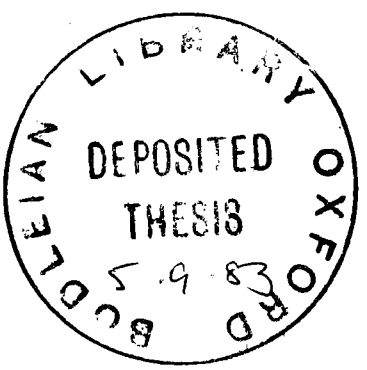
The distribution of cardiac output in the fetus.

A thesis submitted to the Faculty Board of Clinical Medicine, University of Oxford, for the degree of Doctor of Philosophy

by

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Michaelmas 1982

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AESTRACT

The distribution of cardiac output in the fetus

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D.Phil. Thesis, Michaelmas 1982

Hypertension develops in fetal lambs following nephrectomy at ~120 days gestation. The contributions of cardiac output and peripheral resistance to this hypertension were assessed by the microsphere method. Checks on this method showed limitations seldom recognized; the error expected from random distribution of microspheres is usually exceeded and circulatory effects of microspheres were seen following quantities at which others have found none.

The renoprival hypertension was due to decreased peripheral conductance in most of the fetal organs, including the placental cotyledons. Potential mechanisms of vasoconstriction considered were increased concentrations of catecholamines or vasopressin, or alternatively vessel wall thickening. Blood flow to the heart was increased in parallel to its increased workload, and that to the brain was maintained at an appropriate level for the prevailing blood oxygen content.

The alpha adrenergic contribution was assessed by examining the effects of alpha blockade with phentolamine. This indicated some involvement of this mechanism, possibly secondary to hypoxaemia. In addition alpha blockade in control fetuses produced a vasodilatation of the cotyledonary vascular bed not previously described.

Attempts were also made to measure vascular volume changes in nephrectomized fetuses with Evans blue. Plasma volume and possibly also blood volume, was increased in absolute terms, but not in terms of body weight. This, along with generalised oedema suggests increased interstitial fluid volume.

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CHAPTER 1

General introduction

1.1 The fetal circulation

The arrangement of the fetal circulation is illustrated in figure 1.1; its major differences from the adult circulation are adaptations to gas exchange via the placenta rather than the lungs. The whole of right ventricular output does not perfuse the lungs as in the adult, the ductus arteriosus allows passage of pulmonary arterial blood to the descending aorta from where it is distributed to the posterior half of the body and the placenta (fig. 1.1). The umbilical arteries arise from the distal end of the abdominal aorta.

In most species, including man and sheep, umbilical venous return partly passes through the liver and partly directly into the inferior vena cava through the ductus venosus (fig. 1.1). The ductus venosi of the fetal horse and pig disappear early in gestation.

1.1.1 The routes of flow through the central shunts

The course of the various components fetal venous returns through the foramen ovale (fig. 1.1) and heart, was for many years controversial. The development of knowledge in this area has been extensively reviewed by Barclay, Franklin and Prichard (1944) and Dawes (1964), so only the main points will be picked out here. In finding different oxygen contents in fetal carotid and umbilical

Figure 1.1

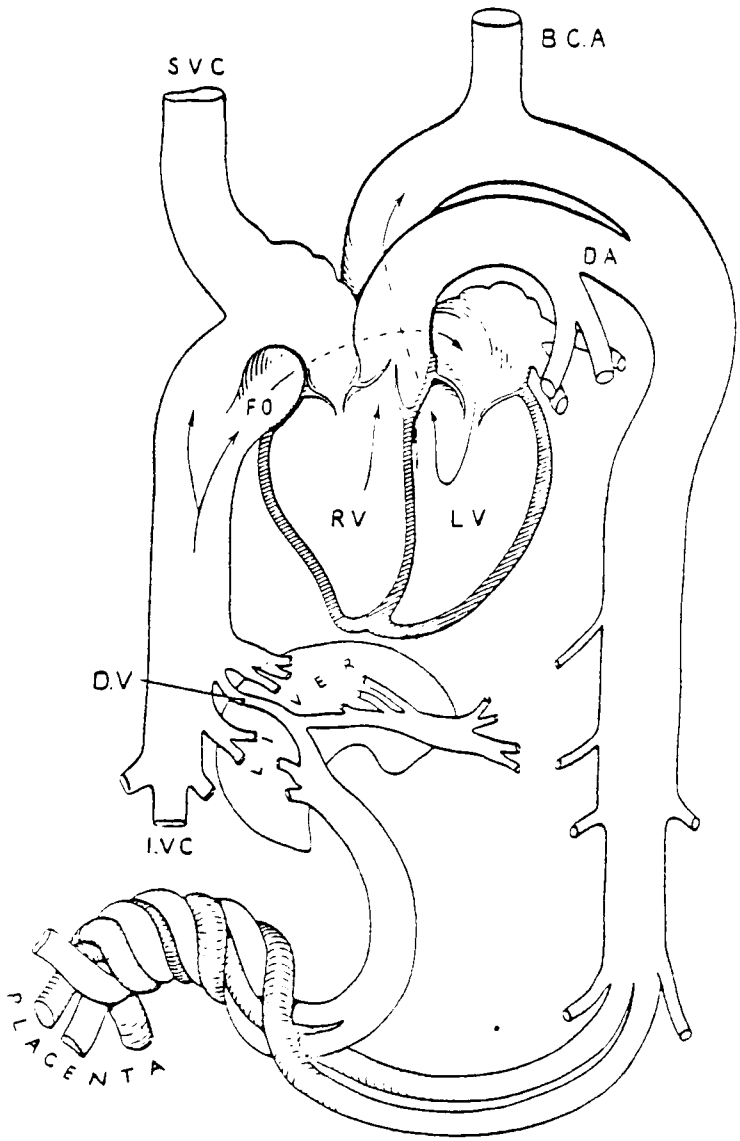


Fig. 1.1. Semi-schematic diagram of the circulation in the fetal lamb, showing the three vascular shunts; the foramen ovale (FO), ductus arteriosus (DA) and ductus venosus (DV). Also shown are the right and left ventricles (RV and LV), superior and inferior venae cavae (SVC and IVC), brachiocephalic artery (BCA) and the umbilical vessels.

arteries, Huggett (1927) established that inferior and superior vena caval returns do not mix completely in the heart. Cineangiographic studies by Barclay, Barcroft, Barron and Franklin (1939) showed that inferior vena caval blood passed into the right atrium, as in the adult, and the left atrium via the foramen ovale. They found no evidence for the passage of superior vena caval blood through the foramen ovale to the left atrium, though their method might miss a small flow if the contrast medium injected into the superior vena cava were sufficiently diluted by inferior vena caval blood. However, injections of radioactive microspheres (Rudolph and Heymann, 1967) or albumin (Dawes, Groom, Mott, Rowlands and Thomas; in Dawes, 1968) into the superior vena cava have confirmed the above conclusions.

There are circumstances, however, under which superior vena caval blood may enter the left atrium. Dawes (1968) reported cineangiographic studies by Adamsons, Dawes and Mott which demonstrated this in asphyxiated or hypoxaemic fetuses, though the production of asphyxia by tying the umbilical cord may have contributed to this result by decreasing inferior vena caval return. The change during hypoxia may be secondary to the bradycardia associated with hypoxia as a superior vena caval to left atrium shunt was found at low heart rates produced by vagal stimulation, but again, the balance of superior and inferior vena caval returns may have been affected. Rudolph and Heymann (1967) also found that superior vena caval blood passed to the anterior body of fetal sheep during asphyxia.

The distribution of coronary sinus blood, which includes a substantial azygos venous contribution in many species (but not man), has not directly been investigated. Its position in relation to the foramen ovale and the crista dividens (a ridge separating the left and right bound streams of inferior vena caval blood) makes it likely that it normally passes into the right atrium. Pulmonary venous return, as in the adult, enters the left atrium only.

