1200-1204.

LITHELL, H.O., McKEIGUE, P.M., BERGLUND, L., MOHSEN, R., LITHELL, U-B. & LEON, D.A. (1996). Relation of size at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50-60 years. *British Medical Journal* **312**, 406-410.

LITTLE, W.A. (1960). The significance of placental/fetal weight ratios. *American Journal of Obstetrics and Gynecology* **79(1)**, 134-137.

LLANOS, A.J., BLOCK, B.S.B., COURT, D.J., GERMAIN, A.M. & PARER, J.T. (1988). Fetal oxygen uptake during uterine contractures. *Journal of Developmental Physiology* **10**, 525-529.

LUCAS, A. & MORLEY, R. (1994). Does early nutrition in infants born before term programme later blood pressure? *British Medical Journal* **309**, 304-308.

LUCAS, A. (1990). Does early diet program future outcome? *Acta Poediatrica Scandinavica Supplement* **365**, 58-67.

LUCAS, A., MORLEY, R., COLE, T.J., GORE, S.M., DAVIS, J.A., BAMFORD, M.F.M. & DOSSETOR, J.F.B. (1989). Early diet in preterm babies and developmental status in infancy. *Archives of Disease in Childhood* **64**, 1570-1578.

LUEDER, F.L., KIM, S-B., BUROKER, C.A., BANGALORE, C.A. & OGATA, E.S. (1995). Chronic maternal hypoxia retards fetal growth and increases glucose utilization of select fetal tissues in the rat. *Metabolism* **44(4)**, 532-537.

LUMBERS, E.R., McCLOSKEY, D.I. & POTTER, E.K. (1979). Inhibition by angiotensin II of baroreceptor-evoked activity in cardiac vagal efferent nerves in the dog. *Journal of Physiology* **294**, 69-80.

MACARA, L., KINGDOM, J.C.P., KAUFMANN, P., KOHNEN, G., HAIR, J., MORE, I.A.R., LYALL, F. & GREER, I.A. (1996). Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery doppler waveforms. *Placenta* **17**, 37-48.

MALEK, A.M., GIBBONS, G.H., DZAU, V.J. & IZUMO, S. (1993). Fluid shear stress differentially modulates expression of genes encoding basic fibroblast growth factor and platelet-derived growth factor B chain in vascular endothelium. *Journal of Clinical Investigation* **92**, 2013-2021.

MALLARD, E.C., WILLIAMS, C.E., JOHNSTON, B.M., GUNNING, M.I., DAVIS, S. & GLUCKMAN, P.D. (1995). Repeated episodes of umbilical cord occlusion in fetal sheep lead to preferential damage to the striatum and sensitize the heart to further insults. *Pediatric Research* **37(6)**, 707-713.

MALONEY, J.E., CANNATA, J., DOWLING, M.H., ELSE, W. & RITCHIE, B. (1977). Baroreflex activity in conscious fetal and newborn lambs. *Biology of the Neonate* **31**, 340-350.

MARSHALL, J.M. (1987). Analysis of cardiovascular responses evoked following changes in peripheral chemoreceptor activity in the rat. *Journal of Physiology* **394**, 393-414.

MARTIN, A.A., KAPOOR, R. & SCROOP, G.C. (1987). Hormonal factors in the control of heart rate in normoxaemic and hypoxaemic fetal, neonatal and adult sheep. *Journal of Developmental Physiology* **9**, 465-480.

MARTIN, W., SMITH, J.A. & WHITE, D.G. (1986). The mechanisms by which haemoglobin inhibits the relaxation of rabbit aorta induced by vasodilators, nitric oxide, or bovine retractor penis inhibitory factor. *British Journal of Pharmacology* **89**, 563-571.

MARTYN, C.N., BARKER, D.J.P. & OSMOND, C. (1996). Mothers' pelvic size, fetal growth, and death from stroke and coronary heart disease in men in the UK. *The Lancet* **348(9)**, 1264-1268.

MARTYN, C.N., BARKER, D.J.P., JESPERSON, S., GREENWALD, S., OSMOND, C. & BERRY, C. (1994). Growth in utero, adult blood pressure, and arterial compliance. *British Heart Journal* **73**, 116-121.

MASAKI, T. (1993). Overview: reduced sensiyivity of vascular response to endothelin. *Circulation* **87(suppl. V)**, V33-V35.

MATSUDA, Y., PATRICK, J., CARMICHAEL, L., CHALLIS, J. & RICHARDSON, B. (1992). Effects of sustained hypoxemia on the sheep fetus at midgestation: endocrine, cardiovascular, and biophysical responses. *American Journal of Obstetrics & Gynecology* **167**, 531-540.

MATSUDA, Y., PATRICK, J., CARMICHAEL, L., FRAHER, L. & RICHARDSON, B. (1992). Recovery of the ovine fetus from sustained hypoxia: effects on endocrine, cardiovascular, and biophysical activity. *American Journal of Obstetrics & Gynecology* **170**, 1433-1441.

MATTHEWS, S.G. & CHALLIS, J.R.G. (1995). Levels of pro-opiomelanocortin and prolactin mRNA in the fetal sheep pituitary following hypoxaemia and glucocorticoid treatment in late gestation. *Journal of Endocrinology* **147**, 139-146.

MATTHEWS, S.G. & CHALLIS, J.R.G. (1995). Regulation of CRH and AVP mRNA in the developing ovine hypothalamus: effects of stress and glucocorticoids. *American Journal of Physiology* **268**, E1096-E1107.

MATTHEWS, S.G., LU, F., YANG, K. & CHALLIS, J.R.G. (1995). Hypothalamic pituitary adrenal function in the sheep fetus. *Reproduction, Fertility & Development* **7**, 509-516.

MAYHEW, T.M. (1991). Scaling placental oxygen diffusion to birthweight: studies on placentae from low- and high-altitude pregnancies. *Journal of Anatomy* **175**, 187-194.

McARDLE, H.J., DOUGLAS, A.J., BOWEN, B.J. & MORGAN, E.H. (1985). The mechanism of iron uptake by the rat placenta. *Journal of Cellular Physiology* **124**, 446-450.

McCANCE, D.R., PETTITT, D.J., HANSON, R.L., JACOBSSON, L.T.H., KNOWLER, W.C. & BENNETT, P.H. (1994). Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype? *British Medical Journal* **308**, 942-945.

McCRABB, G.J., EGAN, A.R. & HOSKING, B.J. (1991). Maternal undernutrition during mid-pregnancy in sheep. Placental size and its relationship to calcium transfer during late pregnancy. *British Journal of Nutrition* **65**, 157-168.

MELKUMYANTS, A.M., BALASHOV, S.A. & KHAYUTIN, V.M. (1995). Control of arterial lumen by shear stress on endothelium. *News in Physiological Sciences* **10**, 204-210.

MELLOR, D.J. & MURRAY, L. (1981). Effects of placental weight and maternal nutrition on the growth rates of individual fetuses in single and twin bearing ewes during late pregnancy. *Research in Veterinary Science* **30**, 198-204.

MELLOR, D.J. & MATHESON, I.C. (1979). Daily changes in the curved crown-rump length of individual sheep fetuses during the last 60 days of pregnancy and effects of different levels of maternal nutrition. *Quarterly Journal of Experimental Physiology* **64**, 119-131.

MEYER, W.W. & LIND, J. (1974). Iliac arteries in children with a single umbilical artery. Structure, calcifications, and early atherosclerotic lesions. *Archives of Disease in Childhood* **49**, 671-679.

MEYERS, R.L., PAULICK, R.P., RUDOLPH, C.D. & RUDOLPH, A.M. (1991). Cardiovascular responses to acute, severe haemorrhage in fetal sheep. *Journal of Developmental Physiology* **15(4)**, 189-97.

MILNER, R.D.G. & GLUCKMAN, P.D. (1996). Regulation of intrauterine growth. In: *Pediatrics and Perinatology. The Scientific Basis,* eds. Gluckman, P.D. & Heymann, M.A. pp.284-289. Arnold.

MIRMIRAN, M. (1995). The function of fetal/neonatal rapid eye movement sleep. *Behavioural Brain Research* **69**, 13-22.

MOLL, W., GIRARD, H. & GROS, G. (1980). Facilitated diffusion of lactic acid in the guinea pig placenta. *Pflügers Archives* **385**, 229-238.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacological Reviews* **43(2)**, 109-142.

MONTEMAGNO, R. & SOOTHILL, P. (1995). Human fetal blood gases, glucose, lactate and amino acids in IUGR. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 3: Growth* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 201-221. Cambridge University Press.

MORLEY, R., LISTER, G., LEESON-PAYNE, C. & LUCAS, A. (1994). Size at birth and later blood pressure. *Archives of Disease in Childhood* **70**, 536-537.

MOSTELLO, D., CHALK, C., KHOURY, J., MACK, C.E., SIDDIQI, T.A. & CLARK, K.E. (1991). Chronic anemia in pregnant ewes: maternal and fetal effects. *American Journal of Physiology* **261**, R1075-R1083.

MUNSON, L., WILHITE, A., BOLTZ, V.F. & WILKINSON, J.E. (1996). Transforming growth factor β in bovine placentas. *Biology of Reproduction* **55**, 784-755.

MUROTSUKI, J., CHALLIS, J.R.G., HAN, V.K.M., FRAHER, L.J. & GAGNON, R. (1996a). Chronic fetal embolization and hypoxemia cause hypertension and myocardial hypertrophy in fetal sheep. *American Journal of Physiology* In press.

MUROTSUKI, J., GAGNON, R., MATTHEWS, S.G. & CHALLIS, J.R.G. (1996b). Effects of long-term hypoxaemia on pituitary-adrenal function in fetal sheep. *American Journal of Physiology* In press.

MURTHY, L.S., AGARWAL, K.N. & KHANNA, S. (1976). Placental morphometric and morphologic alterations in maternal undernutrition. *American Journal of Obstetrics & Gynecology* **124(6)**, 641-646.

NAEYE, R.L. (1987). Do placental weights have a clinical significance? *Human Pathology* **18**, 387-391.

NAKAZAWA, M., MIYAGAWA, S., OHNO, T., MIURA, S. & TAKAO, A. (1988). Developmental hemodynamic changes in rat embryos at 11 to 15 days of gestation: normal data of blood pressure and the effect of caffeine compared to data from chick embryo. *Pediatric Research* **23(2)**, 200-205.

NAKAZAWA, M., MIYAGAWA, TAKAO, A., CLARK, E.B. & HU, N. (1986). Hemodynamic effects of environmental hyperthermia in stage 18, 21, and 24 chick embryos. *Pediatric Research* **20(12)**, 1213-1215.

NAKAZAWA, M., MORISHIMA, M., TOMITA, H., TOMITA, S.M. & KAJIO, F. (1995). Hemodynamics and ventricular function in the day-12 rat embryo: basic characteristics and the responses to cardiovascular drugs. *Pediatric Research* **37(1)**, 117-123.

NATHANIELSZ, P.W. (1992). *Life Before Birth and a Time To Be Born* Promethean Press.

NELSON, R.R., GOBEL. F.L., JORGENSEN, C.R., WANG, K., WANG, Y. & TAYLOR. H.L. (1973). Hemodynamic predictors of myocardial oxygen consumption during static and dynamic exercise. *Circulation* **50**, 1179-1189.

NEWNHAM, J.P., KELLY. R.W., PATTERSON, L. & JAMES, I. (1991). The influence of maternal undernutrition in ovine twin pregnancy on fetal growth and Doppler flow-velocity waveforms. *Journal of Developmental Physiology* **16**, 277-282.

NEWTON, C. (1993). Weighing the placenta. *Nursing Times* **89(17)**, 52-54.

OGINO, Y., OKADA, S. & OTA, Z. (1994). Effects of chronic, urea-induced osmotic diuresis on kidney weight and function in rats. *Diabetologia* **37**, 225-231.

OKA, K., HIRANO, T. & HOMMA, M. (1993). Low-birthweight hypertension via enzyme and receptor imprintings of glucocorticoid. *The Lancet* **342**, 303-304.

OLIVER, M.H., HARDING, J.E., BREIER, B.H., EVANS, P.C. & GLUCKMAN, P.D. (1992). The nutritional regulation of circulating placental lactogen in fetal sheep. *Pediatric Research* **31**, 520-523.

OLIVETTI, G., QUAINI, F., LAGRASTA, C., RICCI, R., TOSINI, P., CAPASSO, J.M. & ANVERSA, P. (1993). Effects of genetic hypertension and nutritional anaemia on ventricular remodelling and myocardial damage in rats. *Cardiovascular Research* **27**, 1316-1325.

OSMOND, C., BARKER, D.J.P., WINTER, P.D., FALL, C.H.D. & SIMMONDS, S.J. (1993). Early growth and death from cardiovascular disease in women. *British Medical Journal* **307**, 1519-1524.

OSOFSKY, H.J. (1975). Relationships between nutrition during pregnancy abd subsequent infant and child development. *Obstetrical and Gynecological Survey* **30(4)**, 227-241.

OWENS, J.A., FALCONER, J. & ROBINSON, J.S. (1987a). Effect of restriction of placental growth on oxygen delivery to and consumption by the pregnant uterus and fetus. *Journal of Developmental Physiology* **9**, 137-150.

OWENS, J.A., FALCONER, J. & ROBINSON, J.S. (1987b). Effect of restriction of placental growth on fetal and utero-placental metabolism. *Journal of Developmental Physiology* **9**, 225-238.

OWENS, J.A., FALCONER, J. & ROBINSON, J.S. (1989). Glucose metabolism in pregnant sheep when placental growth is restricted. *American Journal of Physiology* **257**, R350-R357.

OWENS, J.A., FALCONER, J. & ROBINSON, J.S. (1987). Effect of restriction of placental growth on oxygen delivery to and consumption by the pregnant uterus and fetus. *Journal of Developmental Physiology* **9**, 137-150.

OWENS, J.A., KIND, K.L., CARBONE, F., ROBINSON, J.S. & OWENS, P.C. (1994). Circulating insulin-like growth factors-I and -II and substrates in fetal sheep following restriction of placental growth. *Journal of Endocrinology* **140**, 5-13.

OWENS, J.A., OWENS, P.C. & ROBINSON, J.S. (1995). Experimental restriction of fetal growth. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 3: Growth* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 139-175. Cambridge University Press.

OZOLINS, I.Z., YOUNG, I.R. & McMILLEN, I.C. (1992). Surgical disconnection of the hypothalamus from the fetal pituitary abolishes the corticotrophic response to intrauterine hypoglycemia or hypoxemia in the sheep during late gestation. *Endocrinilogy* **130(5)**, 2438-2445.

PAGE, K.R. (1993). *The Physiology of the Human Placenta.* UCL Press Ltd.

PAPPARELLA, A., BERARD, D. & STONESTREET, B.S. (1994). Circulatory and metabolic effects of anemia in hyperinsulinemic ovine fetuses. *American Jornal of Physiology* **266**, H250-H257.

PARKES, M.J. (1988). Endocrine factors in fetal growth. In: *Fetal and Neonatal Growth,* ed. Cockburn, F. pp. 33-48. John Wiley & Sons.

PECKHAM, C.H. & CHRISTIANSON, R.E. (1971). The relationship between prepregnancy weight and certain obstetric factors. *American Journal of Obstetrics & Gynecology* **111(1)**, 1-7.

PERRY, I.J., BEEVERS, D.G., WHINCUP, P.H. & BAREFORD, D. (1995). Predictors of ratio of placental weight to fetal weight in multiethnic community. *British Medical Journal* **310**, 436-439.

PERSSON, E. & JANSSON, T. (1992). Low birth weight is associated with elevated adult blood pressure in the chronically cathterized guinea-pig. *Acta Physiologica Scandinavia* **145**, 195-196.

PRABHAKAR, N.R. & KOU, Y.R. (1994). Inhibitory sympathetic action on the carotid body responses to sustained hypoxia. *Respiration Physiology* **95**, 67-79.

PRENTICE, A.M., WHITEHEAD, R.G., WATKINSON, M., LAMB, W.H. & COLE, T.J. (1983). Prenatal dietary supplementation of African women and birth-weight. *The Lancet* **1(8323)**, 489-492.

RAFF, H., KANE, C.W. & WOOD, C.E. (1991). Arginine vasopressin responses to hypoxia and hypercapnia in late-gestation fetal sheep. *American Journal of Physiology* **260**, R1077-R1081.

RAFF, H., KANE, C.W. & WOOD, C.E. (1991). Arginine vasopresssin responses to hypoxia and hypercapnia in late-gestation fetal sheep. *American Journal of Physiology* **260**, R1077-R1081.

RASMUSSEN, S. & ØIAN, P. (1993). First- and second-trimester hemoglobin levels. Relation to birth weight and gestational age. *Acta Obstetricia et Gynaecologica Scandinavia* **72**, 246-251.

RAVELLI, G-P., STEIN, Z.A. & SUSSER, M.W. (1976). Obesity in young men after famine exposure in utero and early infancy. *The New England Journal of Medicine* **295(7)**, 349-353.

RAWASHDEH, N.M., RAY, N.D., SUNDBERG, D.K. & ROSE, J.C. (1988). Comparison of hormonal responses to hypotension in mature and immature fetal lambs. *American Journal of Physiology* **255**, R67-R72.

RELLER, M.D., BURSON, M.A., LOHR, J.L., MORTON, M.J. & THORNBURG, K.L. (1995). Nitric oxide is an important determinant of coronary flow at rest and during hypoxemic stress in fetal lambs. *American Journal of Physiology* **269**, H2074-H2081.

RESHETNIKOVA, O.S., BURTON, G.J. & MILOVANOV, A.P. (1993). The effects of hypobaric hypoxia on the terminal villi of the human placenta. *Journal of Physiology* **459**, 308P.

REYNOLDS, T.S., PERKS, C.M., STEVENSON, K.R. & WATHES, D.C. (1995). Expression of insulin-like growth factor-II (IGF-II) mRNA and IGF-binding protein 2 (IGFBP-2) mRNA during early placental development in the ovine uterus. *Journal of Endocrinology* **141(Suppl)**, 41.

REYNOLDS, T.S., STEVENSON, K.R. & WATHES, D.C. (1996). Ovine placentome proliferation by insulin-like growth factor-II (IGF-II) is mediated by which IGF receptor. *Journal of Endocrinology* **143 (Suppl.)**, 93.

RICHARDSON, B., KORKOLA, S., ASANO, H., CHALLIS, J., POLK., D. & FRASER, M. (1996). Regional blood flow and endocrine response to sustained hypoxemia in the preterm ovine fetus. *Pediatric Research* **40(2)**, 337-343.

ROBINSON, J., CHIDZANJA, C., KIND, K., LOK, F., OWENS, P. & OWENS, J. (1995). Placental control of fetal growth. *Reproduction, Fertility & Development* **7**, 333-344.

ROBINSON, J.S., JONES, C.T. & KINGSTON, E.J. (1983). Studies on experimental growth retardation in sheep. The effects of maternal hypoxaemia. *Journal of Developmental Physiology* **5**, 89-100.

ROBSON, S.C. & CHANG, T.C. (1995). Measurement of human fetal growth. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 3: Growth* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 297-325. Cambridge University Press.

ROSE, J.C., MACDONALD, A.A. & HEYMANN, M.A. (1978). Developmental aspects of the pituitary-adrenal axis response to hemorrhagic stress in lamb fetuses in utero. *Journal of Clinical Investigation* **61**, 424-432.

ROSE, J.C., MEIS, P.J. & MORRIS, M. (1981). Ontogeny of endocrine (ACTH, vasopressin, cortisol) responses to hypotension in lamb fetuses. *American Journal of Physiology* **240**, E656-E661.

ROSSO. P., DONOSO, E., BRAUN, S., ESPINOZA, R. & SALAS, S.P. (1992). Hemodynamic changes in underweight pregnant women. *Obstetrics & Gynecology* **79**, 908-912.

RUDOLPH, A.M. & HEYMANN, M.A. (1970). Circulatory changes during growth in the fetal lamb. *Circulation Research* **26**, 289-299.

RUDOLPH, A.M. (1985). Distribution and regulation of blood flow in the fetal and neonatal lamb. *Circulation Research* **57(6)**, 811-821.

RUDOLPH, C.D. (1996). Fetal and postnatal liver blood flow.In: *Pediatrics and Perinatology. The Scientific Basis,* eds: Gluckman, P.D. & Heymann, M.A. pp. 658-660. Arnold.

RURAK, D.W. (1994). Fetal oxygenation, carbon dioxide homeostasis, and acid-base balance. In:*Textbook of Fetal Physiology*, eds. Thorburn, G.D & Harding, R. pp. 131-139. Oxford University Press.

RURAK, D.W., RICHARDSON, B.S., PATRICK, J.E., CARMICHAEL, L. & HOMAN, JACOBUS. (1990). Blood flow and oxygen delivery to fetal organs and tissues during sustained hypoxemia. *American Journal of Physiology* **258**, R1116- R1122.

RUSSEL, A. (1991). Body condition scoring of sheep. In: *Sheep and Goat Practice*, ed.Boden, E. pp. 3-10. Baillière Tindall.

SAITO, S., SAKAKURA, S., ENOMOTO, M., ICHIJO, M., MATSUMOTO, K. & NAKAMURA, T. (1995). Hepatocyte growth factor promotes the growth of cytotrophoblasts by the paracrine mechanism. *Journal of Biochemistry* **117**, 671-676.

SAUM, W.R., BROWN, A.M. & TULEY, F.H. (1976). An electrogenic sodium pump and baroreceptor function in normotensive and spontaneously hypertensive rats. *Circulation Research* **39(4)**, 497-505.

SCHEFFEN, I., KAUFMANN, P., PHILIPPENS, L., LEISER, R., GEISEN, C. & MOTTAGHY, K. (1990). Alterations of the fetal capillary bed in the guinea pig placenta following long-term hypoxia. *Advances in Experimental and Medical Biology* **277**, 779-789.

SCHOENFELD, A., OVADIA, Y., NERI, A. & FREEDMAN, S. (1989). Obstructive sleep apnea (OSA)-implications in maternal-fetal medicine. A hypothesis. *Medical Hypotheses* **30**, 51-54

SCHORK, N.J., JOKELAINEN, P., GRANT, E.J., SCHORK, A.M. & WEDER A.B. (1994). Relationship of growth and blood pressure in inbred rats. *American Journal of Physiology* **266**, R702-R708.

SCHULZE, K., KASHYAP, S. & RAMAKRISHNAN, R. (1993). Cardiorespiratory costs of growth in low birth weight infants. *Journal of Developmental Physiology* **19**, 85-90.

SECKL, J.R., BENEDIKTSSON, R., LINDSAY, R.S. & BROWN, R.W. (1995). Placental 11β-hydroxysteroid dehydrogenase and the programming of hypertension. *Journal of Steroid Biochemistry & Molecular Biology* **5/6**, 447-455.

SEGAR, J.L., HAJDUZOK, G., SMITH, B.A., MERRILL, D.C. & ROBILLARD, J.E. (1992). Ontogeny of baroreflex control of renal sympathetic nerve activity and heart rate. *American Journal of Physiology* **263**, H1819-H1826.

SEGAR, J.L., MERRILL. D.C. & ROBILLARD, J.E. (1994). Role of endogenous ANG II on resetting arterial baroreflex during development. *American Journal of Physiology* **266**, H52-H59.

SEIDMAN, D.S., LAOR, A., GALE, R., STEVENSON, D.K., MASHIACH, S., & DANON, Y.L. (1991). Birth weight, current body weight, and blood pressure in late adolescence. *British Medical Journal* **302**, 1235-1237.

SHAPER, A.G. & ELFORD, J. (1991). Place of birth and adult cardiovascular disease: the British regional heart study. *Acta Poediatrica Scandinavia Supplements* **373**, 73-81.

SHARKEY, A.M., KING, A., JOKHI, P.P., LOKE, Y.W. & SMITH, S.K. (1994). Haematopoietic growth factor expression in the trophoblast. In: *Early Fetal Growth and Development*, , eds. Ward, R.H.T., Smith, S.K. & Donnai, D. pp. 183-196. RCOG Press.

SHEPHERD, L.A., BECKETT, V.A., LACHELIN, G.C.L. & McGARRIGLE, H.H.G. (1996). Absence of 11b-Hydroxysteroid dehydrogenase activity in isolated human placental cytotrophoblast cell preparations. *Journal of Endocrinology* **147(Supplement)**, P108.

SHERER, D.M., CAVERLY, C.B. & ABRAMOWICZ, J.S. (1991). Severe obstructive sleep apnea and associated snoring documented during external tocography. *American Journal of Obstetrics & Gynaecology* **165**, 1300-1301.

SHIELDS, L.E., WIDNESS, J.A. BRACE, R.A. (1993). Restoration of fetal red blood cells and plasma proteins after a moderately severe hemorrhage in the ovine fetus. *American Journal of Obstetrics & Gynecology* **169**, 1472-1478.

SILBERBACH, M., WOODS, L.L., HOHIMER, A.R., SHIOTA, T., MATSUDA, Y. & DAVIS, L.E. (1995). Role of endogenous atrial natriuretic peptide in chronic anaemia in the ovine fetus: effects of a non-peptide antagonist for atrial natriuretic peptide receptor. *Pediatric Research* **38**, 722-728.

SIMMONS, M.A., BATTAGLIA, F.C. & MESCHIA, G. (1979). Placental transfer of glucose. *Journal of developmental Physiology* **1**, 227-243.

SIMPSON, J.W., LAWLESS, R.W. & MITCHELL, A.C. (1975). Responsibility of the obstetrician to the fetus. II. Influence of prepregnancy weight and pregnancy weight gain on birthweight. *Obstetrics & Gynecology* **45(5)**, 481-487.

SMIESCO,V. & JOHNSON, P.C. (1993). The arterial lumen is controlled by flow-related shear stress. *News in Physiological Sciences* **8**, 34-38.

SMITH, F.G., SATO, T., VARILLE, V.A. & ROBILLARD, J.E. (1989). Atrial natriuretic factor during fetal and postnatal life: a review. *Journal of Developmental Physiology* **12**, 55-62.

SNIJDERS, R.J.M., ABBAS, A., MELBY, O., IRELAND, R.M. & NICOLAIDES, K.H. (1993). Fetal plasma erythropoietin concentration in severe growth retardation. *American Journal of Obstetrics & Gynecology* **168**, 615-619.

SOOTHILL, P.W., NICOLAIDES, K.H., RODECK, C.H. & BELLINGHAM, A.J. (1988). The effect of replacing fetal hemoglobin with adult hemoglobin on blood gas and acid-base parameters in human fetuses. *American Journal of Obstetrics & Gynecology* **158**, 66-69.

SOOTHILL, P.W.; NICOLAIDES, K.H., BILARDO, C.M. & CAMPBELL, S. (1986). Relation of fetal hypoxia in growth retardation to mean blood velocity in the fetal aorta. *The Lancet* **2(8516)**, 1118-1120.

SPYER, K.M. (1994). Central nervous mechanisms contributing to cardiovascular control. *Journal of Physiology* **474(1)**, 1-19.

STEIN, C.E., FALL, C.H.D., KUMARAN, K., OSMOND, C., COX, V. & BARKER, D.J.P. (1996). Fetal growth and coronary heart disease in South India. *The Lancet* **348**, 1269-1273.

STEIN, Z. & SUSSER, M. (1975 a). The Dutch famine, 1944-1945, and the reproductive process. I. Effects on six indices at birth. *Pediatric Research* **9**, 70-76.

STEIN, Z. & SUSSER, M. (1975 b). The Dutch famine, 1944-1945, and the reproductive process. II. Interrelations of caloric rations and six indices at birth. *Pediatric Research* **9**, 76-83.

STEVEN, D.H. & MORRISS, G. (1975). Development of the foetal membranes. In: *Comparative Placentation. Essays in Structure and Function.* ed. Steven, D.H. pp. 58-86. Academic Press.

STEVEN, D.H. (1975). *Comparative Placentation. Essays in Structure and Function.* Academic Press.

STEVENSON, , K.R., GILMOUR, R.S. & WATHES, D.C. (1994). Localization of insulin-like growth factor-I (IGF-I) and -II messenger ribonucleic acid and type 1 IGF receptors in the ovine uterus during the estrous cycle and early pregnancy. *Endocrinology* **134(4)**, 1655-1664.

STRUIJKER-BOUDIER, H.A.J., SMITS, J.F.M. & DE MEY, J.G.R. (1995). Pharmacology of cardiac and vascular remodeling. *Annual Reviews in Pharmacology and Toxicology* **35**, 509-539.

SYMONDS, M.E. (1995). Pregnancy, parturition and neonatal development: interactions between nutrition and thyroid hormones. *Proceedings of the Nutrition Society* **54**, 329-343.

TAITTONEN, L., NUUTINEN, M., TURTINEN, J. & UHARI, M. (1996). Prenatal and postnatal factors in predicting later blood pressure among children: cardiovascular risk in young Finns. *Pediatric Research* **40(4)**, 627-632.

TANAKA, M., NATORI, M., ISHIMOTO, H., MIYAZAKI, T., KOBAYASHI, T. & NOZAWA, S. (1994). Experimental growth retardation produced by transient period of uteroplacental ischemia in pregnant Sprague-Dawley rats. *American Journal of Obstetrics & Gynecology* **171**, 1231-1234.

TANGALAKIS, K., LUMBERS, E.R., MORITZ, K.M., TOWSTOLESS, M.K. & WINTOUR, E.M. (1992). Effect of cortisol on blood pressure and vascular reactivity in the ovine fetus. *Experimental Physiology* **77**, 709-717.

TEITEL, D.F. & HOFFMAN, J.I.E. (1996). Myocardial nervous innervation and regulation. In: *Pediatrics and Perinatology. The Scientific Basis,* eds: Gluckman, P.D. & Heymann, M.A. pp. 729-730. Arnold.

TEITEL, D.F. (1988). Circulatory adjustments to postnatal life. *Seminars in Perinatology* **12(2)**, 96-103.

THILAGANATHAN, B., SALVESEN, D.R., ABBAS, A., IRELAND, R.M. & NICOLAIDES, K.H. (1992). Fetal plasma erythropoietin concentration in red blood cell-isoimmunized pregnancies. *American Journal of Obstetrics & Gynecology* **167**, 1292-1297.

THOMPSON, S.B.N. (1993). *Eating Disorders. A guide for health professionals.* Chapman & Hall.