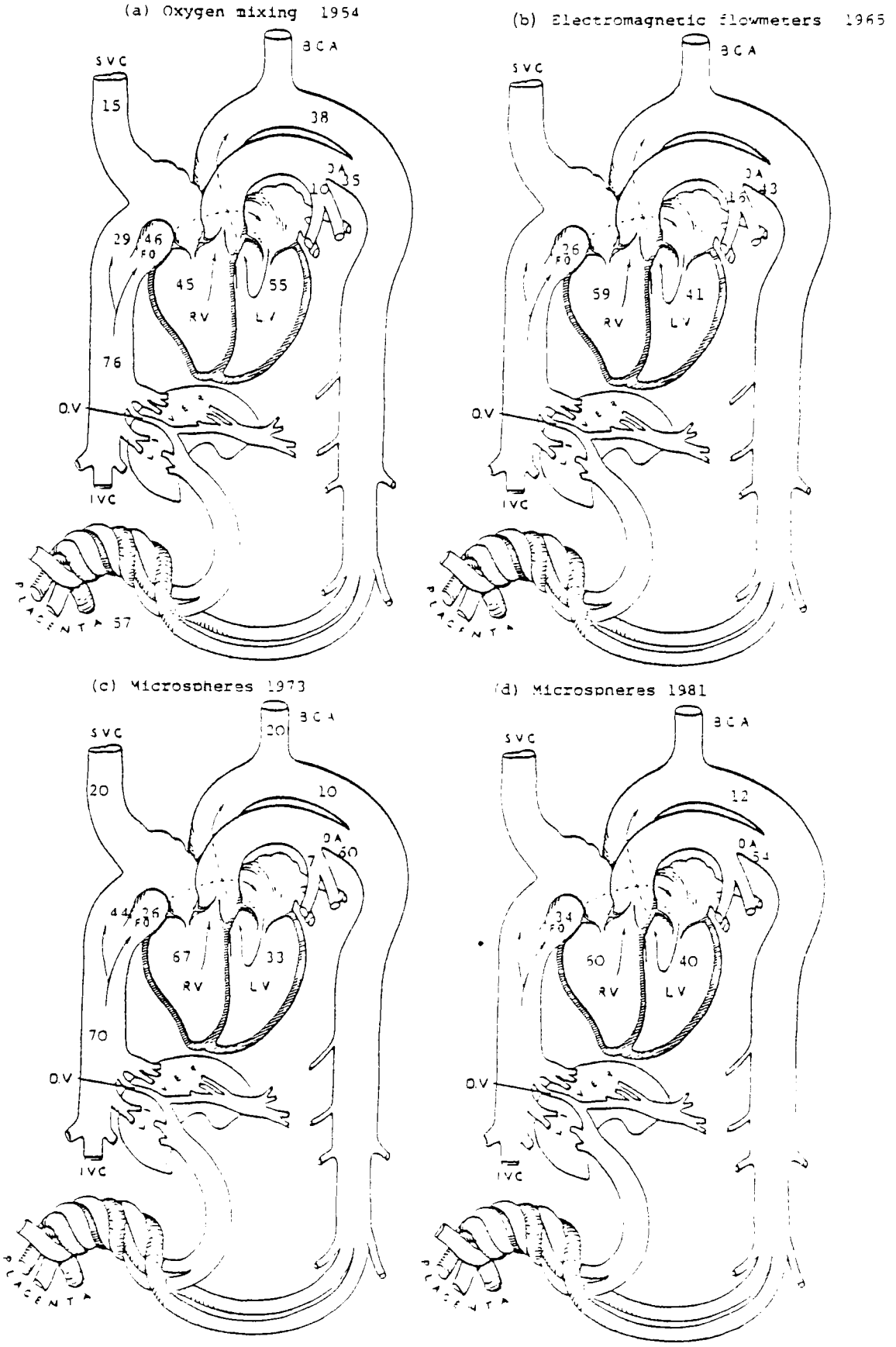
This disposition of these components of fetal venous return results in blood pumped by the left heart being better oxygenated than that pumped by the right heart. The division of right heart output has already been described; left ventricular output passes partly to the anterior body via the brachiocephalic artery and partly to the descending aorta. There are, therefore, three mixtures of blood supplied to the fetal tissues; they are, in descending order of oxygenation; one to the anterior body and heart, one to the posterior body and placenta, and one to the lungs.

1.1.2 Quantification of central shunts

The magnitude of the central shunts was derived from the oxygen contents of eight simultaneously taken blood samples by Dawes, Mott and Widdicombe (1954). Some of the results, obtained in exteriorized open-chest fetuses of sodium pentobarbital anaesthetized ewes, are shown in figure 1.2(a). Some of the assumptions necessary in the calculations can now be seen to be erroneous, however, the

Figure 1.2. Comparative distributions of fetal lamb cardiac output (figures are % combined ventricular output) measured by different methods. (a) Dawes, Mott, and Widdicombe (1954); oxygen contents. (b) Assali, Morris and Beck (1965); electromagnetic flowmeters. (c) Creasy, Heymann and Rudolph (1973); radioactive microspheres. (d) Anderson, Bissonette, Faber and Thornburg (1981); radioactive microspheres.

Figure 1.2


(a) Oxygen mixing 1954
SVC
BCA
15
38
DA
35
10
29
46
FO
45
RV
55
LV
76
O.V
IVC
PLACENTA
57
(b) Electromagnetic flowmeters 1965
SVC
BCA
DA
43
15
36
FO
59
RV
41
LV
O.V
IVC
PLACENTA
(c) Microspheres 1973
SVC
BCA
20
20
10
DA
60
7
44
36
FO
67
RV
33
LV
70
O.V
IVC
PLACENTA
(d) Microspheres 1981
SVC
BCA
12
DA
34
34
FO
60
RV
40
LV
O.V
IVC
PLACENTA

resulting errors are small. Central distribution figures were also obtained by Assali, Morris and Beck (1965) using electromagnetic flowmeters on the aorta, pulmonary artery and ductus arteriosus. This method is considered in more detail in chapter 2, the chief reservation concerns the physical disturbance of and degree of contact of the flowmeters with the vessels. The distribution of cardiac output found is given in figure 1.2(b).

The microsphere method can be used to measure fetal shunt and ventricular flows as indicated by Heymann, Creasy and Rudolph (1973). The findings of these workers and of Anderson, Bissonette, Faber and Thornburg (1981) using similar techniques are given in figures 1.2 (c) and (d). An extensive critique of this method also appears in chapter 2; its accuracy and validity are now well established.

Several points from figure 1.2 are worth highlighting; the bulk of venous return is carried by the inferior vena cava, of which, 1/3 to 1/2 enters the left atrium. Most of the inferior vena caval return mixes with the superior vena caval and coronary return in the right atrium, resulting in a substantially higher right than left ventricular output. Approximately 90% of the right ventricular output passes through the ductus arteriosus, recirculating a large proportion of umbilical venous blood to the placenta. Most of the left ventricular output (about 2/3) passes to the heart and anterior body.

1.1.3 The umbilical circulation

Dawes et al (1954) found that 55% of the cardiac output passed through the umbilical circulation in exteriorized mature fetal lambs. The microsphere method gave an umbilical share of cardiac output of around 40% in mature fetal lambs (120 days to term) in utero with the ewe under spinal anaesthesia (Rudolph and Heymann, 1967). This figure has been confirmed by a number of studies on chronic preparations (e.g. Cohn, Sacks, Heymann and Rudolph, 1974).

The microsphere method has also been used to find figures of 48% in fetal rhesus monkeys (Behrman, Lees, Peterson, de Lannoy and Seeds, 1970); 39% in mature and 50% in immature baboon fetuses (Paton, Fisher, Peterson, de Lannoy and Behrman, 1973).

1.1.4 The ductus venosus

The proportion of umbilical venous blood passing through the ductus venosus was first estimated by Rudolph and Heymann (1967) to be 34 to 91% by injection of radioactive microspheres into an umbilical vein. It was also found that the proportional shunt through the ductus venosus was greater at higher umbilical flows, though the lambs and goats used were of a wide age range, and the association of this effect with age was not investigated. A later paper (Edelstone, Rudolph, and Heymann, 1978) showed no correlation of ductus venosus flow (per kg body weight) with gestational age (over 116-136 days) but found

a good correlation with umbilical flow. The mean proportion of umbilical flow passing through the ductus venosus was 53%; and a small contribution (about 2% of total ductus flow) from the hepatic portal circulation was found.

Behrman et al (1970) found 53% of umbilical flow passed through the ductus venosus in the rhesus monkey. All of these results must be treated with some care, because when microspheres are used to study the distribution of streams of blood which do not pass through the heart, there is some doubt whether mixing is adequate.

There is some evidence that streaming of ductus venosus blood occurs in the thoracic inferior vena cava such that it crosses the foramen ovale in larger proportions than the rest of inferior vena caval blood, thus aiding the distribution of better oxygenated blood to the brain and heart. This was found to occur in rhesus monkeys (Behrman et al, 1970) and baboons (Paton et al, 1973a). In fetal lambs, 36% of ductus venosus blood passed to the anterior circulation and lungs, in contrast to 28% of lower abdominal IVC blood (Edelstone and Rudolph, 1979). The possibility of this being an artefact due to microsphere streaming also affects these results. Though Edelstone and Rudolph (1979) eliminated this possibility, their method of assessing lack of streaming may improve mixing by turbulence caused by the two extra catheters used.

1.1.5 Recirculation in the fetus

As already touched on, these flows through the fetal shunts suggest a high degree of recirculation in the fetus, both of umbilical venous blood back to the placenta without passage through the systemic or pulmonary circulations and vice versa. Reuss and Rudolph (1980) investigated this directly with microspheres in fetal lambs, and found 22% of umbilical venous blood was recirculated to the placenta, though this only includes the portion passing through the ductus venosus.

Approximately another 20% of umbilical venous blood would also be recirculated after having passed through the liver, though whether this is better oxygenated than systemic venous blood is unclear. 53% of distal inferior and 45% of superior vena caval blood are recirculated to the fetal body. In terms of cardiac output, the umbilical recirculation represents 11% and the systemic recirculation 23%. It is only in the shadow of the adult circulation that this appears inefficient. A shunt of this size to the adult lungs would be particularly inefficient because of their effectiveness as gas exchangers; the main result of the system in the fetus is to maintain a high umbilical flow. The importance of this at the expense of recirculation is illustrated by the increase in umbilical recirculation that occurs during hypoxia (Reuss and Rudolph, 1980).

It would also be wrong to overemphasize the importance of the mechanisms which result in the distribution of

better oxygenated blood to the heart and brain. Though on paper these are potentially powerful, in practice they result in only slight differences in oxygenation. The major achievement of the fetal circulation is in pumping nearly half of the combined ventricular output through the placenta rather than the lungs. In its shielded environment the fetus has no need of the highly efficient system of the adult mammal. The fetus must be ready for extrauterine existence as soon as it is born and under these circumstances its circulation is an adequate adaptation of the system which will support it for the bulk of its life.

1.2 Pressures and heart rates

1.2.1 Changes with gestation

The arterial blood pressure and heart rate of the fetus vary with gestational age, blood pressure increases towards term and heart rate decreases; there is also shorter term variability associated with behavioural state and fetal movements.

The changes in mean arterial pressure with gestational age and in the newborn in a number of species are shown in figure 1.3, these curves were all obtained from acute exteriorized preparations with the mother anaesthetized. Boddy et al (1974) found an increase of about 0.5 mm Hg per day insheep fetuses late in gestation.