THORNBURG, K.L. & MORTON, M. (1993). Growth and development of the heart. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 1: The Circulation,* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 1-22. Cambridge University Press.

THORNBURG, K.L. & MORTON, M. (1994). Development of the cardiovascular system. In:*Textbook of Fetal Physiology*, eds. Thorburn, G.D. & Harding, R. pp.95-130. Oxford University Press.

TILKIAN, A.G., GUILLEMINAULT, C., SCHROEDER, J.S., LEHRMAN, K.L., SIMMONS, F.B. & DEMENT, W.C. (1976). Hemodynamics in sleep-induced apnea. Studies during wakefulness and sleep. *Annals of Internal Medicine* **85(6)**, 714-719.

TOMOMATSU, E. & NISHI, K. (1982). Comparison of carotid sinus baroreceptor sensitivity in newborn and adult rabbits. *American Journal of Physiology* **243**, H546-H550.

TREASURE, J.L. & RUSSEKK, G.F.M. (1988). Intrauterine growth and neonatal weight gain in babies of women with anorexia nervosa. *British Medical Journal* **296**, 1038.

TREASURE, J.L. & RUSSELL, G.F.M. (1988). Intrauterine growth and neonatal weight gain in babies of women with anorexia nervosa. *British Medical Journal* **296**, 1038.

TRUDINGER, B.J., STEVENS, D., CONNELLY, A., HALES, J.R.S., ALEXANDER, G., BRADLEY, L., FAWCETT, A. & THOMPSON, R.S. (1987). Umbilical artery flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation. *American Journal of Obstetrics & Gynecology* **157**, 1443-1448.

UEHARA, Y., MINOWA, O., MORI, C., SHIOTA, K., KUNO, J., NODA, T. & KITAMURA, N. (1995). Placental defect and embryonic lethality in mice lacking hepatocyte growth factor / scatter factor. *Nature* **373**, 702-705.

VALLET, J.L. & CHRISTENSON, R.K. (1993). Uterine space affects placental protein secretion in swine. *Biology of Reproduction* **48**, 575-584.

VATNICK, I., SCHOKNECHT, P.A., DARRIGRAND, R. & BELL, A.W. (1991). Growth and metabolism of the placenta after unilateral fetectomy in twin pregnant ewes. *Journal of Developmental Physiology* **15**, 351-356.

VIJAYAKUMAR, M., FALL, C.H.D., OSMOND, C. & BARKER, D.J.P. (1994). Birth weight, weight at one year, and left ventricular mass in adult life. *British Heart Journal* **73**, 363-367.

VILLE, PROUDLER, A., KUHN, P. & NICOLAIDES, K.H. (1994). Aldosterone concentration in normal, growth-retarded, anemic, and hydropic fetuses. *Obstetrics & Gynecology* **84**, 511-514.

WAGMAN, A.J., HU, N. & CLARK, E.B. (1990). Effect of changes in circulating blood volume on cardiac output and arterial and ventricular blood pressure in the stage 18, 24, and 29 chick embryo. *Circulation Research* **67**, 187-192.

WAKATSUKI, A., MURATA, Y., NINOMIYA, Y., MASAOKA, N., TYNER, J.G. & KUTTY, K.K. (1992). Physiologic baroreceptor activity in the fetal lamb. *American Journal of Obstetrics & Gynecology* **167**, 820-827.

WALKER, A.M. (1993). Circulatory transitions at birth and the control of the neonatal circulation. In: *Fetus and Neonate: Physiology and Clinical Applications. Volume 3: The Circulation.* eds. Hanson, M.A., Spencer, J.A.D. & Rodeck, C.H. pp. 160-196. Cambridge University Press.

WALKER, B.R. & WILLIAMS, B.C. (1992). Corticosteroids and vascular tone: mapping the messenger maze. *Clinical Science* **82**, 597-605.

WANGO, E.O., HEAP, R.B. & WOODING, F.B.P. (1992). Regulation of steroid synthesis and metabolism in isolated binucleate cells of the placenta in sheep and goats. *Journal of Reproduction & Fertility* **94**, 203-211.

WANNAMETHEE, S.G., WHINCUP, P.H., SHAPER, G. & WALKER, M. (1996). Influence of fathers' social class on cardiovascular disease in middle-aged men. *The Lancet* **348**, 1259-1263.

WAYNFORTH, H.B. (1980). Vital statistics and miscellaneous information. In: *Experimental and surgical techniques in the rat.* Academic Press.

WEIHE, W.H. 1989. The laboratory rat. In: *The UFAW Handbook on the Care and Management of Laboratory Animals. Sixth Edition,* ed. Poole, T.B. Longman Scientific & Tecnical.

WHEELER, T., ELCOCK, C.L. & ANTHONY, F.W. (1995). Angiogenesis and the placental environment. *Placenta* **16**, 289-296.

WHEELER, T., SOLLERO, C., ALDERMAN, S., LANDEN, J., ANTHONY, F. & OSMOND, C. (1994). Relation between maternal haemoglobin and placental hormone concentrations in early pregnancy. *The Lancet* **343**, 511-313.

WHINCUP, P.H., COOK, D.G. & SHAPER, A.G. (1989). Early influences on blood pressure: a study of children aged 5-7 years. *British Medical Journal* **299**, 587-591.

WHINCUP, P.H., PERRY, I.J. & SHAPER, A.G. (1994). Regional differences in blood pressure in developed countries: fetal influences. In: *Textbook of Hypertension,* ed: Swales, J.D. pp. 36-45. Blackwell Scientific Publications.

WIDDOWSON, E.M. & McCANCE, R.A. (1975). A review: new thoughts on growth. *Pediatric Research* **9**, 154-156.

WILKENING, R.B. & MESCHIA, G. (1989). Effect of umbilical blood flow on transplacental diffusion of ethanol and oxygen. *American Journal of Physiology* **256(3)**, H813-H820.

WILKENING, R.B., BOYLE, D.W. & MESCHIA, G. (1989). Fetal neuromuscular blockade: effect on oxygen demand and placental transport. *American Journal of Physiology* **257**, H734-H738.

WINICK, M. (1969). Malnutrition and brain development. *Journal of Pediatrics* **74(5)**, 667-679.

WINICK, M., COSCIA, A. & NOBLE, A. (1967). Cellular growth in human placenta. I. Normal placental growth. *Pediatrics* **39(2)**, 248-251.

WOOD, C.E. & CHEN, H-G. (1989). Acidemia stimulates ACTH, vasopressin, and heart rate responses in fetal sheep. *American Journal of Physiology* **257**, R344-R349.

WOOD, C.E. (1989). Sinoaortic denervation attenuates the reflex responses to hypotension in fetal sheep. *American Journal of Physiology* **256**, R1103-R1110.

WOOD, C.E. (1995). Baroreflex and chemoreflex control of fetal hormone secretion. *Reproduction, Fertility & Development* **7**, 479-489.

WOOD, C.E., CHEN, H-G, & BELL, M.E. (1989). Role of vagosympathetic fibers in the control of adrenocorticotropic hormone, vasopressin, and renin responses to hemorrhage in fetal sheep. *Circulation Research* **64**, 515-523.

WOODING, F.B.P. & FLINT, A.P.F. (1994). Placentation. In: *Marshall's Physiology of Reproduction. Volume 3. Pregnancy and Lactation. Part One. Ovulation and early pregnancy* **Fourth Edition**, ed. Lamming, G.E. pp. 233-460. Chapman & Hall.

WOUDSTRA, B.R., DE WOLF, B.T.H.M., SMITS, T.M., NATHANIELSZ, P.W., ZIJLSTRA, W.G. & AARNOUDSE, J.G. (1995). Variability of continuously measured arterial pH and blood gas values in the near term fetal lamb. *Pediatric Research* **38(4)**, 528-532.

XAVIER-NETO, J. & KRIEGER, E.M. (1995). Viscoelastic behaviour of "in situ" aortic wall during hemorrhagic hypotension. *American Journal of Physiology* **268**, H1773-H1780.

XIE, P., CHAPLEAU, M.W., McDOWELL, T.S., HAJDUCZOK, G. & ABBOUD, F.M. (1990). Mechanism of decreased baroreceptor activity in chronic hypertensive rabbits. Role of endogenous prostanoids. *Journal of Clinical Investigation* **86**, 625-630.

YARDLEY, R.W., BOWES, G., WILKINSON, M., CANNATA, J.P., MALONEY, J.E., RITCHIE, B.C. & WALKER, A.M. (1983). Increased arterial pressure variability after arterial baroreceptor denervation in fetal lambs. *Circulation Research* **52**, 580-588).

YATES, J.R.W. (1988). Genetics of fetal and postnatal growth. In: *Perinatal Practice, volume 5. Fetal and Neonatal Growth*, ed. Cockburn, F. pp.1-10. John Wiley & Sons.

YAU, J.L.W., OLSSON, T., MORRIS, R.G.M., MEANEY, M.J. & SECKL, J.R. (1995). Glucocorticoids, hippocampal corticosteroid receptor gene expression and antidepressant treatment: relationship with spatial learning in young and aged rats. *Neuroscience* **66(3)**, 571-581.

ZAHALKA, E.A., SEIDLER, F.J. & SLOTKIN, T.A. (1993). Dexamethasone treatment *in utero* enhances neonatal cholinergic nerve terminal development in rat brain. *Research Communications in Chemical Pathology and Pharmacology* **81(2)**, 191-198.

ZHOU, J. & BONDY, C.A. (1993). Placental glucose transporter gene expression and metabolism in the rat. *Journal of Clinical Investigation* **91**, 845-852.

ZHU-S-T., CHEN, Y-F., WYSS, J.M., NAKAO, K., IMURA, H., OPARIL, S. & CALHOUN, D.A. (1996). Atrial natriuretic peptide blunts arterial baroreflex in spontaneously hypertensive rats. *Hypertension* **27**, 297-302.

ZUREIK, M., BONITHON-KOPP, C., LECOMTE, E., SIEST, G. & DUCIMETIÈRE, P. (1996). Weights at birth and in early infancy, systolic pressure, and left ventricular structure in subjects aged 8 to 24 years. *Hypertension* **27(1)**, 339-345.

APPENDICES

APPENDIX 1
General Biological Data

Human

Basic physiological data

Adult

Body weight (kg)	70
Rectal temperature (°C)	37
Heart rate (bpm)	70
MAP (mmHg)	100
Respiratory frequency (breaths min^{-1})	15-20
Tidal volume (ml)	400
Haemoglobin (g/dl)	14.5
Life span (years)	70

Neonate

Weight at birth (kg)	3.5

Basic data on reproduction

Age at sexual maturity (years)	12-15
Length of oestrous cycle (days)	28
Gestation (month)	9

Rat (from Weihe, 1989).

Basic physiological data

Adult

Body weight (g)	240-480
Rectal temperature (°C)	38-39
Heart rate (bpm)	320-480
MAP (mmHg)	100
Respiratory frequency (breaths min^{-1})	85-110
Tidal volume (ml)	1.6
Haemoglobin (g dl^{-1})	12.8
Life span (years)	2-3.5

Neonate

Weight at birth (g)	5-6
Age at weaning (day)	21
Weight at weaning (g)	45

Basic data on reproduction

Age at sexual maturity (day)	40-50
Length of oestrous cycle (days)	4-6
Gestation (day)	21
Average litter size (young)	11

Sheep (from Harrison, 1989)

Basic physiological data

Adult

Body weight (kg)	45-100
Rectal temperature (°C)	39.5
Heart rate (bpm)	70-80
MAP (mmHg)	100
Respiratory frequency (breaths min^{-1})	15-25
Tidal volume (ml)	1.4
Haemoglobin (g/dl)	10.3
Life span (years)	6-8

Neonate

Weight at birth (kg)	4.5-5.5

Basic data on reproduction

Age at sexual maturity (months)	8-12
Length of oestrous cycle (days)	16
Gestation (day)	147

APPENDIX 2
Average Gestational Length of Various Species

Species	Length of gestation (days)
Chicken	21
Cow	280
Guinea pig	68
Horse	335
Human	280
Llama	345
Pig	115
Rabbit	32
Rat	21
Sheep	147

APPENDIX 3
Drugs

ANAESTHETICS

Intraval Sodium (Rhône Mérieux Ltd.)

Thiopentone Sodium BP, which is a mixture of 100 parts w/w of the monosodium derivative of 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid and 6 parts w/w of dried sodium carbonate.

Uses

Induction and maintenance of anaesthesia. In the context of this thesis "Intraval' was only used for the induction of anaesthesia.

Dosage and administration

5 g of Intraval was dissolved in 50 ml isotonic saline to make a solution that contained 10 g/ml (10% w/v). 12 ml (1.2 g) of Intraval solution was administered to the ewe i.v.

Fluothane (ICI Pharmaceuticals)

Chemically it is 2-bromo-2-chloro-1, 1, 1-trifluorethane stabilised with thymol 0.01%.

Uses

Fluothane is an inhalational anaesthetic, that may be used for all types of veterinary surgery.

Dosage and administration

Fluothane was administered in oxygen via a vapouriser. In this thesis it was used only for the maintenance of anaesthesia (not induction) at a concentration of about 2% in oxygen (2 l/min).

ANTIBIOTICS

Crystapen (Brittania Pharmaceuticals Ltd.)

Sodium benzylpenicillin (penicillin G)

Uses

Bacteriacidal activity against penicillin-sensitive organisms, particularly streptococci, pneumococci. meningococci, gonococci and staphylococci (except penicillinase-producing strains).

Dosage and administration

Surgery : 600 mg Crystapen was dissolved in 4 ml isotonic saline and administered intra-amniotically.

5 post-operative days: 600 mg Crystapen was dissolved in 4 ml isotonic saline and administered 2 ml (300 mg) was administered i.v. to the ewe, 1 ml (150 mg) i.v. to the fetus, and a further 1 ml (150 mg) intra-amniotically. .

Cidomycin (Roussel)

Gentamicin sulphate.

Uses

Bacteriacidal activity against many Gram-positive and Gram-negative bacteria, particularly streptococci, pneu,ococci, meningococci, gonococci and staphylococci (except penicillinase-producing strains). Gentamicin is also often active against strains that are resistant to other antibiotics e.g. streptomycin, and is effective against penicillin-resistant Staphyloccocci.

Dosage and administration

Surgery: 2 ml (80 mg) Cidomycin was administerd intra-amniotically.

5 post-operative days: 1 ml (40 mg) was administered i.v. to the ewe and 1 ml (40 mg) intra-amniotically.

Cidomycin was never administered directly to the fetus because of its ototoxicity and nephrotoxicity.

Streptopen (Pitman-Moore)

Procaine penicillin G (250 mg) in a solution of dihydrostreptomycin sulphate (250 mg).

Uses

Active against Gram-positive and Gram-negative bacteria.

Dosage and administration

Surgery: 4 ml Streptopen i.m. to the ewe.

EUTHANASIA

Euthatal (Rhône Mérieux)

Pentobarbitone sodium (200 mg/ml)

Uses

For the euthanasia of animals.

Dosage and administration

40 ml (ca. 130 mg/kg) of Euthatal was injected i.v. to the ewe.

MISCELLANEOUS

Heparin (CP Pharmaceuticals Ltd.)

Heparin sodium 5000 iu/ml

Uses

Heparin is an anticoagulant. It acts by inhibiting thrombin and potentiating the naturally occurring inhibitors of factor X.