The most conspicuous feature of fetal arterial pressure is that it is low compared with that in the adult.

Figure 1.3

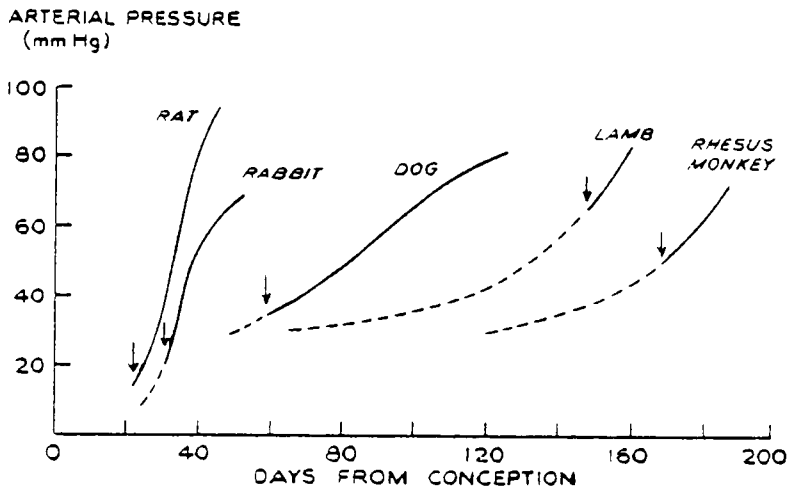


Figure 1.3. Mean arterial pressure in the fetuses (- - - -) and newborns (———) of several species. The measurements were all made in acute experiments under anaesthesia. From Dawes (1968) (with permission).

1.2.2 Sources of variability

Slow records of arterial pressure in mature fetal lambs show a variation over a range of about 10 mm Hg, heart rate too shows considerable variation, it is likely this is due to several factors.

Arterial blood pressure in the adult is a notoriously labile variable. Continuous records of human arterial pressure show variations in association with various behavioural activities (Pickering, 1968). The fetus is not subject to many of the various stimuli and responses found in the adult, but it does exhibit different behavioural states which are associated with cardiovascular changes.

In mature fetuses, the electrocorticogram (ECoG) shows differentiation into low and high voltage episodes which alternate in periods of increasing duration as gestation proceeds. Fetal breathing movements occur during much, but not all, of the low voltage episodes (Dawes, Fox, Leduc, Liggins and Richards, 1972). Fetal limb and body movements of varying character have been observed during all phases of the electrocorticogram (Ruckebusch, Gaujoux, and Eghbali, 1977; Natale, Clewlow and Dawes, 1981). All these three features of fetal behaviour might be expected to be associated with changes in the cardiovascular system.

1.2.2.1 Electrocortical activity

The effects of ECoG state on heart rate and blood pressure in 135-145 day fetal lambs were investigated by

Jost, Quilligan, Yeh and Anderson (1972); both were higher during high voltage ECoG activity than during low voltage activity. Monitoring was only continued for three days post-operation so the effects of surgery cannot be excluded. The heart rate was 10-30 beats.min^{-1} higher during high voltage activity. It is not clear whether arterial blood pressure was corrected for amniotic pressure, indeed the values are high compared with the observations of other workers, ranging from 50 to 67 mm Hg. The difference in pressure between ECoG states varied from 0-10 mm Hg but was generally 2-5 mm Hg.

Similar differences were observed by Mann, Duchin and Weiss (1974), but again the reference point for arterial pressure is not stated. The monitoring periods were longer but the results from chronic preparations are combined with some from acute experiments because they were similar. They also found that the change to low voltage activity preceded the cardiovascular changes, whereas the reverse occured for the change from low voltage to high voltage activity.

Clapp, Szeto, Abrams, Larrow, and Mann (1980) found arterial pressures of about 59 mm Hg during high voltage ECoG activity and about 54 mm Hg during low voltage activity; heart rates were about 180 and 160 beats.min^{-1} respectively. Blood gas tensions and pH were similar in both states though the samples were taken 2-5 minutes after the ECoG change which is probably shorter than the time required to reach a steady circulatory state.

1.2.2.2 Fetal breathing movements

The changes described above appear to be associated with the ECoG changes themselves; in addition there are differences in the patterns of breathing and other movements occuring during the different ECoG states which give rise to more transient variations. Dawes, Fox, Leduc, Liggins and Richards (1972) found that the onset of rapid irregular breathing movements was often associated with a rise in blood pressure of variable magnitude, sometimes heart rate was also increased.

Dalton, Dawes and Patrick (1977) examined the patterns of heart rate variability in fetal lambs over long periods. In contrast to other workers,they found no difference in heart rate between periods of high voltage activity and low voltage activity when breathing was absent. Breathing movements were associated with either an increase or decrease in heart rate and increased variability as assessed by beat-to-beat differences of heart period or the standard deviation of heart period. A two to four hour rhythm of heart period of about 20 msec amplitude (about 5%) was also observed, along with a 1-4 hour rhythm of heart rate variabilty; these two rhythms often being asynchronous. Diurnal rhythms of both variables were also seen, with the peaks occuring late at night and the troughs in the mid-morning, the amplitude of the heart rate rhythm being about 8%.

1.2.2.3 Body and limb movements

The changes in heart rate associated with body movements were studied by Ruckebusch et al (1977). During high voltage activity, tachycardia, sometimes followed by bradycardia was often found, with complex movements (movements made up of several components). Similar changes were seen during low voltage activity when rapid eye movements (REM) were absent, during REM phases of low voltage activity, heart rate changes were only seen in association with breathing movements.

1.2.3 Variability of blood flows

It is technically more difficult to make continuous measurements of blood flows and so their variability is less well documented. Jost et al (1972) found no differences in carotid arterial flow measured by electromagnetic flowmeters between low voltage and high voltage states. Common umbilical arterial flow, however, has been found to be 5-6% higher during high voltage activity (Clapp et al, 1980). This can largely be explained by the differences in arterial pressure described by the same workers.

1.2.4 Control haemodynamic data

A considerable body of work has now accumulated on the chronic fetal lamb preparation, in which control observations of cardiovascular variables have been made. Arterial blood pressure, heart rate and combined ventricular output from a number of these studies is given

in table 1.1. The measurements of combined ventricular output were all made using the microsphere method. Comparison of this with other methods is made in chapter 2. Most of this work has been on mature fetuses (about 120 days onwards) in which the mean arterial pressure is 40-50 mm Hg, usually around 45 mm Hg. Heart rate is 150-170 beats.min^{-1}, and CVO about 500 ml.min^{-1}.kg^{-1}, varying from about 400-600 ml.min^{-1}.kg^{-1} between studies.

It is difficult to arrive at a suitable measure of cardiac output to compare with the adult, but if either systemic flow (about 60% of CVO) or half CVO is used, it is clear that fetal cardiac output is high compared with that in the adult (e.g. 120 ml.min^{-1}.kg^{-1} derived from Hales, 1973).

As already indicated, fetal systemic arterial pressure is lower than that in the adult, largely because it forms one circuit, replacing the dual circuit of the adult consisting of low and high pressure components. These two findings imply high conductance in the fetal cardiovascular system.

Systemic flow is probably higher in the fetus than the adult because of the lower oxygen content of fetal blood. In addition, many species show a higher systemic oxygen consumption in the fetus than in the adult. A compilation of comparative figures by Battaglia and Meschia (1978) suggests that fetal oxygen consumption is similar in many species (at about 7-8 ml.min^{-1}.kg^{-1}) but adult oxygen consumption (per unit weight) is inversely related to body

Reference	Age days	Pressure ± SE mmHg	Heart rate ± SE Beats.min^{-1}	CVO ± SE ml.min^{-1}.kg^{-1}
Rudolph & Heymann (1970) Acute experiments in utero	60-85	39 ± 2	-	485 50
	86-100	45 ± 3	-	377 29
	101-110	43 ± 3	-	549 ± 48
	111-120	58 ± 1	-	497 ± 42
	121-140	53 ± 3	-	527 ± 42
	141-150	55 ± 2	-	548 ± 20
Kirkpatrick, Covell & Friedman (1973)	124-146	63 ± 2	161 ± 4	-
Faber, Green & Thornburg (1974)	110-145	43 ± 2	176 ± 6	-
Cohn, Sacks, Heymann & Rudolph (1974)	122-142	60 ± 6	162 ± 12	481 ± 50
Longo, Wyatt, Hewitt & Gilbert (1978)	134-146	53 ± 2	151 ± 5	467 ± 30
Iwamoto, Rudolph, Keil & Heymann (1979)	123-140	47 ± 2	174 ± 4	474 ± 35
Iwamoto & Rudolph (1979)	116-123	47 ± 2	171 ± 11	541 ± 39
Mott (1980)	111-125	45 ± 2	162 6	
Reuss & Rudolph (1980)	121-129	43 ± 1	184 ± 6	505 ± 36
Lorijn & Longo (1980)	129-141	45 ± 3	184 ± 8	538 ± 22
Gilbert (1980)	130-145	46 ± 1	163 ± 5	592 ± 28
Toubas, Silverman, Heymann & Rudolph (1981)	128-147	51 ± 2	186 ± 6	610 ± 58
Iwamoto & Rudolph (1981)	120-131	46 ± 2	172 ± 6	526 ± 32
Reuss, Parer, Harris & Krueger (1982)	125-138	42 ± 2	165 ± 8	450 ± 69
Itskowitz & Rudolph (1982)	122-139	45 ± 2	186 ± 8	-
Tabsh, Nuwayhid, Murad, Ushioda, Erkkola, Brinkman & Assali (1982)	> 100	49 ± 4	146 ± 16	-

Table 1.1

Measurements of arterial pressure, heart rate and combined ventricular output (CVO) in chronic fetal lamb preparations

weight. Thus in larger species (eg. sheep and cattle) fetal oxygen consumption is higher than in the adult, approximately equal in others (rhesus monkey), and adult greater than fetal in small species (guinea pig).