Dosage and administration

Surgery: 1000 iu was mixed with ca. 450 ml isotonic saline to form a solution containing about 2 iu/ml of heparin. This was then used to flush the vascular catheters.

Thereafter: 5000 iu was mixed with 500 ml of sterile saline to form a solution of 10 iu/ml of heparin. This solution was used daily for flushing catheters.

APPENDIX 4
Blood gas machine and CO-Oximeter

BLOOD GAS MACHINE (BGE, INSTRUMENTATION LABORATORIES)

Sample volume - 240 µl

Measured parameters	Calculated parameters
PCO_2	HCO_3^-
Na^+	Base excess (BE)
Ca^{++}	Temperature corrections for PCO_2, PO_2 and pH
pH	
PO_2	
K^+	
Haematocrit	

Calculations

Calculated parameters

HCO_3^-

$\log_{10}\{HCO_3^-] = pH + \log_{10} PCO_2 - 7.604$

BE

$BE = (1 - 0.014)\ [Hb])\ ([HCO_3^-] - 24 + (1.43\ [Hb] + 7.7)\ (pH - 7.4)$

Temperature corrections

To obtain an accurate reflection of *in vivo* conditions, pH, PCO_2 and PO_2 values were corrected to 39.5°C, the core temperature of the sheep.

pH

$\Delta pH/\Delta T = -0.0147 + 0.0065\ (7.4 - pH)$

PCO_2

$\Delta \log_{10} PCO_2 / \Delta T = 0.019$

PO_2

$\Delta \log_{10} PO_2 / \Delta T = (5.49 \times 10^{-11}\ PO_2^{3.88} + 0.071) / (9.72 \times 10^{-9}\ PO_2^{3.88} + 2.30)$

CO-Oximeter (IL 482, INSTRUMENTATION LABORATORIES)

The co-oximeter is interfaced to the blood gas machine.

Sample volume - 85 µl

Measured parameters	Calculated parameters
Total haemoglobin (Hb)	Oxygen content
	Oxygen saturation

Calculations

Calculated parameters

Oxygen content

$CaO_2 = \{Hb \times 1.39 \times (\%O_2aHb / 100)\} + \{0.0031 \times PaO_2)$

Oxygen saturation

$SaO_2 = \{(\%O_2Hb) / 100 - (\%COHb + \%\ MetHb)\} \times 100$

APPENDIX 5
Hormone Assays

CATECHOLAMINES

This assay was performed by Dr. Clare Smith, Department of Pharmacology and Medicine, University College London.

2 ml aliquots of plasma were treated with 20 mg alumina and 400 µl of 2M Tris buffer, and 5 pmol (25 µl) DHBA internal standard. Catecholamine (adrenaline And noradrenaline) concentration was determined using HPLC with electrochemical detection. The HPLC system consisted of a Waters "resolve" C18 reversed-phase column (3.9mm x 150 mm, particle 5µM), a waters Model 460 electrochemical detector with glossy carbon electrode set ata potential of +0.58v vs. KCl and a sensitivity of 0.5 nA and a waters model 510 HPLC pump. Data was recorded using a Waters 740 Data Module (Waters, Division of Millipore Ltd., Middlesex, U.K.). Separation was achieved using an isocratic solvent system that consisted of an acetate-citrate buffer that contained sodium octane sulphonate (5.8 mmol l^{-1}), EDTA (3 mmol l^{-1}) and 14% methanol, with the flow rate maintained at 1 ml min^{-1}.

Adrenaline and noradrenaline recoveries were more than 80% and the sensitivity of the assay was 0.1 pmol per 70 µl injection into the HPLC column. Interassay coefficients of variation were 13% for adrenaline and 8% for noradrenaline, and the intraassay coefficients of variation were 5% and 4% respectively.

CORTISOL

This assay was performed by Dr. H.H.G. McGarrigle, Department of Obstetrics and Gynaecology, University College London.

Plasma cortisol concentrations were measured by radio-immuno assay (RIA) using ^{125}I cortisol as a tracer. Duplicate 50 µl aliquots of plasma were mixed with an equal volume of sodium bicarbonate solution (1.7 M, pH 7.5) and extracted with 2 ml of diethyl ether. After freezing, the ether was decanted

and evaporated, and the residue was reconstituted in 500 µl of phosphate buffered saline (pH 7.4).

For the RIA procedure, aliquots were removed and mixed with phosphate-buffered saline, to make up a total volume of 400 µl, and incubated with 16000 dpm[1,2,6,7-3H]cortisol (Amersham International, Aylesbury, U.K.) and 100 ml of anti-cortisol antiserum (Steranti Ltd., St. Albans, U.K.). Bound and free cortisol were separated using dextran-coated charcoal and, after centrifugation, a 500 µl aliquot was removed and the radioactive content measured.

The assay recovery was 90%. The sensitivity of the assay was 30 fmol ml^{-1}.

THYROXINE (T_4)

This assay was performed by Dr. L.Clarke and Dr. M. Symonds, School of Animal and Microbial Sciences, University of Reading.

T_4 was assayed using "double antibody" RIA. Standards (6.3 - 400 nM, Sigma Chemical Company, Poole, Doreset, U.K.) were prepared in iodothyronine-free plasma which was obtained by treating sheep plasma with charcoal (100g charcoal / 500 ml plasma) at 4°C for 24 hours.Barbital buffer (0.075 M) was used, to which was added 0.05% 8-anilo-1-naphthalenesulphonic acid (ammonium salt) to block interference by thyroid hormone binding proteins. 100 µl specific antisera for T_4, developed in rabbits, (final dilution 1:1000 in 0.01 M phosphate buffer, pH 7.4, Sigma Chemical Company, Poole, Dorset, U.K.) was incubated at 37°C for 1 hour with 5 µl of sample/standard and 200 µl of ^{125}I-T_4 (Amersham International, Aylesbury, U.K.). 100 µl of sheep anti-rabbit gamma globulin 6% (1:40 dilution in phosphate buffer), 100 µl 0.1 M EDTA and 500 µl polyethylene glycol 6000 was then added. This was then incubated for 24 hours at 4°C. Following centrifugation the supernatant was aspirated and the radioactivity in the precipitate measured in an automatic gamma counter (LKB-Wallac 1260 multigamma 11 counter).

The interassay coefficient of variation was 7.7.%.

APPENDIX 6
Abstracts

THE SOCIETY FOR THE STUDY OF FETAL PHYSIOLOGY

20TH ANNUAL MEETING

May 16th-19th, 1993. Plymouth, U.K.

P2

THE EFFECTS OF ANAEMIA IN UTERO AND PRE-WEANING ON HEART SIZE AND BLOOD PRESSURE IN NEONATAL RATS

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Anaemia in early pregnancy has been suggested to result in a greater placental to birthweight ratio, and this has been reputed to be associated with high blood pressure and cardiovascular disease later in life. We are using an animal model for studying the mechanism of this phenomenon. Nutritional anaemia was induced in female rats prior to mating, and their pups were studied on post-natal days 17-20. Anaemic pups (haemoglobin 4.49 g/dl $\pm$ 0.29 vs. 8.61 g/dl $\pm$ 0.78; $p < 0.05$ from age-matched controls) had significantly lower body and liver weights than control animals, but the anaemic pups had hearts that were larger (0.51 g $\pm$ 0.03; $p < 0.05$). This suggests an alteration in the cardiovascular development of these pups, in support of Barker's ideas. However, we found that the systolic blood pressure, measured non-invasively in the unanaesthetised pups, was significantly lower in the anaemic rats than in the control animals (127 mmHg $\pm$ 5.64 vs. 145 mmHg $\pm$ 2.92; $p < 0.05$).

1. Godfrey KM, Redman CWG, Barker DJP, Osmond C. Br J Obstet Gynaecol 1991; 98:886-891

XXXII CONGRESS OF THE INTERNATIONAL UNION OF PHYSIOLOGICAL SCIENCES

August 1st - 6th, 1993. Glasgow, U.K.

214.10/P

THE EFFECTS OF ANAEMIA IN UTERO AND PRE-WEANING ON HEART SIZE AND BLOOD PRESSURE IN NEONATAL RATS

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Anaemia in early pregnancy has been suggested to result in a greater placental to birthweight ratio, and this has been reputed to be associated with high blood pressure and cardiovascular disease later in life (Godfrey, K.M. et al. (1991) Br J Obstet Gynaecol 98, 886-891). We are using an animal model for studying the mechanism of this phenomenon. Nutritional anaemia was induced in female rats prior to mating, and their pups were studied on post-natal days 17-20. Anaemic pups (haemoglobin 4.49 g/dl $\pm$ 0.29 vs. 8.61 g/dl $\pm$ 0.78; $p < 0.05$ from age-matched controls) had significantly lower body and liver weights than control animals. However, the anaemic pups had hearts which were larger (0.51 g $\pm$ 0.02 vs. 0.30 $\pm$ 0.03; $p < 0.05$). This suggests an alteration in the cardiovascular development of these pups, in support of Barker's ideas. We have not yet, however, been able to find a significant difference in the systolic blood pressures between anaemic and control animals, measured non-invasively in the unanaesthetised pups.

European Society for the

STUDY and PREVENTION

of INFANT DEATHS

THIRD EUROPEAN CONGRESS

August 26th - 29th, 1993. Oxford, U.K.

THE EFFECTS OF ANAEMIA IN UTERO AND PRE-WENING ON HEART SIZE AND BLOOD PRESSURE IN NEONATAL RATS

Crowe, C., Dandekar, P., Bennet, L., Hanson, M.A.

Department of Obstetrics and Gynaecology, University College London, London, U.K.

Anaemia in early pregnancy has been suggested to result in a greater placental to birthweight ratio, and this has been reputed to be associated with high blood pressure and cardiovascular disease later in life. We are using an animal model for studying the mechanism of this phenomenon. Nutritional anaemia was induced in female rats prior to mating, and their pups were studied on post-natal days 17-20. Anaemic pups (haemoglobin 4.49 g/dl ± 0.29 vs. 8.61 g/dl ± 0.78; $p < 0.05$ from age-matched controls) had significantly lower body and liver weights than control animals, but the anaemic pups had hearts that were larger (0.51 g ± 0.03 vs. 0.30 ± 0.03; $p < 0.05$). This suggests an alteration in the cardiovascular development of these pups, in support of Barker's ideas. However, we found that the systolic blood pressure, measured non-invasively in the unanaesthetised pups, was significantly lower in the anaemic rats than in the control animals (127 mmHg ± 5.64 vs. 145 mmHg ± 2.92; $p < 0.05$).

REFERENCE

Godfrey, K.M., Redman, C.W.G., Barker, D.J.P., Osmond, C. (1991) *Br J Obstet Gynaecol* **98**, 886-891.

THE SOCIETY FOR THE STUDY OF FETAL PHYSIOLOGY

21st ANNUAL MEETING

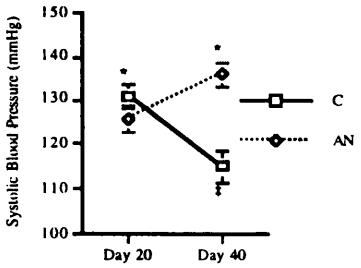
July 30th - August 3rd, 1994. Cairns, Australia.

A42

THE EFFECTS OF SEVERE ANAEMIA IN UTERO AND PRE-WEANING ON HEART SIZE, PLACENTAL TO BIRTH WEIGHT RATIO AND BLOOD PRESSURE IN RATS

CROWE,C., DANDEKAR,P., FOX,M., DHINGRA,K., BENNET,L., HANSON,M.A.
Department of Obstetrics and Gynaecology, University College London, London, U.K.

Anaemia in early pregnancy has been suggested to result in a greater placental to birth weight ratio which is associated with high blood pressure and cardiovascular disease in later life (Godfrey et al. 1991). We have attempted to duplicate these findings in the rat. Nutritional anaemia was induced in female rats prior to mating and maintained throughout pregnancy and until weaning. Fetuses (n = 104) were studied at 20 days of gestation, and the pups on post-natal days 20 (n = 139) and 40 (n = 86), having been weaned onto normal rat chow at 21 days of age. Anaemic pups (haemoglobin ,anaemic [AN]: 4.5 ± 0.312 vs. control [C]: 8.5 ± 0.308g/dl; $p < 0.001$) were found to have significantly lower placental to body weight ratios (AN: 0.140 ± 0.003 vs. C: 0.155 ± 0.004; $p < 0.005$) and significantly lower body weights at all ages. At 20 days the heart weights of AN were almost twice that of C (AN: 0.537 ± 0.018 vs. C: 0.300 ± 0.016g; $p < 0.001$), suggesting an alteration in their cardiovascular development. It was of particular interest to find that at 20 days the systolic blood pressure, measured non-invasively in the unanaesthetised pups, was significantly lower in AN than in C. We have now confirmed these observations and extended them by studying pups at 20 and at 40 days of age.



* $p < 0.05$, C vs. AN ‡ $p < 0.001$, AN Day 20 vs. Day 40

Whilst mean systolic pressure was significantly lower in AN at day 20, it increased to greater levels than in C at day 40.

These findings complement those of Davis and Hohimer (1991) who found that the anaemic sheep fetus has a lower arterial blood pressure than controls. It may be that the rise in blood pressure associated with maternal anaemia in pregnancy (Godfrey et al. 1991) develops postnatally, and in the rat this can occur without a greater placental to body weight ratio.

Supported by the Wellcome Trust.

REFERENCES

1. GODFREY,K.M., REDMAN,C.W.G., BARKER,D.J.P. OSMOND,C. (1991) *Br J Obstet Gynaecol* **98**: 886-891
2. DAVIS,L.E., HOHIMER,A.R. (1991) *Am. J. Physiol.* **261**: R1542 - R1548

THE THORBURN SYMPOSIUM

August 5th - 9th, 1994. Hamilton Island, Australia

A53

CARDIOVASCULAR DEVELOPMENT IN FETAL SHEEP: HYPOXAEMIA, WEIGHT, AND GLUCOSE

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Department of Obstetrics and Gynaecology, University College London, London, U.K.

Intrauterine growth and environment have been implicated in affecting cardiovascular development. We examined whether acute isocapnic hypoxaemia repeated for 1 hour each day for 14 days produced changes in fetal mean arterial blood pressure (MAP), heart rate (FHR), weight, and blood glucose or lactate. Fetuses were instrumented at 105 - 109 days gestation with carotid, jugular and amniotic catheters, and precordial ECG electrodes. Seven fetuses underwent repeated hypoxaemia (1 hour normoxia, 1 hour P_aO_2 11 - 13mmHg, 1 hour recovery), starting on day 110-114 of gestation. Five control fetuses were studied repeatedly in normoxaemia (3 hours) also over a 14 day period.

For the hypoxia and control fetuses taken as a whole over the 14 day period, there were no significant differences in ABP, FHR, blood glucose or lactate, crown -rump length (CRL), or in body, liver, heart, lung, and kidney weight. However, examination of hypoxia and control fetuses together revealed that there were two distinct groups: A - those which showed a consistent rise in ABP over the 14 days (4 hypoxia, 2 control; 3 male, 3 females; 3 singletons, 3 twins), and B - those in which ABP did not show a clear increase over the 14 days (3 hypoxia, 3 control; 4 males, 2 females; 4 singletons, 2 twins). Regressiion analysis of MAP vs. age showed these groups to be significantly different ($p < 0.01$). Body, heart, and kidney weights were significantly greater in group A (Body weight, A: 4083 ± 276 vs. B: 3057 ± 324g, $p < 0.05$; heart weight, A: 35 ± 3 vs. B: 23 ± 2g, $p < 0.01$; kidney weight, A:15.0 ± 0.3 vs. B: 12.0 ± 0.9g, $p < 0.05$). Liver and lung weights were not different, nor was CRL. There was no difference between the groups in blood glucose or lactate over the 14 day period.