1.3 Arterial pressure control in the fetus

1.3.1 Arterial baroreceptors

Brinkman, Ladner, Weston, and Assali (1969) concluded that the carotid sinus baroreceptors were functional in near term fetal lambs. However, no systemic pressor response to gradual carotid occlusion was found until the occlusion was complete, and no heart rate change was seen. It is possible that this was a mild central nervous ischaemic response. No details of the isolated carotid sinus preparations are given, nor the changes in pressures used to elicit systemic responses. Systemic pressure responses to sinus pressure changes were small and control pressures were high (about 70 mm Hg mean). Heart rate did not fall during increased sinus pressure.

A better study by Shinebourne, Vapaavouri, Williams, Heymann and Rudolph (1972) using balloon inflation in the descending aorta, showed that though the baroreflex was present in the fetus, its sensitivity was low. The slope of the heart period to systolic pressure relationship increased from about 0.5 msec.mm Hg^{-1} at 85-100 days gestation, to about 1.5 msec.mm Hg^{-1} at 101-120 days and about 2 msec.mm Hg^{-1} at 121 days to term. Responses were not always found to balloon inflation, the proportion of

positive responses increased with gestational age.

Using a similar method of increasing pressure; Maloney, Cannata, Dowling, Else and Ritchie (1977); found greater sensitivity, about 4.5 msec.mm Hg^{-1}, from about 100 days to near term. Higher sensitivity was found when arterial pressure was increased by phenylephrine, 9.1 msec.mm Hg^{-1}. A similar sensitivity was found to pressure increases caused by phenylephrine by Ismay, Lumbers and Stevens (1979) at 8.4 msec.mm Hg^{-1}. In contrast, pressure increases by angiotensin II injections caused no, or at most transient, increases in heart period. This was attributed to central inhibition of parasympathetic activity to the heart rather than sympathetic stimulation, as bradycardia still did not occur after the administration of the beta-blocker propanolol. There was no evidence for a direct chronotropic action of angiotensin II on the heart. The basis for a central action of angiotensin II was established by Scroop and Lowe (1969) in anaesthetized greyhounds.

The differences in heart period sensitivity to pressure increases by phenylephrine and balloon inflation were again found by Dawes, Johnston and Walker (1980). In fetuses from 118-142 days gestation the slope of the heart period to arterial pressure curve was 1.35 msec.mm Hg^{-1} over the pressure range 48-90 mm Hg. This is comparable with that found by Shinebourne et al (1972) though not significantly different from zero. Dawes et al (1980) pooled their data for each fetus whereas Shinebourne et al

(1972) considered each inflation separately, which may explain why their result was statistically significant.

Denervation of the arterial baroreceptors and chemoreceptors by carotid sinus stripping and aortic nerve section at 120-135 days gestation in fetal lambs, abolished the bradycardia associated with the pressor response produced by phenylephrine (Itskowitz and Rudolph, 1982). Resting arterial pressure was not compared in intact and denervated fetuses but may have been decreased by about 3 mm Hg. This suggests only a minimal role of the baroreceptors in the maintenance of arterial pressure.

In assessing the maturity of the baroreflex, the mechanical methods of increasing arterial pressure are to be preferred. Drugs, such as phenylephrine, may also stimulate venous receptors by increasing venous pressure; may have a modulatory effect on the walls of the carotid sinus; and may have central effects. The bulk of the evidence suggests that although the baroreflex is functional in the mature fetal lamb, its sensitivity is low and its threshold above the normal arterial pressure. The maturation of the peripheral vascular component of the baroreceptor reflex is uncertain in the fetus.

At what stage in the reflex pathway the insensitivity occurs is not clear; Biscoe, Purves and Sampson (1969) found that pressure pulse related activity could be recorded from the carotid sinus nerves of term fetal lambs which followed changes in mean pressure. The arterial pressures in their preparations (exteriorized under

maternal or fetal sodium pentobarbitone) were high, about 60 mm Hg and no systematic attempt was made to relate the level of activity to pressure.

1.3.2 The renin-angiotensin system

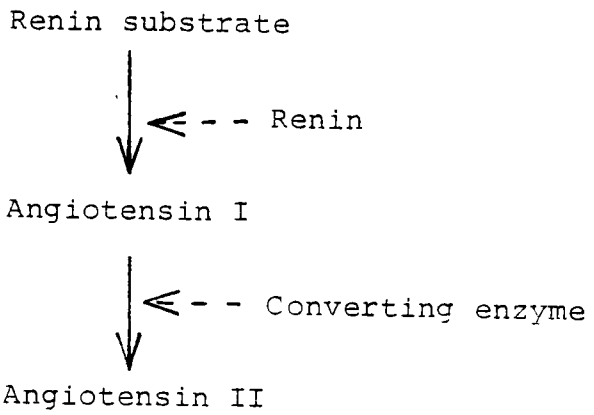
Experiments in newborn rabbits suggest the immature mammal is as well able to regulate its blood pressure as the adult in the face of haemorrhage (Mott,1965). The kidney appears to be largely responsible for this, while in the adult it is the arterial baroreceptors (Mott, 1969). It is likely that it is the renin-angiotensin system that lies behind this function of the kidneys. The renin-angiotensin system consists of several components, summarized in figure 1.4, which vary in the fetus from the adult in different ways.

Plasma renin activity (PRA), which is the endogenous rate of production of angiotensin I, is higher in the fetal lamb than adult sheep (Broughton Pipkin, Lumbers and Mott, 1974; Fleischmann, Oakes, Epstein, Catt and Chez, 1974; and Smith, Lupu, Barajas, Bauer and Bashore, 1974). Carver and Mott (1975) found the renin substrate concentration in the ewe was twice that in the fetus, indicating that differences in renin enzyme concentration or its kinetics lie behind the differences in PRA.

Converting enzyme is present and active in the lungs of fetal lambs near term but was not detected in a single 100 day old fetus (Hebert, Fouron, Boileau and Biron, 1972).

Figure 1.4

Diagram illustrating the main components of the renin-angiotensin system.



Despite the higher fetal PRA, angiotensin II levels are similar in the fetal lamb and ewe (Broughton Pipkin et al, 1974). Robillard, Gomez, Van Orden and Smith (1982) found clearance of angiotensin II from the fetal circulation was approximately five times that from the adult, which may explain the similar angiotensin concentrations at different PRA values.

The renin-angiotensin system in the mature fetus is responsive to many of the factors which stimulate the system in the adult. Furosemide, a diuretic which increases adult plasma renin activity has been shown to increase plasma renin concentration (Trimper and Lumbers, 1972) and PRA (Fleischmann et al, 1975) in mature sheep fetuses, Siegel and Fisher (1980) found that PRA did not change following administration[a] of furosemide to a group of 95-106 day fetal lambs while the response was present, though less than that in newborn lambs, in a 123-142 day group.

Robillard, Gomez, Meernik, Kuehl and Van Orden (1982) found 16% haemorrhage increased PRA from 103-119 days, whereas 8% haemorrhage was sufficient to produce an increase in 132-144 day old fetuses. Plasma angiotensin II maintained the same relationship to PRA in both groups, only increasing with haemorrhage at higher PRA levels such as found in the older fetuses. Arterial blood pressure was well maintained during haemorrhage in the mature fetuses whereas it fell in the younger group. Bilaterally nephrectomized mature fetuses were unable to

maintain their arterial pressure during haemorrhage, indicating the role of the kidneys in the response.

Broughton Pipkin et al (1974) and Smith et al (1974) also found PRA was increased by haemorrhage, and the latter group observed an increase following aortic constriction proximal to the kidneys.

Attempts to determine whether angiotensin II exerts a tonic influence on the fetal circulation have met with variable results. Broughton Pipkin and O'Brien (1978) observed variable pressure responses to the infusion of the angiotensin II antagonist saralasin ([sar^1] ,[ala^8]-angiotensin II). There were some indications that the response was dependent on AII concentration, with decreases in pressure being seen at higher levels of AII. Rankin and Phernetton (1978) found no change in arterial pressure in response to another angiotensin II antagonist, [sar^1], [ile^8]-angiotensin II. Their control and experimental measurements were carried out on different groups of fetuses, making the comparison relatively insensitive.

A more extensive study of the circulatory effects of angiotensin II blockade was made by Iwamoto and Rudolph (1979). They found arterial pressure fell from 47 to 41 mm Hg, umbilical resistance was unchanged, while the resistance of the carcass and skin fell, resulting in a shift of cardiac output from the umbilical circulation to the fetal body. This indicates a tonic influence of angiotensin II on the fetal systemic circulation,

maintaining arterial pressure and umbilical flow.

The circulatory effects of infused angiotensin II present an interesting contrast to the effects of blockers (Iwamoto and Rudolph, 1981). Infusions producing a 10-15% increase in arterial pressure resulted in a 20% increase in combined ventricular output, total peripheral resistance being unchanged. The whole of the flow increase was directed to the fetal body, umbilical resistance was increased. Combined with the blocking studies these results suggest the circulation of the fetal body is normally under maximal influence from angiotensin II whereas the umbilical circulation is below threshold.