Thus, repeated acute isocapnic hypoxia of this degree does not produce any changes in the development of ABP or FHR in late gestation fetal sheep. However, it is clear from this study that the ABP can follow different trajectories in individual fetuses over this period. This appears to be related to fetal weight, including heart and kidney weight, and not to be influenced by repeated hypoxaemia or correlated with blood glucose/lactate levels.

Supported by the Wellcome Trust

SOCIETY FOR THE STUDY OF FETAL PHYSIOLOGY

22nd ANNUAL MEETING

June 11th - 14th, 1995. Malmö, Sweden.

A35

NUTRITIONAL PLANE IN EARLY PREGNANCY AND FETAL CARDIOVASCULAR DEVELOPMENT IN THE SHEEP

Crowe C., Kreindler JR, Bennet L., Hanson MA.
Dept. Obstetrics & Gynaecology, University College London, UK.

Maternal undernutrition has been suggested to result in alterations in placental and fetal growth and development. We have now studied the effect of maternal nutritional plane in early pregnancy on the fetal and placental size, and fetal cardiovascular development in late gestation.

Ewes were designated as either Heavy (He; n=7) or Light (Li; n=12), depending on their weight at mating ($p<0.005$) and their nutritional plane in early pregnancy. From 30 days gestation ewes from both groups were fed *ad libitum* and weighed on days 30,60 and 90. At 105-106 days, 5 He and & 4 Li fetuses were instrumented under halothane anaesthesia for blood pressure (MAP) and heart rate (FHR) measurement. Crown-rump length (CRL), abdominal circumference (AC) and femur length (FL) were also measured. After a 5 day recovery period all fetuses were monitored until 128-131 days gestation. Then, all ewes (instrumented and non-instrumented) were sacrificed and a post-mortem was carried out.

Fetuses in both groups showed an increase in MAP and a decrease in FHR between about 110 and 130 days. However, Li tracked at a higher MAP than He ($p<0.005$). Li had a lower haemoglobin than He ($p<0.005$). At post-mortem fetal body and organ weights, CRL, AC, FL, placental weights, and blood glucose levels were not different for both groups, nor was the increase in CRL, AC or FL different between the two groups. Thus, the nutritional plane of the ewe in early gestation affects the subsequent MAP of the fetus. This effect can occur in the absence of changes in fetal and placental growth.

Supported by the Wellcome Trust.

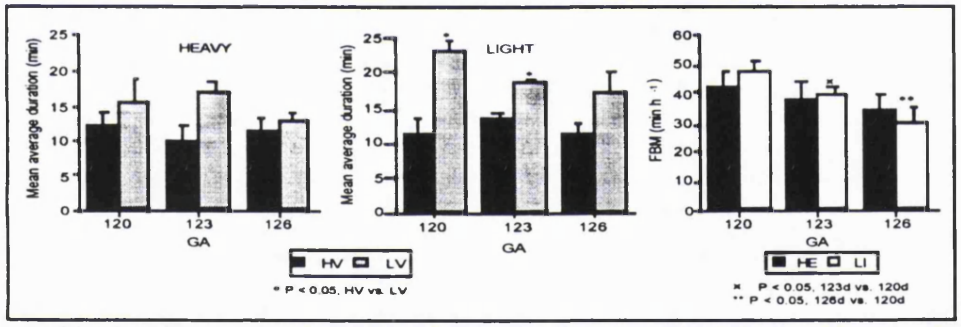
SOCIETY FOR GYNECOLOGIC INVESTIGATION

43rd Annual Meeting

March 20th - 23rd, 1996. Philadelphia, U.S.A.

THE EFFECT OF NUTRITIONAL PLANE IN EARLY PREGNANCY ON THE DEVELOPMENT OF FETAL BEHAVIOUR C. Crowe* & M.A. Hanson*, Dept. of Obstetrics and Gynaecology, University College London, UK. (SPON: G.C.L. Lachelin)

The aim of this study was to investigate the effect of nutritional plane in early pregnancy on the development of fetal behaviour in late gestation. Ewes were divided into two groups, Heavy (HE: body weight 62±1.9 kg) and light (LI: 49±1.6 kg; $p< 0.001$ vs. HE), on the basis of liveweight and body score at mating. Singleton fetuses were instrumented at about 105 days gestation with biparietal electrodes to measure the incidence (min/hour) and duration of low (LV) and high voltage (HV) electrocortical activity (ECoG), tracheal pressure, and diaphragm EMG to measure the incidence of fetal breathing movements (FBM), arterial, venous and amniotic pressure and ECG. They were studied in normoxia between 110-129 days. As previously reported (Crowe et al., 1995. Society for Study of Fetal Physiology, Abstract A35) LI fetuses tended to be smaller than HE fetuses, and had a significantly higher mean arterial blood pressure (MAP) than HE, though they did not show the consistent rise in MAP that was seen in the HE group. There were also differences between the groups in chemo- and baroreflex development.



In LI the incidence and duration of LV was greater than HV at 120 and 123 days. In contrast, there were no differences between these variables in HE. The incidence of FBM decreased from 120-126 days in LI, but there was no decrease in duration. However, in HE there was a decrease in duration but not in incidence, of FBM from 120-126 days. We conclude that nutrition in early gestation perturbs the subsequent development of fetal behaviour.

Supported by the Wellcome Trust.

FETAL AND NEONATAL PHYSIOLOGICAL SOCIETY

23rd ANNUAL MEETING

August 14th - 19th, 1996. Arica, Chile.

11

NUTRITIONAL PLANE IN EARLY PREGNANCY: EFFECT ON FETAL AND PLACENTAL SIZE IN LATE GESTATION.

Crowe, C.[1], Hawkins[1], P., Saito[1], T., Stratford, L.[1], Noakes, D.E.[2] & Hanson, M.A.[1]

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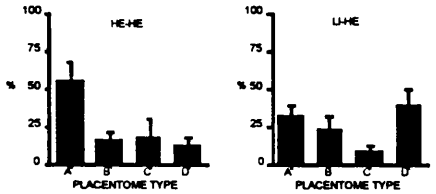
Maternal undernutrition in early pregnancy has been suggested to result in alterations in placental and fetal growth (DeBarro *et al.*, 1992). Our previous work showed that there were also alterations in the development of cardiovascular reflexes and blood pressure. In this study we set out to examine in detail the effect of such a nutritional insult on fetal size and gross placental morphology in late gestation.

Clun ewes were randomly assigned to one of two groups, Heavy-Heavy (HE-HE) or Light-Heavy (LI-HE). HE-HE were fed an unrestricted diet prior to mating and throughout gestation. LI-HE were put on a restricted diet (15% reduction in total intake) one month prior to mating and up until 30 days gestation, after which they were fed an unrestricted diet. All ewes were weighed weekly. At about 130 days gestation the ewes were sacrificed (HE-HE, n = 6; LI-HE, n = 6) and an extensive post-mortem was carried out. Maternal and fetal body weights, fetal crown-rump length (CRL), abdominal circumference (AC) , femur length and organ weights were recorded. Individual placentomes were weighed, and their morphology, depth and diameter determined.

LI-HE ewes lost a significant amount of weight while on the restricted diet. HE-HE ewes did not lose weight over the equivalent 2-month period. We found that fetal body weight, organ weights, CRL, AC and femur length and total placental weight were comparable in the two groups. This is similar to our previous report to this society last year (Crowe *et al.*, 1995). The distribution of the different morphological types of placentome (A, B, C, D, from Vatnick *et al.*, 1991) was, however, strikingly different between the two groups. HE-HE had significantly more A-type (59±13%) than D-type (10±5%) placentomes ($p < 0.05$), whereas LI-HE had a similar number of D-type (37±11%) and A-type (31±7%) placentomes (not significant) both in terms of placentome numbers and when expressed as a proportion of total placental weight ($p < 0.0001$, χ^2 test) (see figure).



These results show that a nutritional insult during the periconceptual period and in the first month of pregnancy results in a significant change in placental morphology in the sheep, which is not, however, accompanied by a decrease in fetal size in late gestation. Our working hypothesis is that such an insult produces a degree of placental compensation, adequate to preserve fetal growth but producing changes in subsequent fetal cardiovascular development.

1. DeBarro, T.M., Owens, J., Earl, C.R., Robinson, J.S. (1992). *Aust. Nutr. Soc.* **abstract**
2. Crowe, C., Kreindler, J.R., Bennet, L., Hanson, M.A. (1995). *SSFP* **A35**
3. Vatnick, I., Schoknecht, P.A., Darrigrand, R., Bell, A.W. (1991). *J.Dev. Physiol.* **15**, 351-356.

Supported by the Wellcome Trust.

APPENDIX 7
Papers

4032 Journal of Physiology (1995), 488.2, pp.515–519 515

The effects of anaemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats

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Department of Obstetrics & Gynaecology, University College London, London, UK

1. Reports that maternal anaemia in pregnancy is associated with a greater placental : birth weight ratio, which predisposes towards high postnatal blood pressure in the human, led us to examine the effects of maternal anaemia during pregnancy on placental size, fetal and neonatal growth, and blood pressure development in the rat.

2. Nutritional anaemia was induced in female rats prior to mating and maintained throughout pregnancy and up until weaning of the pups. Fetuses were studied at 20 days of gestation (E20). Pups were studied on postnatal days 20 (P20) and 40 (P40), having been weaned onto normal rat chow at 21 days.

3. In the anaemic group placental : fetal body weight ratios were lower compared with controls. Body weights at all ages were lower in the anaemic group than in controls, despite a greater rate of growth in the anaemic group between P20 and P40.

4. At P20 heart weights of the anaemic group were almost twice that of controls, suggesting an alteration in their cardiovascular development. However, paradoxically, the systolic blood pressure of the anaemic group was lower than that of controls.

5. By P40 the systolic blood pressure of the anaemic group (136 ± 3 mmHg) had increased and was greater than that in control pups (126 ± 3 mmHg).

6. In conclusion, we have shown that there is a pronounced postnatal rise in systolic blood pressure associated with maternal anaemia during pregnancy, which is not related to a greater placental : birth weight ratio. Before weaning, anaemic pups have a lower systolic blood pressure than controls and there is an important association between the rate of postnatal growth and blood pressure.

The development of hypertension in later life may be the result of events that occurred *in utero*, and there is an association between large placental weight, low birth weight and blood pressure in later life (Barker, Bull, Osmond & Simmonds, 1990). We do not know whether placental growth directly affects the development of blood pressure, or whether other factors such as nutrition and oxygen supply are decisive determinants. Lucas & Morley (1994) showed that early nutrition in low birth weight, preterm infants did not affect blood pressure measured at 7.5–8 years of age, focusing attention on prenatal factors. It has been suggested (Godfrey, Redman, Barker & Osmond, 1991) that anaemia in early pregnancy results in a high placental : birth weight ratio, which may subsequently *predispose* towards high blood pressure. As anaemia is known to produce fetal growth restriction and relatively greater placental size (Beischer, Sivasamboo, Vohra, Silpisornkosol & Reid, 1970; Hebbel, Berger & Eaton, 1980), this served to provide an experimental model with which to investigate the role of fetal and placental size on blood pressure development in neonates.

In this study we examined the effects of maternal anaemia during pregnancy on placental and fetal weight, postnatal body and organ growth of the pups, and the development of their blood pressure postnatally.

METHODS

Female Sprague–Dawley rats were fed either a low-iron diet (Special Diet Services, iron < 6 p.p.m.) or normal rat chow from 3–4 weeks prior to mating, by which time haemoglobin levels of those on the low-iron diet had dropped from 17 ± 2 to 11 ± 2 g dl^{-1}. After mating the rats were split into three groups, each comprising anaemic and control dams. They were studied as follows.

The first group of animals (anaemic dams, $n = 4$; control dams, $n = 4$; anaemic fetuses, $n = 47$; control fetuses, $n = 59$) were killed on day 20 of gestation (E20) and fetal and maternal

Table 1. Haemoglobin levels, body and placental weights, and placental : body weight ratio in the anaemic group and controls at E20, P20 and P40

	Haemoglobin (g dl^{-1})		Body weight (g)		Placental weight (g)		Placental : body weight ratio	
	Control	Anaemic	Control	Anaemic	Control	Anaemic	Control	Anaemic
E20	8·2 ± 0·3	5·4 ± 0·5***	3·30 ± 0·05	3·54 ± 0·04***	0·57 ± 0·02	0·47 ± 0·01***	0·155 ± 0·004	0·140 ± 0·003**
P20	10·1 ± 0·4	4·4 ± 0·9***	48·0 ± 1·0	28·0 ± 0·7***	—	—	—	—
P40	14·6 ± 0·5	13·3 ± 0·4*	157·3 ± 2·9	103·0 ± 3·5***	—	—	—	—

Note that placental to body weight ratio was less in the anaemic group than controls. * $P < 0·05$, anaemic *vs.* control; ** $P < 0·005$, anaemic *vs.* control; *** $P < 0·001$, anaemic *vs.* control.

haemoglobin, body weights and placental weights were recorded. For fetal haemoglobin measurements, each fetus was decapitated and blood was collected into a capillary tube, the blood from up to four fetuses being pooled so as to obtain an adequate volume for the assay. Maternal blood samples were collected by puncture of the tail vein.

Rats in the second group (anaemic dams, $n = 4$; control dams, $n = 6$; anaemic pups, $n = 18$; control pups, $n = 39$) were allowed to litter and at postnatal day 20 (P20) systolic blood pressure was recorded in the conscious mother and pups using a rat tail blood pressure monitor (Harvard Apparatus Ltd., Edenbridge, Kent, UK). At least three recordings were obtained for each animal. The pups were then killed and their haemoglobin levels, body and organ weights recorded, as well as maternal haemoglobin levels and body weights. Blood samples for both mothers and pups were obtained from a tail vein as described above.

The final group of rats (anaemic dams, $n = 8$; control dams, $n = 7$; anaemic pups, $n = 80$; control pups, $n = 64$) were also allowed to litter and neonatal and maternal systolic blood pressure (at least three recordings for each animal), haemoglobin levels (tail vein blood sample) and body weights were recorded at P20. Both anaemic and control pups were weaned onto normal rat chow at P21 and studied again at P40, at which time systolic blood pressure (at least three recordings per animal), neonatal haemoglobin (tail vein), body and organ weights were measured.

Blood pressure values for each animal were taken as a mean of the recordings obtained. When more than three readings were made, three were selected at random to obtain the mean. These values were then pooled for each group, as were all other results. Statistical differences between values for anaemic and control animals in each group were analysed by Student's unpaired t test. Data are expressed as means ± standard error of the mean (S.E.M.).