1.4 Hypertension in fetal lambs

It was with the aim of investigating the role of the kidneys in fetal arterial pressure regulation that Dutton, Mott and Valdes-Cruz (1978) discovered that hypertension developed 2-9 days following bilateral nephrectomy in fetal lambs, with pressures of 62-98 mm Hg being reached. Hypertension was also produced by tying both ureters. It was later found that only about 80% of the nephrectomized fetal lambs developed hypertension, but the reasons for this were unclear (Mott, 1980). Heart rate was slightly higher than in control fetuses, haematocrit was lower, body weight higher and there were external signs of oedema, the ratio of girth to crown-rump length being increased (Mott, 1980). Cardiac enlargement was also associated with the hypertension. It was also found that the plasma sodium and potassium concentrations were increased in both ewe and

fetus (Dutton and Mott, 1979). Aldosterone concentration was also higher in nephrectomized fetal lambs, showing a positive correlation with plasma potassium and a negative correlation with plasma sodium (Dutton and Mott,1980).

Hyman, Levin, Rudolph and Heymann (1975) described a fetal renovascular hypertension apparently similar to that in the adult, following constriction of one renal artery, the opposite kidney was untouched. The operation was carried out at 110-130 days gestation, and a 50% higher arterial pressure developed within 8 days. The maximum arterial pressures developed were 60% higher than in control lambs. The mechanism of this hypertension has not been investigated, but is likely to involve stimulation of the renin-angiotensin system.

1.5 Objectives

The main objective of the work described in this thesis was to establish whether the fetal renoprival hypertension described above was associated with increased peripheral resistance or increased cardiac output, and which organs were involved in the changes. It was also possible to investigate the contribution of alpha adrenergic vasoconstriction to the hypertension. As the fall in haematocrit suggests changes in vascular volumes, these were also investigated.

CHAPTER 2

Measurement of fetal cardiac output and blood flows

2.1 Introduction

2.1.1 Comparison of methods

2.1.1.1 Cardiometer

Fetal cardiac output was first measured by Barcroft, Flexner and McClurkin (1934) in the exteriorized fetal goat. They used a glass cardiometer, sealed around the great vessels, to follow the volume changes of the heart. This technique excludes coronary flow and the blood entering the atria during ventricular contraction. In adult guinea pigs and rabbits it was shown this method underestimated cardiac output by 15% when compared with the Fick method, so values obtained in the fetus were corrected for this error. However,it is likely that the cardiometer would mechanically interfere with the heart. Corrected combined ventricular output (CVO) was fairly constant at around 150 $ml.min^{-1}.kg^{-1}$ in fetuses from 89 days gestational age to term. Barcroft and Torrens (1946) applied the same technique to fetal lambs, observing a fall in CVO from 360-395 $ml.min^{-1}.kg^{-1}$ body weight at 95-108 days to 190-285 $ml.min^{-1}.kg^{-1}$ at 129-141 days. These values are higher than those from the goat fetus and are comparable with those found by a number of more sophisticated but less direct methods (table 2.1).

Method	Reference	Maternal Anaesthesia	Fetus Ext./i.u.	Gestational Age days	CVO $ml.min^{-1}.kg^{-1}$ Mean ± SD
Cardiometer (corrected)	*Barcroft et al (1934)	Yes, type unstated	Ext.	89-170	150 ± 36
Cardiometer (corrected)	Barcroft and Torrens (1946)	Chloral/ Chloral & spinal	Ext.	95-108 120-141	382 ± 19 243 ± 46
O_2 consumption and contents	Dawes et al (1954)	Pentobarb.	Ext.	134-143	220
Umbilical electromagnetic flowmeter and O_2 contents as above	Dawes (1968)	Pentobarb.	Ext.	134-143	315
Electromagnetic flowmeters	Assali et al (1965)	Spinal	Ext.	?	235 ± 72
Electromagnetic flowmeters	Assali et al (1974)	Pentobarb.	Ext.	120-147	292 ± 34
"	"	Spinal	Ext.	"	258 ± 22
"	"	Spinal	i.u.	"	250 ± 29
"	"	None	i.u.	"	266 ± 22
Dye dilution	Mahon et al (1966)	Chlor.	Ext.	138-147	261 ± 76
Microspheres & antipyrine	Rudolph and Heymann (1967)	Spinal	i.u.	95-108 115-138	660 ± 259 368 ± 76
Microspheres & antipyrine	Rudolph and Heymann (1970)	Spinal	i.u.	60-85 86-100 101-110 111-120 121-140 141-150	485 ± 95 377 ± 77 549 ± 118 497 ± 114 527 ± 111 548 ± 63
Microspheres & reference sample	Cohn et al (1974)	None	i.u.	122-142	481 ± 30(SE?)

Table 1.1

Measurements of combined ventricular output per kg body weight (CVO) by different methods in fetal sheep (* and goats)

Abbreviations: Pentobarb. sodium pentobarbitone; Chlor. chloralose; Ext. = exteriorized; i.u.= in utero

2.1.1.2 Oxygen consumption and mixing technique

Dawes, Mott and Widdicombe (1954) obtained measurements of total oxygen consumption which in conjunction with their figures for distribution (see section 1.1.2) gave an estimated combined ventricular output of 230 $ml.min^{-1}.kg^{-1}$. Dawes (1968), taking umbilical flow as 180 $ml.min^{-1}.kg^{-1}$ body weight, used the distribution figures to estimate CVO as 315 $ml.min^{-1}.kg^{-1}$. Umbilical flow was estimated by critical examination of measurements made by several methods on exteriorized preparations. This figure is confirmed by both electromagnetic flowmeter and indicator dilution techniques (table 2.1).

2.1.1.3 Electromagnetic flowmeters

Assali, Morris and Beck (1965) obtained continuous measurements of flow in the main pulmonary artery, ascending aorta and ductus arteriosus using cuff-type electromagnetic flowmeters in exteriorized fetal lambs of ewes under spinal anaesthesia. They expressed fetal cardiac output as the sum of ascending aortic and ductus arteriosus flows; this they called '"effective" cardiac output', and ideally it would have included coronary flow. The preference of "effective" cardiac output because combined ventricular output includes pulmonary flow twice (Assali et al, 1974) seems illogical. All cardiac outputs quoted from these papers have been recalculated as combined ventricular output, but still exclude coronary flow. Assali et al (1965) observed a CVO of 235 $ml.min^{-1}.kg^{-1}$

In contrast to Dawes et al (1954), Assali et al found a ratio of 41:59 for left:right ventricular output. Assali et al (1965) compared control flows measured by electromagnetic flowmeters under different experimental circumstances. There were four groups of animals; ewes under pentobarbital or spinal anaesthesia with exteriorized fetuses, ewes under spinal anaesthesia with fetuses in utero, and chronically instrumented fetuses of unanaesthetized ewes from one day to four weeks after implantation of catheters and flowmeters. The most noticeable difference between the groups was the slightly higher CVO in the fetuses of pentobarbital anaesthetized ewes. The chronically prepared fetuses had similar CVOs to the fetuses of spinally anaesthetized ewes; the left:right distribution was also comparable, however, foramen ovale flow was notably higher and pulmonary flow slightly lower.

The main criticism of this technique is that of mechanical interference with the vessels and heart, and the possibility of disturbance of neural pathways by implanting up to three electromagnetic flowmeters in the confined space of the thorax. The close spacing of the flowmeters also includes the possibilty of mutual electrical interference. The relative flow to the lungs is high in comparison with other techniques (fig.1.2), perhaps because of direct or indirect constriction of the ductus arteriosus.

2.1.1.4 Dye dilution

The methods described so far all involved thoracotomy, and presumably in the case of the flowmeter experiments a substantial preparation time. To circumvent these problems, Mahon Goodwin and Paul (1966) measured fetal ventricular outputs by dye dilution. Dye was injected into the ventricles and blood sampled from the brachiocephalic artery or the ductus arteriosus to obtain the dilution curves for right and left ventricular outputs respectively. All the catheters were positioned via peripheral vessels in about 40 minutes. The lambs were of 138-147 days gestational age and exteriorized with the ewes under chloralose anaesthesia. Neither blood gas tensions nor blood pressures were measured. The mean heart rate was 252 $\pm$ 31 beats.min^{-1}, which is high, and the possibility of altered magnitude of cardiac output and the contributions of each ventricle cannot be excluded.

Left and right ventricular outputs were 182 $\pm$ 51 and 180 $\pm$ 56 ml.min^{-1}.kg^{-1} (means $\pm$ SD). The large variability of the measurements may hide differences in ventricular outputs. The CVO is larger than by the other methods so far described (see table 2.1). This was attributed to the shorter preparation time as measurements continued for over an hour showed a progressive fall in both ventricular outputs, though the statistical significance of this relationship was not assessed.

Dawes (1968) reported work of a similar nature where ^{131}I albumin was used as the indicator. These results

showed a large spread (110-260 $ml.min^{-1}.kg^{-1}$) with no significant difference between ventricles. Checks against the direct Fick method in 3 week old lambs showed underestimation of ventricular output by an average of 22%.

2.1.1.5 Radioactive microspheres

In 1967 Rudolph and Heymann described a method using radioactively labelled plastic spheres of 50 μm diameter (microspheres) to simultaneously measure fetal cardiac output and its distribution to major organs. Radioactively labelled plastic microspheres are injected such that they become thoroughly mixed with the blood and are distributed in proportion to blood flow. The spheres are trapped in precapillary arterioles, the relative radioactivity in different organs receiving blood from the same source represents their relative flows. If the flow to one organ is measured simultaneously it is possible to calculate the other flows.

As with other methods the application of this method to the fetus is more complex than in the adult because of the incomplete mixing of venous return in the heart. Microspheres injected into the inferior vena cava are distributed at different concentrations to the anterior, posterior and pulmonary circulations. Rudolph and Heymann (1967) used the umbilical blood flow, measured by the steady state antipyrine technique, as a reference flow for the hind body organs, allowing calculation of their flows using the standard equation relating organ activity to blood flow

$$Q_o = \frac{Q_r \cdot A_o}{A_r} \qquad (2.1)$$

where Q_o is the flow to an organ containing trapped microsphere activity A_o, and Q_r and A_r are the flow and activity of the reference organ.

The mean CVO measured two to three hours after replacing the fetuses in utero, with the ewe under spinal anaesthesia, is given in table 2.1. Rudolph and Heymann (1970), using the same techniques, showed no statistically significant differences in CVO per kg body weight in fetuses from 60 to 150 days gestation (see table 2.1). The microsphere method shows a substantially greater CVO, around 500 $ml.min^{-1}.kg^{-1}$ body weight, than obtained by earlier methods (table 2.1).

Instead of measuring the flow to one organ, Makowski, Meschia, Droegemuller and Battaglia (1968) obtained a reference flow by withdrawing arterial blood at a known rate during, and for a short period after, the microsphere injection. They found good agreement between umbilical blood flow measured by this technique and by the antipyrine technique. The advantages of the reference sample technique are that it is simultaneous with the microsphere injection and obviates the equilibration period required by the antipyrine technique.

Heymann, Creasy and Rudolph (1973) used reference samples from the carotid, femoral and pulmonary arteries to simplify the calculation of flows. Comparison of the CVO

obtained as in the original method (Rudolph and Heymann, 1967), using only the hind body reference flow, with this method showed the older method underestimated anterior flows. Measurement of pulmonary flows made using a pulmonary arterial reference sample showed close agreement with measurements made by injection of spheres into the superior vena cava aswell as the inferior vena cava. Collection of three reference samples also allows calculation of flows in the ventricles and fetal shunts. The right ventricular contribution to CVO (67%) was higher than found by other methods, as was the proportion of right ventricular output flowing through the ductus arteriosus (fig. 1.2).

Archie (1974) described equations to calculate right and left ventricular outputs and the flows in the great vessels and fetal shunts, using SVC and IVC injections of microspheres, with anterior, posterior and pulmonary sampling. This technique allows repeated measurements using the same two isotopes each time. However the number of measurements possible is limited by the disturbance of the circulation by large numbers of microspheres.

2.1.1.6 Four-way thermodilution

A four-way thermodilution method for measuring fetal cardiac output was described by Gilbert, Power, Schroder and Longo (1980). Dilution curves were recorded from thermistors in the brachiocephalic artery and descending aorta following successive injections of ice-cold glucose solution into the superior and inferior venae cavae.

Cardiac output (CVO) was calculated from the following equation.

$$CVO = H \cdot \frac{A_s + B_i - A_i - B_s}{A_s.B_i - A_i.B_s} \qquad (2.2)$$

Where A_s and B_s are the integrals of the temperature time curves recorded in the brachiocephalic artery and descending aorta respectively, following injection of indicator into the superior vena cava; and A_i and B_i are the integrals of the curves recorded following inferior vena caval injection of the cold solution. H is the amount of heat injected, corrected for warming of the solution during its passage through the catheter.

The derivation of equation (2.2) given in the paper is invalid, relying on the model of the fetal circulation shown in fig. 2.1 , which does not include pulmonary blood flow. The validity of the equation itself is demonstrated in Appendix 1.

It is well established in the adult that there is little recirculation of indicator by the systemic circulation (Goodyer, Huvos, Eckhardt and Ostberg, 1959). In this application of thermodilution it is important that the fetal lungs also do not allow recirculation of indicator. The adult lungs allow the passage of nearly all the cold indicator but as this is because they are air-filled (Fegler,1954; Goodyer et al, 1959; Hosie, 1962) it seems reasonable to assume the fetal lungs behave like systemic organs in this respect. The results were

Figure 2.1

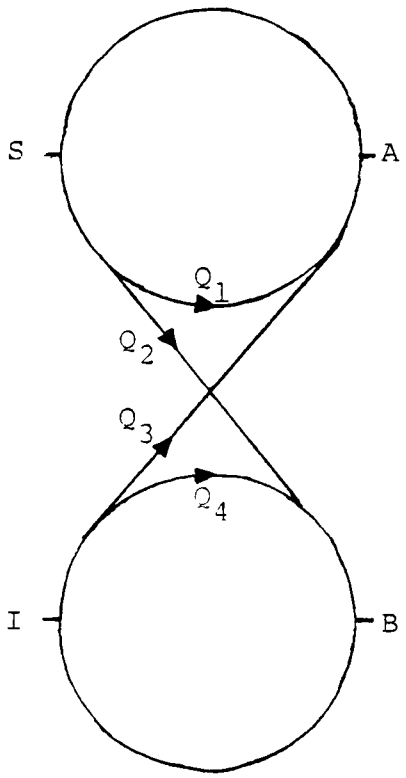


Figure 2.1. Model of the fetal circulation used by Gilbert et al (1980) in the derivation of their four-way thermodilution equation. Points S and I represent the injection sites in the superior and inferior venae cavae respectively. A and B are the recording sites in the brachiocephalic artery and descending aorta. Q_1 to Q_4 are used to represent the heat flows between these sites. The model and derivation of the equation are invalid because these flows correspond to different real flows for anterior and posterior injections.

not presented in a suitable form for inclusion in table 2.1, but reasonable agreement was found with electomagnetic flowmeter measurements in acute fetal lamb preparations and with the microsphere method in both acute and chronic preparations (Gilbert et al, 1980).

Table 2.1 shows estimates of fetal cardiac output have gradually increased as newer methods have been introduced. The most likely explanation for this is the decreasing interference with the cardiovascular system. All except the microsphere and thermodilution techniques involve some disturbance of the heart and/or great vessels and their innervation.

The two most useful methods are the microsphere and thermodilution techniques, both are technically complex though if the dilution curve integration is automated then the thermodilution method is simpler in execution. Other advantages of this method are its repeatability and economy. For long-term studies however, the stability of thermistor sensitivity and response time require investigation.

One of the chief advantages of the microsphere method is in the greater amount of information it provides. The blood flow to almost all discrete organs can be measured, allowing an analysis of the cardiovascular system as an integrated whole. The method is repeatable to a limited extent. The radioactive counting procedures must be carefully controlled and, even in their routine application, are time consuming, and microspheres are

relatively expensive. A degree of interference with the circulation is inevitable, though it can be kept at acceptable levels.

Though the absolute validity of the results may be called into question (Assali et al, 1974), electromagnetic flowmeters are valuable in certain applications where comparative information is adequate. They allow continuous recording which allows almost indefinitely repeatable measurements and removes some variability due to lability of flow.

In order to obtain information about the involvement of different organs, as well as cardiac output in the hypertension described in chapter 1 the microsphere method was used, a more detailed consideration of which follows.

2.1.2 Verification of the microsphere technique

There are three requirements for the validity of the microsphere method. Firstly, microspheres must be evenly mixed with the blood flowing to each organ and its respective reference sample. Secondly, all the spheres must be trapped on their first passage through a capillary bed. Lastly, the blocking of vessels by microspheres must not affect the function of the animal.

2.1.2.1 Uniform mixing of spheres and blood.

2.1.2.1.2 Macrocirculation

Two factors which give rise to uneven distribution of microspheres are; failure of spheres and blood to mix completely before branching of the arterial system, and selective streaming of spheres induced by their rheological behaviour.

There is good evidence of microsphere streaming; Phibbs, Wyler and Neutze (1967) observed axial accumulation of 50 um spheres in cross-sections of rapidly frozen rabbit femoral arteries. The distribution of 7.5 - 10 μm spheres approached uniformity across the vessel crossection, 12.5 - 17.7 μm spheres were also reasonably well dispersed (Phibbs and Dong, 1970). The axial accumulation was greater with spheres of larger diameter (up to 80 μm).

Streaming of microspheres does not imply their maldistribution to or within organs; in assessing this the random variability of microsphere distribution has to be taken into account. The number of microspheres received by an organ varies around the mean determined by its flow, according to the binomial distribution. The larger the number of spheres injected the closer their distribution can be expected to represent the distribution of flow. Buckberg Luck, Payne, Hoffman, Archie and Fixler (1971) showed that the expected variation in the difference of microsphere concentrations received by two reference samples is dependent on the total number of spheres in the

samples and their relative flows. However, in dogs and sheep they found the variability in microsphere concentrations of paired reference samples exceeded the theoretical predictions. This indicates an additional source of variation to the random distribution errors. However, as there was no systematic difference between vessels, apart from between the carotid and femoral arteries in sheep, this is probably due to incomplete mixing rather than streaming.

Neutze, Wyler and Rudolph (1968) found no systematic difference between carotid and femoral concentrations of 50 μm spheres in rabbits but did not consider distribution errors. Bartrum, Berkowitz and Hollenberg (1974) found the variability between femoral arterial concentrations of 15 μm spheres in rabbits was as expected, whereas greater, though still random variability was found between carotid and femoral concentrations.

Using 25 μm spheres in the fetal lamb, Makowski et al (1968) found 1.7 $\pm$ 0.9% difference in activity of left and right umbilical artery samples, it is unlikely that this is greater than expected. Makowski, Schneider, Tsoulos, Colwill, Battaglia and Meschia (1972) observed 8.6 $\pm$ 2.4% difference between left and right transverse scapular artery concentrations, though sphere numbers were not given.

Similar checks can be made by comparing the activity of paired organs, e.g. left and right kidneys or cerebral hemispheres, which may reasonably be assumed to have equal

flows. Rudolph and Heymann (1967) showed comparatively large variability of the left:right renal activity ratio in fetal lambs (mean 1.03 ± 0.31 SD). In rabbits Neutze et al (1968) found close agreement of right and left kidney flows. Forsyth, Nies, Wyler, Neutze and Melmon (1968) (50 μm spheres in monkeys) and Sasaki and Wagner (1971) (50 μm spheres in rats) found similar flows for left and right kidneys; but the crucial factor, the variabilty of the left:right distribution, was not assessed. Buckberg et al (1971) found more variability in the left:right kidney distribution than explained by random distribution errors, as did Bartrum et al (1974) for 15 μm spheres in rabbits. Warren and Ledingham (1974) found extremely close agreement of left and right renal flows in rabbits, it is unlikely that these exceed expectation.