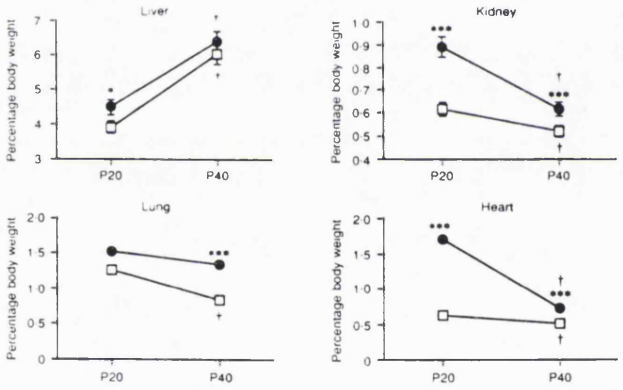

Figure 1. Liver, lung, kidney and heart weights expressed as a percentage of body weight in anaemic and control animals

Whilst there is an increase in liver weight as a percentage of body weight from P20 to P40 in both anaemic (●) and control (□) animals, lung, kidney and heart weights show a decrease in both groups. In the anaemic group the decrease in the percentage kidney and heart weights is far more pronounced than in controls, suggesting that the growth of these two organs is disproportionally less than body growth in anaemic animals. Where not shown, S.E.M.s are contained within the symbols. * $P < 0·05$, anaemic *vs.* control; *** $P < 0·001$, anaemic *vs.* control; † $P < 0·001$, P20 *vs.* P40.

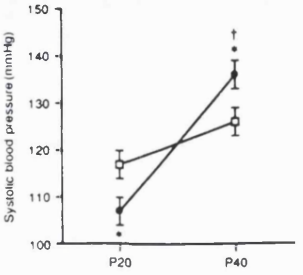

Figure 2. The change in systolic blood pressure from P20 to P40 in anaemic and control pups from group 3

Systolic blood pressure in the anaemic group (●) was significantly lower than that of controls (□) at P20. However, by P40 anaemic animals were hypertensive compared with controls. * $P < 0.05$, control vs. anaemic; † $P < 0.001$, P20 vs. P40.

RESULTS

Anaemic fetuses and pups had lower haemoglobin levels than controls at all ages and both anaemic and control animals showed a rise in haemoglobin between P20 and P40; however the rise was steeper in the anaemic group so that by P40 values for anaemic animals approached those of controls (Table 1).

Fetal body and placental weights were less in anaemic fetuses, and placental : body weight ratio was also lower compared with controls (Table 1). Body weights of the anaemic group were also less than controls at P20 and P40. However, the percentage increase in body weight between P20 and P40 was greater in anaemic than in control animals (see Table 1). Anaemic pups thus showed a greater rate of growth than controls after weaning.

Expressed as a percentage of body weight, liver weight increased from P20 to P40 in both groups, and kidney, lung and heart weights decreased (Fig. 1). Expressed as a percentage of body weight, the kidneys and hearts of the anaemic group showed a much steeper decrease than those of control between P20 and P40, indicating that these organs displayed a lower rate of growth over this period in anaemic animals than in controls.

Systolic blood pressure in anaemic pups at P20 was lower than control pups. However, by P40 systolic blood pressure was higher in anaemic than in control pups, having risen dramatically in the anaemic group between P20 and P40, but only slightly in the controls (Fig. 2). There was no significant difference in maternal systolic blood pressure levels between the anaemic group and controls for any of the three groups.

DISCUSSION

Anaemic rats had heavier hearts (Fig. 1) and lower systolic blood pressure (Fig. 2) than controls at P20. This is consistent with the finding of Davis & Hohimer (1991) that anaemic sheep fetuses had larger hearts, greater combined ventricular output and lower mean arterial blood pressure than controls. Arterial blood pressure is determined by cardiac output and total peripheral resistance and although we did not measure combined ventricular output in our rats, from the work of Davis & Hohimer (1991) it seems probable that it was increased. At first sight, such an increase in combined ventricular output in anaemic animals may be explained by a reflex initiated by hypoxia at the peripheral chemoreceptors or the brainstem, resulting in an increase in heart rate and myocardial contractility. However, as systolic blood pressure was lower in anaemic animals in our study and in that of Davis & Hohimer (1991), we then have to postulate that any increase in combined ventricular output was accompanied by a considerable fall in total peripheral resistance. This is feasible for several reasons. Firstly, decreased viscosity due to the severe anaemia, as has been observed in rats by Olivetti *et al.* (1993), would decrease impedance directly (Poiseuille's law). Secondly, Dawson & Hudlicka (1993) have shown that there is increased capillary growth in response to a long-term increase in blood flow, postulating that increased shear stress and/or capillary wall tension may be the stimulus responsible for initiating this growth. It has also been demonstrated in young rats that ventricular hypertrophy as a result of anaemia is accompanied by a significant amount of myocardial capillary proliferation (Olivetti *et al.* 1989). Thus, angiogenesis could occur in response to an increase in blood flow in several tissues and would result in a decrease in blood pressure, as the impedance to flow would be greatly decreased according to Kirchhoff's law concerning tubes in parallel. Lastly, there may have been a release of endothelial vasodilator factors such as nitric oxide or prostacyclin, again in response to increased shear stress at the endothelium (Smiesco & Johnson, 1993) or in response to hypoxaemia (Busse, Mülsch, Fleming & Hecker, 1993).

By P40 the anaemic group had developed a higher blood pressure than the controls (Fig. 2). Schork, Jokelainen, Grant, Schork & Weder (1994) showed in the rat that the rise in blood pressure with age is due predominantly to increases which occur concomitantly with growth spurts. Thus the rapid rise in systolic blood pressure seen in the anaemic group may be linked to their much faster growth than controls between P20 and P40, but, since anaemic rats were smaller than controls at all ages, it appears that

growth rate and not body size is the factor which influences blood pressure. The effect may of course be related to the relative growth or function of an individual organ. For example, we found that the anaemic group had heavier kidneys than controls, which could be related to a change in renal function. This is interesting as it has been suggested that in obesity renal sodium reabsorption may be increased as a result of hyperinsulinaemia, activation of the renin–angiotensin system, increased sympathetic activity, or histological changes in the renal medulla (Hall, 1994), and that this leads to hypertension associated with increased body weight. It may be that this was also the case with the increase in weight in our anaemic rats.

It is also possible that the processes determining the increase in total peripheral resistance may have been initiated prenatally. Langille (1993) found that during the perinatal period in fetal sheep there is a rapid accumulation of aortic elastin and collagen which may serve to preadapt vessels to the large increases in pressure which occur after birth. He also observed a strong relationship between blood flow and arterial growth during the postnatal period. Thus we can see from Langille's observations that the normal maturation in the level of blood pressure could be affected if either pre- or postnatal growth and vascular development are perturbed. So the changes in blood pressure development that occurred in the anaemic group may have been the result of both pre- and postnatal events.

Turning to the relation between birth weight and placental weight, our results share similarities with previous reports but also reveal differences from them. Our anaemic group showed a fall in fetal body weight, placental weight and placental : body weight ratio. Early growth of the anaemic fetuses was probably impaired as a result of hypoxaemia, since in sheep chronic hypoxaemia reduces fetal growth (Owens, Falconer & Robinson, 1987) and in the fetal rat Hebbel *et al.* (1980) found that growth was impaired in response to increased maternal oxygen affinity. Hebbel *et al.* (1980) also reported an increase in placental : birth weight ratio, as did Godfrey *et al.* (1991) in anaemic women. Whilst this may be partly due to reduced fetal growth, there also appears to be a contribution from enhanced placental growth, possibly in response to hypoxia. There are several studies which support this idea, for example the finding that the placenta is larger in women resident at high altitude (Krüger & Arias-Stella, 1970) and that of Alexander (1964) that reduction in the number of caruncles in sheep was compensated for during pregnancy by an increase in individual cotyledon weight. However, our finding was that placental weight decreased in the anaemic group. This may have been due to the severity of the anaemia, which may have been too great for the compensatory growth associated with moderate anaemia to have taken place. Lastly, there is the problem that we cannot distinguish between cause and effect in our results. It is likely, however, that the small placentae of the anaemic group resulted in a reduction in fetal growth, as studies in fetal sheep have shown an association between small placentae and intrauterine growth restriction (Harding, Jones & Robinson, 1985). It is clear that further work is required if we are to understand the interacting influences which are important in determining placental and fetal growth.

In conclusion, our study shows that there is a pronounced postnatal rise in systolic blood pressure associated with maternal anaemia during pregnancy, and that this is preceded by a low systolic blood pressure before weaning in anaemic pups. We did not find that the effect was related to greater placental to birthweight ratio, rather that there is an important association between the rate of postnatal growth and blood pressure.

ALEXANDER, G. (1964). Studies on the placenta of the sheep (*Ovis aries* L.). Effect of surgical reduction in the number of caruncles. *Journal of Reproduction and Fertility* **7**, 307–322.

BARKER, D. J. P., BULL, A. R., OSMOND, C. & SIMMONDS, S. J. (1990). Fetal and placental size and risk of hypertension in adult life. *British Medical Journal* **301**, 259–262.

BEISCHER, N. A., SIVASAMBOO, R., VOHRA, S., SILPISORNKOSAL, S. & REID, S. (1970). Placental hypertrophy in severe pregnancy anaemia. *Journal of Obstetrics and Gynaecology of the British Commonwealth* **77**, 398–409.

BUSSE, R., MÜLSCH, A., FLEMING, I. & HECKER, M. (1993). Mechanisms of nitric oxide release from the vascular endothelium. *Circulation* **87**, suppl. V, V18–25.

DAVIS, L. & HOHIMER, A. R. (1991). Hemodynamics and organ blood flow in fetal sheep subjected to chronic anemia. *American Journal of Physiology* **261**, R1542–1548.

DAWSON, J. M. & HUDLICKA, O. (1993). Can changes in microcirculation explain capillary growth in skeletal muscle? *International Journal of Experimental Pathology* **74**, 65–71.

GODFREY, K. M., REDMAN, C. W. G., BARKER, D. J. P. & OSMOND, C. (1991). The effect of maternal anaemia and iron deficiency on the ratio of fetal weight to placental weight. *British Journal of Obstetrics and Gynaecology* **98**, 886–891.

HALL, J. E. (1994). Renal and cardiovascular mechanisms of hypertension in obesity. *Hypertension* **23**, 381–394.

HARDING, J. E., JONES, C. T. & ROBINSON, J. S. (1985). Studies on experimental growth retardation in sheep. The effects of a small placenta in restricting transport to and growth of the fetus. *Journal of Developmental Physiology* **7**, 427–442.

HEBBEL, R. E., BERGER, E. M. & EATON, J. W. (1980). Effect of increased maternal hemoglobin oxygen affinity on fetal growth in the rat. *Blood* **55**, 969–974.

KRÜGER, H. & ARIAS-STELLA, J. (1970). The placenta and the newborn infant at high altitudes. *American Journal of Obstetrics and Gynecology* **106**, 586–591.

LANGILLE, B. L. (1993). Remodeling of developing and mature arteries: endothelium, smooth muscle, and matrix. *Journal of Cardiovascular Pharmacology* **21**, suppl. 1, S11–17.

LUCAS, A. & MORLEY, R. (1994). Does early nutrition in infants born before term programme later blood pressure? *British Medical Journal* **309**, 304–308.

OLIVETTI, G., LAGRASTA, C., QUAINI, F., RICCI, R., MOCCIA, G., CAPASSO, J. M. & ANVERSA, P. (1989). Capillary growth in anemia-induced ventricular wall remodeling in the rat heart. *Circulation Research* **65**, 1182–1192.

OLIVETTI, G., QUAINI, F., LAGRASTA, C., RICCI, R., TOSINI, P., CAPASSO, J. M. & ANVERSA, P. (1993). Effects of genetic hypertension and nutritional anaemia on ventricular remodelling and myocardial damage in rats. *Cardiovascular Research* **27**, 1316–1325.

OWENS, J. A., FALCONER, J. & ROBINSON, J. S. (1987). Effect of restriction of placental growth on oxygen delivery to and consumption by the pregnant uterus and fetus. *Journal of Developmental Physiology* **9**, 137–150.

SHORK, N. J., JOKELAINEN, P., GRANT, E. J., SHORK, M. A. & WEDER, A. B. (1994). Relationship of growth and blood pressure in inbred rats. *American Journal of Physiology* **266**, R702–708.

SMIESKO, V. & JOHNSON, P. C. (1993). The arterial lumen is controlled by flow-related shear stress. *News in Physiological Sciences* **8**, 34–38.

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Blood Pressure and Cardiovascular Reflex Development in Fetal Sheep. Relation to Hypoxaemia, Weight, and Blood Glucose

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Abstract. We measured mean arterial blood pressure (MAP), fetal heart rate (FHR), the baroreflex, weight, blood gases, blood glucose and lactate in chronically instrumented fetal sheep for 14 days from Day 105 to Day 109 gestation, with or without daily administration of 1 h of acute isocapnic hypoxaemia (arterial O_2 partial pressure, PaO_2, 11–13 mm Hg; 1 mm Hg = 133 Pa). Fetuses subjected to hypoxia showed no significant differences in MAP or FHR *v.* control fetuses. However, examination of all fetuses together revealed that there were two distinct groups: those showing a rise in MAP over the 14 days ('pressure up' group, PU), and those in which blood pressure did not increase or showed only a slight decrease ('pressure down' group, PD). PU fetuses were proportionately larger than PD fetuses. In contrast to PU fetuses, PD fetuses had lower blood glucose concentration, arterial O_2 saturation (SaO_2), PaO_2, total haemoglobin, haematocrit and oxygen content, and higher lactate concentration, pH and $PaCO_2$. PU fetuses showed a shift of the baroreflex MAP/R–R interval curve to the right, however, the PD group showed a shift upwards from Day 1 to Day 14. The PD group responded to hypoxia with a greater increase in MAP than the PU group.

Thus, repeated acute moderate isocapnic hypoxia does not affect development of MAP or FHR in late gestation fetal sheep. However, MAP follows different trajectories in individual fetuses, related to fetal size and the availability of oxygen and/or glucose. Cardiovascular chemoreflexes and baroreflexes are also different, depending on the MAP trajectory. These data indicate an important association between growth and blood pressure development, and also show that differences in growth are associated with changes in cardiovascular control.

Introduction

Intrauterine environment and fetal growth have been implicated in affecting cardiovascular development (Barker *et al.* 1990). In both the human (Economides *et al.* 1989) and the sheep (Robinson *et al.* 1983), intrauterine growth-retarded (IUGR) fetuses are hypoxaemic and have low amino acid concentrations. As acute hypoxaemia causes an increase in MAP (Giussani *et al.* 1994), the purpose of this study was to follow the development of MAP, FHR and cardiovascular reflexes in late gestation sheep, in the presence or absence of repeated acute hypoxaemia. Although our data do not show any effect of hypoxaemia, they do reveal important effects of fetal growth on blood pressure and chemoreflex and baroreflex development.

Materials and Methods

Surgical Methods

Fetuses (14) between 105 and 109 days gestation were instrumented under halothane (2% in O_2) anaesthesia with carotid artery, jugular vein and amniotic catheters, precordial wire electrodes for ECG recording, and a Transonic flow probe around a femoral artery. A maternal pedal vein was catheterized for drug administration. Catheters and electrodes were exteriorized through the ewe's flank. Antibiotics were given to the ewe as follows: 4 mL Streptopen (Pitman and Moore, Crewe Hall, Crewe, Cheshire, UK) administered intramuscularly, and 300 mg Crystapen (Glaxo, Greenford, UK) and 40 mg Cidomycin (Roussel, Uxbridge, UK) administered intravenously (i.v.). Antibiotics were given to the fetus as follows: 150 mg Crystapen administered i.v. and 150 mg Crystapen and 40 mg Cidomycin administered intra-amniotically). All antibiotics were given once daily for 5 days post-operatively.