Thus, there is evidence that systematic streaming between organs and reference samples can occur but is rare. A more important finding is that the theoretical variability of microsphere distribution is often exceeded.

2.1.2.1.2 Microcirculation

Streaming in the microcirculation has been assessed mainly by comparison of the distribution of different size spheres. At this level the distinction between erythrocyte and plasma flows also has to be recognized.

In dog kidneys, McNay and Abe (1970) found no difference in the distribution of 19, 27 or 35 μm spheres. In contrast, Katz, Blantz, Rector and Seldin (1971) using a

wider range of sphere sizes (7-10, 15, 35, 50 and 80 μm), found larger spheres were preferentially distributed to the cortical layers receiving highest flow; the effect was less marked between 7-10 and 15 μm spheres. Slotkoff, Logan, Jose, D'Avella and Eisner (1971) found similar sizes of spheres in different layers. Warren and Ledingham (1975) found no size differences in different renal cortical layers for spheres of nominal 15 μm diameter in adult rabbits; but for 25 μm spheres in adults and 15 μm in young rabbits, larger spheres were found in the outer quarter of cortex.

In the heart, though there is a greater fractional distribution of all size microspheres to the subendocardial layers than to the subepicardial layers, this is greater for larger spheres (Domenech, Hoffman, Noble, Saunders, Henson, and Subijanto, 1969; Fortuin, Kaihara, Becker and Pitt, 1971). This seems partly to arise from the anatomy of the coronary microcirculation and partly because larger spheres overestimate the flow in regions receiving higher flows (Yipintsoi, Dobbs, Scanlon, Knapp and Bassingthwaighte, 1973; Utley, Carlson, Hoffman, Martinez and Buckberg, 1974). The distribution of even 9 and 15 μm spheres differs from that of diffusible indicators, though which more closely resemble the distribution of flow is not clear (Yipintsoi et al,1973 and Utley et al 1974).

2.1.2.2 Recirculation of microspheres

Though considerations of accuracy and disturbance of the circulation require that spheres be as small as

possible a lower limit is imposed on sphere size by the need to avoid recirculation.

Rudolph and Heymann (1967) found no evidence of shunting of 50 μm spheres by the posterior circulation of fetal lambs. Even if the hepatic arterial contribution is totally ignored, the activity in the liver represented a neglible level of shunting by the umbilical circulation.

Neutze et al (1968) found less than 1% shunting of 50 μm spheres by the systemic circulation of anaesthetized and conscious, normotensive and hypertensive rabbits. Shunting of this order was also observed by Bartrum et al (1974) in anaesthetized rabbits. Similarly, systemic shunting of 50 μm spheres has been found to be insignificant in rhesus monkeys (Forsyth et al, 1968), and rats (Sasaki and Wagner, 1971; Mendell and Hollenberg, 1971). This was usually assessed by the activity in the lungs following abdominal aortic injection of the spheres. The efficiency of the lungs as a trap for systemically shunted spheres was verified by Sasaki and Wagner (1971).

The same approach was used by Kaihara, Van Heerden, Migita and Wagner (1968) who found 5-10% systemic shunting of 15 μm spheres in sodium pentobarbital anaestheized dogs, compared with 1% for 50 μm spheres. Pentobarbital anaesthesia was found to increase the proportion of 15 μm spheres injected into the left ventricle from 3% to 17%, though these values include the bronchial circulation.

Though total shunting may be insignificant, it may hide

high levels of shunting by individual organs, and this must be assessed when organ flows are being considered.

Fortuin et al (1971) found less than 1% shunting of 15 μm spheres by the coronary circulation of pentobarbital anaestheized dogs. Utley et al (1974) found 0-3.8% coronary shunting of 15 μm spheres and 0-2.2% of 25 μm spheres. During maximal coronary vasodilation with acetylcholine, 0.8-1.1% shunting occured.

Several groups have investigated the shunting by the canine renal circulation. Less than 1% of 15, 25 and 35 μm spheres were shunted by the kidneys of dogs under control conditions (Urdaneta, Gilsfdorf, Scarpino and Delaney, 1969).Less than 0.5% renal shunting of 19-35 μm spheres was observed in anaesthetized dogs (McNay and Abe,1970). However, in this investigation venous sampling was only carried out for 30 seconds post injection. Slotkoff et al (1971), using 15 μm spheres showed 0.2% shunting by the renal circulation over 30 seconds following the injection. Katz, Blantz, Rector and Seldin (1971) also found no significant renal shunting of spheres greater than 10 μm in diameter.

Warren and Ledingham (1975) assessed the shunting by individual organs of the conscious rabbit. No significant shunting was found for 15-50 μm spheres in the liver, kidneys or gut. However, the skinned hind limb shunted 7% of 50 μm, 13% of 25 μm and 18% of 15 μm spheres and over 80% of all three sizes passed through the ear. Following left atrial injection around 12% of 15 μm and 25 μm spheres

were found in the lungs but only ~3% of 50 μm spheres, suggesting substantial shunting by the systemic circulation as a whole.

Archie, Fixler, Ullyot, Hoffman, Utley and Carlson (1973) found 1.4% of 15 μm spheres were not trapped in the kidneys of anaesthetized lambs. No spheres over 9 μm were found in blood sampled from the renal and coronary venous effluents and the pulmonary artery.

Makowski et al (1968), found 0.28% of 25 μm and 0.15% of 15 μm spheres escaped trapping in the umbilical circulation. Cohn, Sacks, Heymann and Rudolph (1974) found no significant activity in the liver following injection of 15 μm spheres into an umbilical artery. Though the liver does not receive all the umbilical venous blood, it receives enough (~ 50%; Edelstone, Rudolph and Heymann, 1978) to imply insignificant shunting by the umbilical circulation. The cerebral circulation of the fetal lamb also traps virtually 100% of 15 μm spheres, as determined by sagittal sinus sampling (Makowski et al, 1972).

Microspheres smaller than 15 μm do not appear to be generally suitable for total cardiac output measurements but may still be valid in some organs and species (Buckberg et al, 1971; Marcus, Heistad, Ehrhardt and Abboudt, 1976; Fan, Schuessler, Chen and Chien, 1979).

2.1.2.3 Effects of microspheres on circulatory function

An unavoidable consequence of the microsphere method is its disturbance of the animal, through the blocking of vessels, potentially affecting the circulation and the function of the tissues. However, this disturbance can be kept to a level which may be safely ignored. Intuitively, it might be expected that the degree of interference is influenced by sphere size, numbers of spheres relative to animal size, and the different vascular patterns and densities of different organs. In addition, though resting function may be unimpaired, the degree to which potential function is affected is also important.

Rudolph and Heymann (1967) injected 10-25 $x10^3$ 50 μm spheres (~3-25 $x10^3$/kg) into fetuses from 85 days to term and found no changes in arterial pressure, heart rate, umbilical flow or blood gas tensions. Second and third injections were similarly distributed to the first.

Makowski et al (1972) found cerebral flow was 20% higher for a second injection of an unstated number of 15 μm spheres in acute preparations. One of two chronic preparations showed 70% higher flow for the second injection. A larger group of chronic preparations showed a significant decrease in arterial PO_2 following microsphere injections, which may explain the changes in flow. Heymann et al (1977) stated that no significant effects were seen following up to five injections of over 1 million spheres in fetal lambs, though no details are given.

In dogs, Domenech et al (1969) found no changes in heart rate, arterial pressure, cardiac output or coronary flow over six injections of 7-100 $x10^3$ 50 μm spheres (0.2-3 $x10^3$ /kg). Fortuin et al (1971) examined the effect of 15 μm spheres on dogs in more detail; they measured cardiac output, heart rate, arterial pressure, resting and peak reactive hyperaemic coronary flow (e.m. flowmeter). No significant changes were found until 4 $x10^6$ (0.1-2.5 $x10^6$ /kg) were given, which caused increased arterial pressure. Other variables were unchanged by up to $12x10^6$ spheres (0.5-0.8 $x10^6$ /kg). However, total peripheral resistance was, by implication, raised.

Two studies of the effects of microspheres on dog kidneys have been made. Katz et al (1971) showed no significant effect on renal function of approximately 0.2-0.6 x 10^6 spheres per kg. Slotkoff et al (1971) found four injections of 0.2 x 10^6 15 μm spheres per kg had no effects, while injections of 2 x 10^6 /kg produced progressively greater decreases in renal blood flow and glomerular filtration rate.

Both of the preceding studies used anaesthetized dogs; Roth et al (1970) observed significant and disturbing neurological effects of 0.2 x 10^6 50 μm spheres on conscious dogs. It is, however, difficult to relate this to numbers of smaller spheres.

Rabbits are more sensitive to microspheres. Although 0.03 x 10^6 15 μm spheres did not change cardiac output or its distribution (Warren and Ledingham, 1974) and 0.1 x 10^6

(~0.04 x 10^6 /kg) did not affect renal flow or GFR, 0.2 x 10^6 (~0.08 x 10^6 /kg) reduced renal flow by 12% and 0.5 x 10^6 (~0.2 x 10^6 /kg) by 40%, other measures of renal function were significantly reduced (Warren and Ledingham, 1975).

Gregory (1975) found no changes in cardiac output, blood pressure, organ flows or resistances following 3 injections of 0.1 x 10^6 15 μm spheres (~0.04 x 10^6 /kg) in rabbits, though there was a 12% increase in heart rate following the third injection when compared to control.