Experimental Protocol

For 14 days (after a 5-day recovery period), 7 fetuses were subjected to daily hypoxaemia (arterial O_2 partial pressure, PaO_2, 13 mm Hg; 1 mm Hg = 133 Pa) by lowering maternal inspired oxygen (FiO_2) for 1 h of a 3-h study period, and control fetuses were studied for 3 h in normoxia. Maternal FiO_2 was manipulated by altering the gas mixture delivered into a polythene bag secured over the ewe's head. During the course of each 3-h experiment, 5 fetal blood samples (0·5 mL each) were taken at 30 min, 70 min, 100 min, 135 min and 165 min for the measurement of pH, blood gases, haematocrit, haemoglobin, blood glucose and lactate.

On Day 1, before experimentation, and on Day 14, at the end of the last experiment, each fetus was given a single bolus dose (75–100 μg i.v.) of the α_1 agonist phenylephrine, to elevate blood pressure so that the baroreflex could be measured. The fetus was allowed to recover (i.e. blood pressure, heart rate and flow measurements returned to basal values) from the phenylephrine before the experiment began. The baroreflex was assessed by plotting the mean R-R interval of animals in each group, measured at 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50 and 60 s after the injection of phenylephrine.

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against the MAP measured at the same times (i.e. while the pressure was rising). The curve that resulted was taken to represent the baroreflex (see Fig. 4).

At the end of the 14-day experimental period, ewes were killed by an overdose of pentobarbitone (40 mL Euthatal i.v.; Rhône Merieux, Spire Green Centre, Harlow, Essex, UK) and a post-mortem was carried out in which fetal bodyweight, fetal heart, lung, liver and individual kidney weights, and crown–rump length (CRL) were recorded.

All experiments were carried out in accordance with UK Home Office regulations (Animals Scientific Procedures Act 1986).

Data Collection

Pressures were recorded using pressure transducers and amplifiers (Gould, Hainault, Essex, UK); heart rate was recorded by passing the ECG signal through an amplifier and tachometer (Gould Biotach); femoral blood flow was measured by flow meter (Transonic Systems, T206). All signals were recorded onto disk using MacLab/8 hardware and data acquisition software. MAP and FHR were calculated by averaging blood pressure and heart rate, respectively, over 1 min, at 15-min intervals between 0 min and 60 min, then at 65, 70 and 75 min, and at 15-min intervals until 180 min. MAP presented on any day was calculated as the mean of values at 15, 30 and 45 min for all animals in each group.

Blood gases, pH and haematocrit were measured on a blood gas analyser (Instrumentation Laboratory, BGE, values corrected to 39.5 °C). A haemoximeter was used for measurement of haemoglobin (CO-oximeter 482; Instrumentation Laboratory, Warrington, Cheshire, UK), and glucose and lactate were measured by glucose–lactate analyser (YSI, 2300 STAT PLUS).

Statistical Analysis

Initially, measurements were compared between control and hypoxia animals. Subsequently, they were split into two groups, designated as 'pressure up' (PU) and 'pressure down' (PD), shown to be different by regression analysis of MAP v. age (PU: $r = 0{\cdot}965$; PD: $r = -0{\cdot}818$; $P < 0{\cdot}005$, PU v. PD) and by two-way ANOVA ($P < 0{\cdot}01$) using day as a repeated measure. For each group, weights, weight as a percent of bodyweight, lengths, and gestational age were compared by unpaired *t*-test. Differences between the means of the other parameters measured in the two groups from Day 1 to Day 14 were compared using ANOVA. $P < 0{\cdot}05$ was considered significant.

Results

Hypoxaemic fetuses did not differ from normoxaemic fetuses in terms of development of MAP and FHR (measured in normoxia) over the 14-day period, blood glucose and lactate concentrations, CRL, or body, heart, lung, liver and kidney weights. However, *post-hoc* analysis showed that the fetuses fell into two distinct

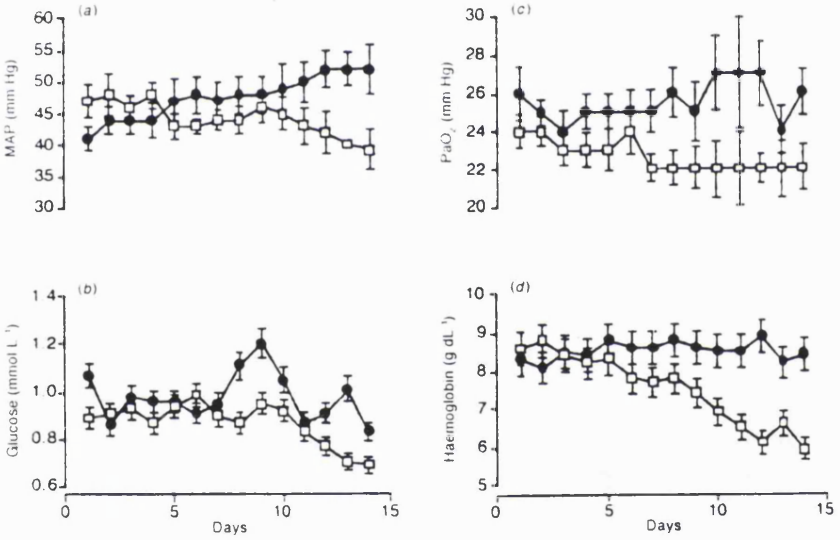

Fig. 1. Trends in MAP, glucose concentration, PaO_2 and haemoglobin during normoxia for 'pressure up' (PU) fetuses (●; $n = 7$) and 'pressure down' (PD) fetuses (□, $n = 7$). *(a)* MAP decreased from Day 1 to Day 14 in PD fetuses (not significant), whereas MAP increased in PU fetuses ($P < 0{\cdot}05$). The upward trend of PU fetuses compared with the downward trend of PD fetuses was shown to be significant ($P < 0{\cdot}01$). *(b)* Glucose concentrations were lower ($P < 0{\cdot}01$) in PD fetuses. *(c)* PaO_2 was lower in PD fetuses ($P < 0{\cdot}001$), decreasing from Day 1 to Day 7 and remaining lower than that in PU fetuses for the remaining 7 days. *(d)* Haemoglobin decreased over the 14-day period in PD fetuses ($P < 0{\cdot}001$), but remained fairly constant in PU fetuses. Values are mean ± S.E.M.

Table 1. Number of hypoxia (H), control (C), male (M), female (F), twin (T) and singleton (S) fetuses and bodyweight, crown–rump length (CRL), organ weights as a percentage of bodyweight and gestational ages at the start and end of the study period in 'pressure up' and 'pressure down' fetuses

Values are mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.005$

	'Pressure up' ($n = 7$)	'Pressure down' ($n = 7$)
Hypoxia, control	4H, 3C	3H, 4C
Sex	4M, 3F	5M, 2F
Twin, singleton	3T, 4S	3T, 4S
CRL (cm)	48·4±1·4	44·4±1·4
Bodyweight (g)	4000±218	2942±297*
Heart weight (g)	34±2	22±2**
Lung weight (g)	121±8	91±13
Liver weight (g)	125±12	99±11
Kidney weight (g)	14±0·3	12±0·7**
Heart % bodyweight	0·86±0·03	0·76±0·03
Lung % bodyweight	3·03±0·09	3·02±0·19
Liver % bodyweight	3·11±0·19	3·41±0·28
Kidney % bodyweight	0·36±0·01	0·43±0·05
Gestational age (days) on Day 1	114±1·2	113±1·1
Gestational age (days) on Day 14	129±1·5	127±0·6

groups: those that showed the expected increase in MAP over this period of gestation (PU fetuses) (Kitanaka *et al.* 1989), and those that showed either no increase or a decrease in MAP (PD fetuses) (Fig. 1*a*). Both groups showed a decrease in heart rate, consistent with the findings of Kitanaka *et al.* (1989). As illustrated in Table 1, the groups were similarly distributed with respect to whether fetuses had been exposed to hypoxia or were control fetuses, and for sex, twin or singleton, and gestational age. We did not have any information on maternal history, but all ewes were from the same flock and were fed the same diet of hay and concentrates *ad libitum* when in our animal house.

PU fetuses were larger than PD fetuses and had heavier hearts, lungs, livers and kidneys (Table 1). There was no significant difference between the two groups when organ weights were expressed as a percentage of bodyweight (Table 1). The crown–rump length of PU fetuses tended to be longer than that of PD fetuses, although the difference between the two groups did not reach significance (Table 1). Thus, PU fetuses tended to be proportionately larger than PD fetuses.

Normoxaemic plasma glucose concentrations (Fig. 1*b*) were lower in the PD group, and it is striking that normoxaemic PaO_2 (Fig. 1*c*), total haemoglobin (Fig. 1*d*), haematocrit (PU: 28±1% on Day 1, 28±1% on Day 14; PD: 29±1% on Day 1, 22±3% on Day 14; $P < 0.001$ PU *v.* PD), and thus arterial O_2 saturation (SaO_2) (PU: 82·0±2·6% on Day 1, 82·3±1·5% on Day 14; PD: 77·9±2·3% on Day 1, 73·2±3·0% on Day 14; $P < 0.001$ PU *v.* PD) and oxygen content (PU: 9·1±0·5 vol. % on Day 1, 9·2±0·4 vol. % on Day 14; PD: 8·6±0·4 vol. % on Day 1, 6·6±0·5 vol. % on Day 14; $P < 0.001$ PU *v.* PD), were all also lower in the PD group, decreasing from Day 1 to Day 14. The PD group showed higher concentrations of lactate (Fig. 2*a*) and $PaCO_2$ (Fig. 2*b*), but no significant difference in pH (Fig. 2*c*).

Femoral flow increased in both PU and PD fetuses from Day 1 to Day 14, but the increase was greater in PU fetuses (Fig. 3*a*). Femoral vascular resistance, on the other hand, was similar for both groups (Fig. 3*b*).

The cardiovascular responses of these two groups of fetuses were also found to be different. By the end of the 14-day period, the baroreflex curve of the PU group had shifted upwards and to the right (Fig. 4*a*) showing resetting to the higher pressures and lower FHR in this group on Day 14. The PD group, however, showed no such shift to the right, but the curve was merely displaced upwards on Day 14 (Fig. 4*b*) consistent with the fall in FHR seen at this age. In the animals which were exposed to repeated acute hypoxaemia (PaO_2: PU, 12±0·2 mm Hg; PD, 12±0·2 mm Hg; $PaCO_2$: PU, 44·9±0·6 mm Hg; PD, 44·1±0·6 mm Hg), the PD fetuses showed a greater increase in MAP over basal levels after 15 min of hypoxia than did the PU fetuses (Fig. 5). Both groups showed a bradycardia after 5 min of hypoxia, but there was no significant difference in the magnitude of this response between the two groups.

Discussion

Our results show that repeated isocapnic hypoxia of this degree does not affect the development of either MAP or FHR in late gestation fetal sheep. MAP increased from 43±2 mm Hg to 47±3 mm Hg, and FHR decreased from 192±5 beats min^{-1} to 168±6 beats min^{-1} over the 14-day

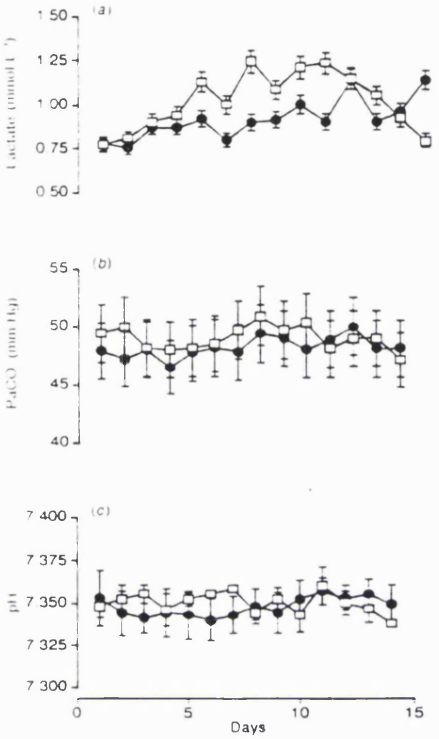

Fig. 2. Trends in lactate concentration, $PaCO_2$ and pH during normoxia for 'pressure up' (PU) fetuses (●; n = 7) and 'pressure down' (PD) fetuses (□; n = 7) fetuses. *(a)* Lactate concentrations were higher in PD fetuses than in PU fetuses and increased from Day 1 to Day 10, after which they decreased below the values seen in PU fetuses. *(b)* $PaCO_2$ values followed a similar trend to lactate concentration, levels being higher in PD fetuses than in PU fetuses until Day 10, after which there was a decrease similar to that seen in PU fetuses ($P < 0.05$). *(c)* There was no significant difference in the pH values between PD fetuses and PU fetuses. Values are mean ± S.E.M.

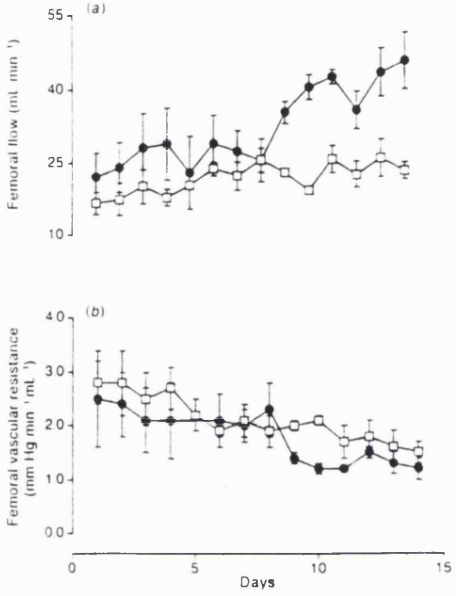

Fig. 3. Femoral flow and femoral vascular resistance in 'pressure up' (PU) fetuses (●) and 'pressure down' (PD) fetuses (□). *(a)* PU fetuses had a greater increase in femoral flow from Day 1 to Day 14 than PD fetuses ($P < 0.001$); however, *(b)* when expressed as femoral vascular resistance, there was no difference between PD fetuses and PU fetuses.

study period, and these measurements compare well with those previously reported for the chronically-catheterized fetal sheep over this period (Kitanaka *et al.* 1989). The lack of effect of hypoxia is consistent with the findings of Gagnon *et al.* (1994) who found that daily placental embolization did not produce maintained changes in FHR or MAP, and those of Kitanaka *et al.* (1989) who found that continuously reduced PaO_2 for three weeks did not affect FHR or MAP. However, it is clear that our fetuses fell into two distinct groups: PU and PD. The PU fetuses showed the well documented rise in MAP over this period of gestation, however, the PD fetuses clearly followed a different trajectory (Fig. 1*a*). What is the mechanism for the failure of MAP to increase in the PD fetuses? One possibility is that the effect is related to growth as PD fetuses were proportionately smaller than PU fetuses and had lower plasma glucose concentration (Fig. 1*b*).

Poor maternal nutrition also may be one mechanism for the failure of MAP to increase in PD fetuses, either at mating (T. M. DeBarro, J. A. Owens, C. R. Earl and J. S. Robinson, unpublished observations) or in mid-pregnancy (Newnham *et al.* 1991), as both result in reduced fetal weight and influence placental growth. Growth retardation in carunclectomized fetal sheep is associated with reduced glucose concentration (Owens *et al.* 1989), chronic hypoxaemia and higher lactate and $PaCO_2$ (Robinson *et al.* 1983). Indeed, carunclectomized hypoxaemic animals have also been reported to have a lower MAP than control animals (Robinson *et al.* 1983). It is, therefore, possible that reduced placental capacity

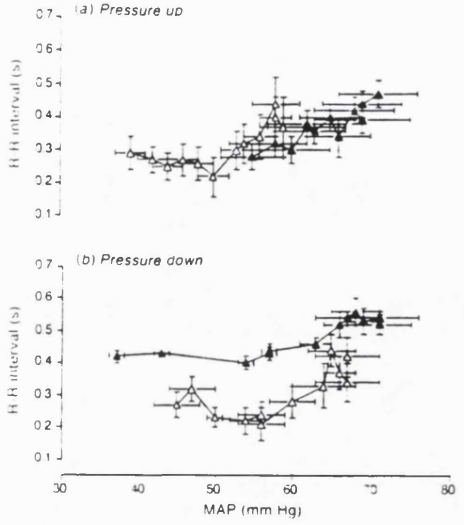

Fig. 4. Baroreflex curves for 'pressure up' (PU) fetuses ($n = 5$) and 'pressure down' (PD) fetuses ($n = 4$) on Day 1 (□) and Day 14 (▲). *(a)* There was a shift of the curve to the right and up from Day 1 to Day 14 in PU fetuses, consistent with the rise in pressure ($P < 0.001$) and the drop in FHR, which results in an increase in R–R interval ($P < 0.05$). *(b)* The curve for PD fetuses shifts upwards from Day 1 to Day 14, which is indicative of the decreased FHR and thus increased R-R interval ($P < 0.001$). Values are mean ± s.e.m.

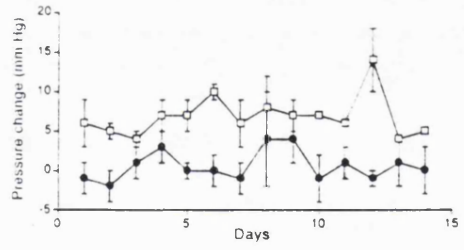

Fig. 5. The change in blood pressure in response to hypoxia, measured after 15 min of hypoxia, in 'pressure up' (PU) fetuses (●; $n = 4$) and 'pressure down' (PD) fetuses (□; $n = 3$). PD fetuses showed a more pronounced rise in arterial blood pressure than PU fetuses ($P < 0.001$). Values are mean ± s.e.m.

may be an important consideration in our PD fetuses, as they had lower PaO_2 and glucose levels (Figs 1*b* and 1*c*), and higher lactate and $PaCO_2$ levels by comparison with PU. However, we did not record placental weights or measure transplacental transport.

We also found that PD fetuses had lower haemoglobin and haematocrit levels. We do not know whether this was because they responded poorly to the repeated blood sampling, or whether they had a reduced rate of red cell production. It is also possible that in PD fetuses there was a greater increase in plasma volume than in PU fetuses. This may have occurred if there were increased levels of vasopressin in PD fetuses, perhaps due to the lower PaO_2 levels in these animals.

Results from the PD fetuses agree with data on the effect of anaemia in pregnancy on neonatal blood pressure in rats (Crowe *et al.* 1995) where anaemic pups at 20 days of age were found to have lower systolic blood pressures than controls. Davis and Hohimer (1991) also found that anaemic late gestation fetal sheep had a lower MAP than controls. In the study of Crowe *et al.* (1995) the MAP of the anaemic pups increased rapidly above that of controls after weaning. This may mirror the findings of Godfrey *et al.* (1991) that maternal anaemia in pregnancy predisposes to higher blood pressure postnatally.

The reduced SaO_2 observed in PD fetuses is the result of the lower PaO_2 which, together with the lower haemoglobin levels, accounts for the decreased oxygen content seen in these fetuses. Hence, it is likely that oxygen delivery to many tissues is substantially reduced. We do not know whether this and/or the reduced glucose levels mentioned above are responsible for the difference in growth in these fetuses. Clearly, however, growth has important effects on cardiovascular development.

The failure of the baroreflex curve in the PD fetuses to shift to higher pressures on Day 14 contrasts with the observation that the discharge frequency of the carotid baroreceptors for a given pressure decreased with advancing gestational age (Blanco *et al.* 1988). This finding along with their heightened increase in MAP in response to an acute hypoxic challenge (Fig. 5) leads us to question the normality of the development of these PD fetuses. Clearly there were developmental changes in the chemoreflex and baroreflex responses of these fetuses which could have important consequences later, for example, during birth or postnatally.

Acknowledgments

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References

Barker, D. J. P., Bull, A. R., Osmond, C., and Simmonds, S. J. (1990). Fetal and placental size and risk of hypertension in adult life. *Br. Med. J.* **301**, 259–62.

Blanco, C. E., Dawes, G. S., Hanson, M. A., and McCooke, H. B. (1988). Carotid baroreceptors in fetal and newborn sheep. *Pediatr. Res.* **24**, 342–6.

Crowe, C., Dandekar, P., Fox, M., Dhingra, K., Bennet, L., and Hanson, M. A. (1995). The effects of anaemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. (1995). *J. Physiol. (Lond.)* (In press.)

Davis, L. E., and Hohimer, A. R. (1991). Hemodynamics and organ blood flow in fetal sheep subjected to chronic anemia. *Am. J. Physiol.* **261**, R1542–R1548.

Economides, D. L., Nicolaides, K. H., Gahl, W. A., Bernardini, I., and Evans, M. I. (1989). Plasma amino acids in appropriate-and small-for-gestational-age fetuses. *Am. J. Obstet. Gynaecol.* **161**, 219–27.

Gagnon, R., Challis, J., Johnston, L., and Fraher, L. (1994). Fetal endocrine responses to chronic placental embolisation in the late gestation ovine fetus. *Am. J. Obstet. Gynaecol.* **170**, 929–38.

Giussani, D. A., Spencer, J. A. D., and Hanson, M. A. (1994). Fetal cardiovascular reflex responses to hypoxaemia. *Fetal Maternal Med. Rev.* **6**, 17–37.

Godfrey, K. M., Redman, C. W. G., Barker, D. J. P., and Osmond, C. (1991). The effect of maternal anaemia and iron deficiency on the ratio of fetal weight to placental weight. *Br. J. Obstet. Gynaecol.* **98**, 886–91.

Kitanaka, T., Alonso, J. G., Gilbert, R. D., Siu, B. L., Clemons, G. K., and Longo, L. D. (1989). Fetal responses to long-term hypoxemia in sheep. *Am. J. Physiol.* **256**, R1348–R1354.

Newnham, J. P., Kelly, R. W., Patterson, L., and James, I. (1991). The influence of maternal undernutrition in ovine twin pregnancy on fetal growth and Doppler flow-velocity waveforms. *J. Dev. Physiol.* **16**, 277–82.

Owens, J. A., Falconer, J., and Robinson, J. S. (1989). Glucose metabolism in pregnant sheep when placental growth is restricted. *Am. J. Physiol.* **257**, R350–R357.

Robinson, J. S., Jones, C. T., and Kingdom, E. J. (1983). Studies on experimental growth retardation in sheep. The effects of maternal hypoxaemia. *J. Dev. Physiol.* **5**, 89–100.

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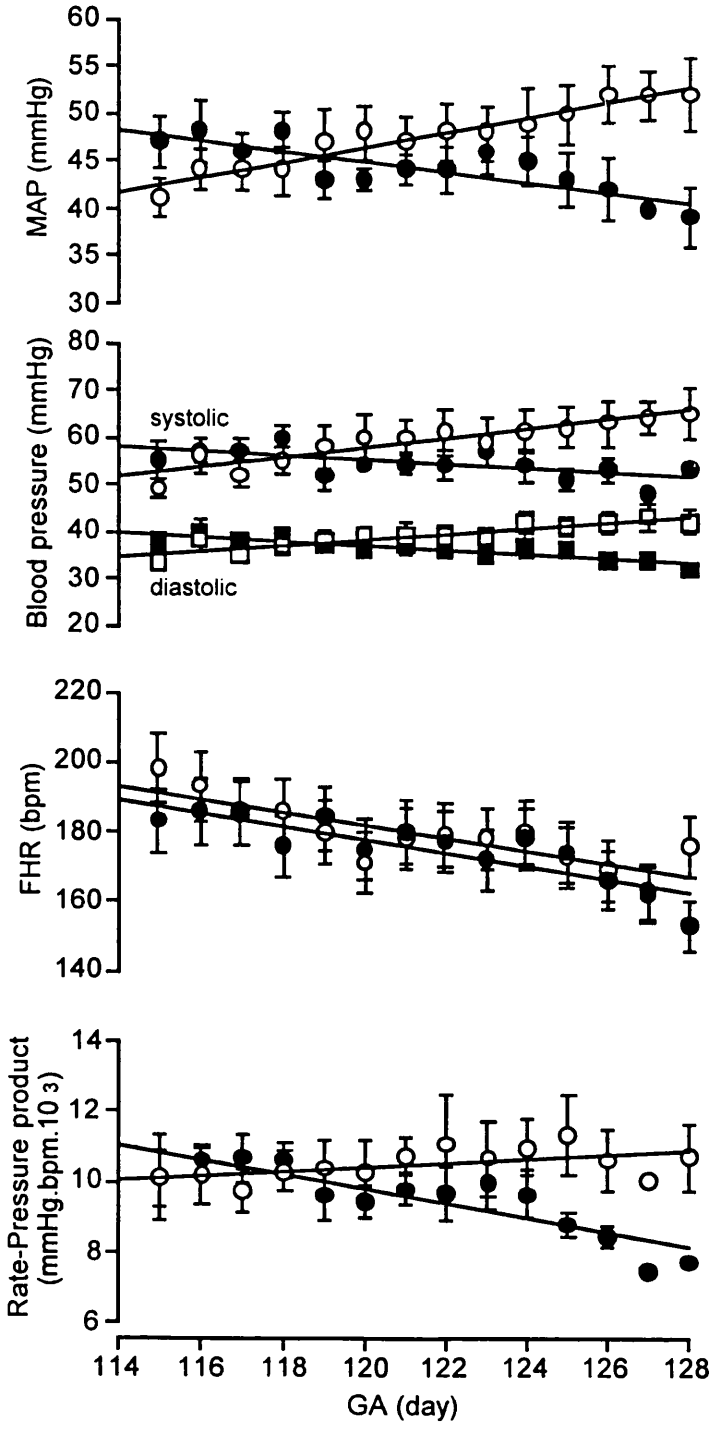
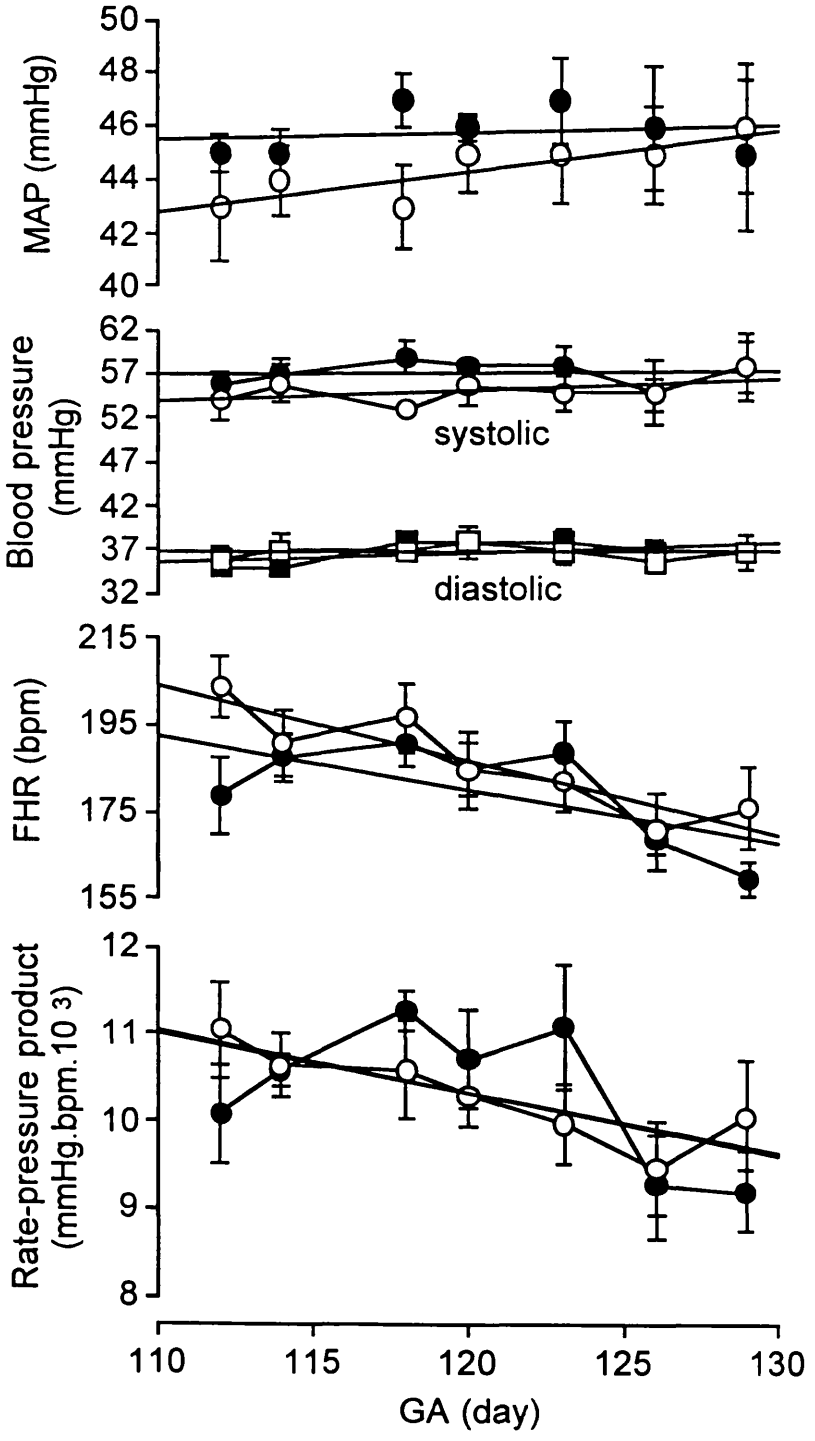
APPENDIX 8
BP and FHR trajectories



The trend in basal MAP, SBP and DBP, FHR and rate-pressure product for PU (o/□) and PD (●/■) fetuses. The linear curve fit through each set of values shows more clearly the different trajectories for PU and PD fetuses. Values are mean ± S.E.M. (See also Fig. 5.2).



MAP, SBP and DBP, FHR and rate-pressure product in HE (o) and LI (•) fetuses over the course of the study. † $P < 0.05$, 112 d vs. 129 d. Values are mean ± S.E.M. (See also Fig. 6.3)