Hoffbrand and Forsyth (1969) gave 5-10 x 10^3 50 μm spheres to rhesus monkeys. they found no significant differences between measurements of cardiac output, total peripheral resistance, blood pressure or heart rate made 24 hours apart. They did find some significant changes during the course of four injections over two hours, notably an increased share of cardiac output received by the heart.

2.2 Methods

The standard experimental procedures are described here, variations in protocol will be given where appropriate.

2.2.1 Surgery

Pregnant ewes at 117 to 125 days gestation were starved for 24 hours prior to induction of anaesthesia with 1g thiopentone sodium (Intraval, May and Baker), an

endotracheal tube was inserted and anaesthesia maintained with halothane (May and Baker) in oxygen. Following standard aseptic procedures, the uterus was exposed through a ventral mid-line incision and catheters for insertion into the fetus were passed through a stab wound made in the ewe's flank. In multiple pregnancies, the most suitably placed fetus was selected for catheterization and the head and neck delivered through an incision in the uterus, the edges of which were clamped to the fetal skin to avoid loss of amniotic fluid. The left carotid artery and a jugular vein were ligated and vinyl catheters (2mm o.d., 1mm i.d.) inserted approximately 8 cm towards the heart. The head was returned to the uterus and the incision closed, taking care to appose amnion to amnion.

The hind-quarters were located and delivered as for the head. In 7 fetuses both kidneys were removed through retroperitoneal incisions; they were cleared of fat, the renal artery and vein and ureter tied as one bundle and cut; the fascia and skin were closed separately. In one fetus both ureters were tied close to the renal pelvices, the renal circulation was left intact. A vinyl double lumen catheter (2mm o.d, 2 x 1mm i.d.; Dural Plastics, Australia) was inserted into the right tarsal vein proximal to the heel and advanced 1.5 times the heel-toe length, to position the tip in the inferior vena cava, around the level of the diaphragm. Both femoral arteries were ligated and catheters (as for neck) inserted 4-6 cm to position the tips near the abdominal aortic bifurcation. Either at this stage, or before closing the first uterine

incision, a vinyl catheter (3mm o.d, 2mm i.d.) with side holes for ~10 cm at the tip, was passed towards the chest and sutured to the skin.

The fetus was returned to the uterus, the incision closed and the uterus restored to its original position. The peritoneum was closed, an antibiotic spray (Dispray, Stuart Pharmaceuticals) applied to the subcutaneous space and the skin sutured. The catheters were secured with a purse string suture around the flank incision.

Simultaneously with this procedure, catheters (3mm o.d., 2mm i.d.) were inserted into a maternal carotid artery and jugular vein. All catheters were ended in three-way taps, which were kept sterile with plugs and rubber caps. The catheters were tunnelled through the wool and kept in plastic bags on the ewe's back. Typically the ewe was anaesthetized for 3-3.5 hours, recovery was generally swift, most were standing one hour post-operation and accepted food and water enthusiastically. The ewe was kept in a metabolism cage and prevented from turning around by a collar chained to the front of the cage.

2.2.2 Daily maintenance

Following recovery, one femoral arterial catheter, one lumen of the inferior vena caval catheter and the amniotic catheter were connected to sterilized pressure transducers (Bell and Howell, 4-422-0001) kept with the remaining fetal catheters in a covered perspex box at the side of the cage. The arterial and venous pressures were electronically

corrected for amniotic pressure by differential amplifiers (built in the electronic workshop of the Nuffield Institute). Heart rate was obtained from an instantaneous ratemeter (Devices, type 2751) triggered by the arterial pressure pulse. Mean arterial (2 second time constant), venous and amniotic pressures and heart rate were displayed on a Cambridge Instruments slow recorder. Sterile heparinized saline (250 units per ml) was infused (at 3.8 ml per day) through each of the vascular recording catheters.

The calibration of the recording system was checked daily, or less frequently when stable, using calibration signals matched for each transducer. These signals were checked between experiments with a mercury manometer.

Antibiotics were given to ewe and fetus for three days post-operation ,twice daily, in the following doses: 300 mg penicillin (Crystapen, Glaxo) each to the fetus (i.v.) and amniotic cavity; 0.5 or 1g streptomycin (Glaxo) and 600mg penicillin (i.m.) to the ewe. All catheters without a continuous infusion were flushed daily with heparinized saline.

0.5-1.0 ml blood samples were taken from fetal femoral and maternal carotid arteries and their PO_2, PCO_2 and pH measured in a Corning blood gas analyser (Model 165). It was found that the samples were being analysed at a temperature of 36.5°C rather than the 39°C indicated. All values have been corrected to 39°C, the body temperature of the fetal lamb, according to the formulae given in appendix

2. The haematocrit of the samples was measured on a microhaematocrit centrifuge (Hawksley). In some cases haemoglobin concentration was measured; a 50 µl sample was added to 10ml 0.4% v/v NH_4OH and at 20 minutes, its optical density read at 540 nm on a Dow colorimeter. Concentration was obtained from a haemoglobin standard curve (all haemoglobin analyses were performed by G. Haines).

Where PO_2, pH and haematocrit were known it was possible to calculate oxygen content. Oxygen saturation was calculated using the dissociation curve described by Cassin, Dawes, Mott, Ross and Strang (1964). Where haemoglobin concentration was not measured it could be calculated by multiplying the haematocrit by a mean cellular haemoglobin concentration of 4.58 mM (obtained in a number of control and nephrectomized fetuses). Oxygen capacity (mM) was obtained by multiplying haemoglobin concentration by 4. Oxygen content is the product of saturation and oxygen capacity.

2.2.3 Microsphere protocol

Measurement of cardiac output and organ flows was performed 5-15 days post operation (mostly at 7-9 days). At least one day prior to the experiment the preparation was connected to a Schwarzer polygraph to record arterial, central venous and perfusion pressures. Mean perfusion pressure was obtained by electronically subtracting central venous pressure from arterial pressure. The records were approximately calibrated using the electrical signals, a

definitive calibration was performed with a mercury manometer at the end of the experiment.

PO_2, PCO_2, pH and haematocrit were measured on 0.5-1ml samples taken from the fetal carotid and femoral arteries, and the catheters connected to the withdrawal system shown in figure 2.2. No taps or other obstructions which might trap microspheres were included in the withdrawal lines. Microspheres, nominally 15 μm in diameter, labelled with ^{46}Sc or ^{85}Sr and suspended in 10% dextran with Tween 80 were used (3M company or New England Nuclear). Before use a sample was examined microscopically and the sizes of at least 100 spheres measured, checks were also made for aggregations and broken spheres. The fraction of the suspension activity in the supernatant was also determined. Having previously been well shaken and ultrasonicated, the vial containing the spheres was given a final shake, ~0.5ml of the suspension was withdrawn into a 2ml syringe and a 21 gauge cannula attached. The syringe and cannula were weighed and a few drops of the suspension put into a preweighed counting vial. Throughout, air was kept in the syringe to aid keeping the spheres in suspension by agitating the syringe. The cannula was connected directly to the free lumen of the inferior vena caval catheter and the withdrawal pumps switched on. Once the reference samples were flowing smoothly, the microspheres were injected into the catheter and flushed into the circulation with 3-4ml heparinized saline. At least 90 seconds later, the pumps were switched off, the catheters flushed with 5ml heparinized saline and the tubing of the arterial side of

Figure 2.2

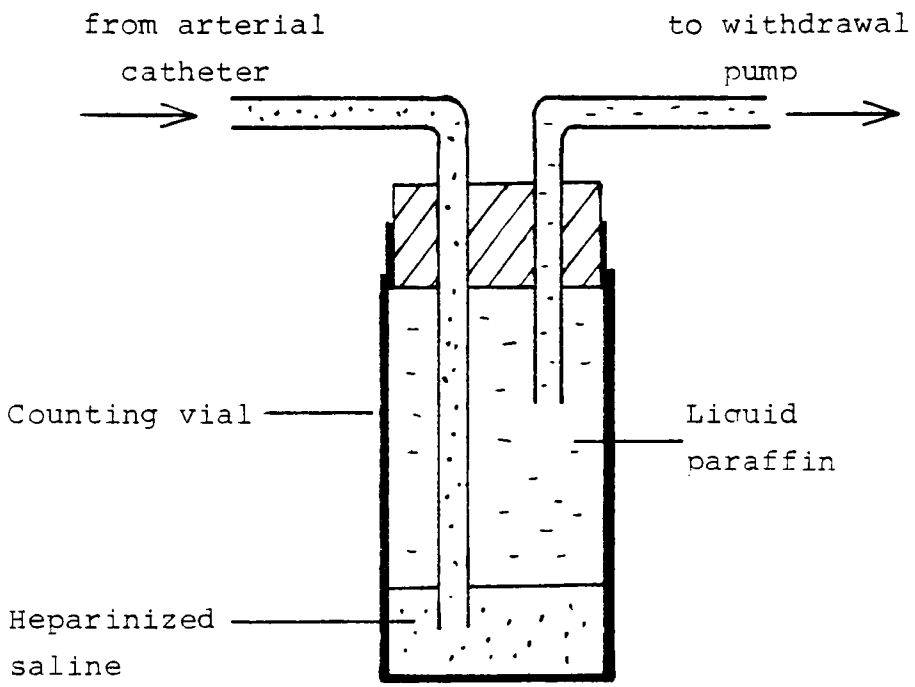


Figure 2.2. System for collection of arterial reference samples directly into counting vials. Care was taken to exclude air from the system on setting up.

each withdrawal system rinsed into a counting vial. During the sampling and injection procedure, the recorder chart was run fast enough to distinguish individual beats, so any cardiac arrhythmia could be seen and heart rate measured. The injection syringe and vial containing the injectate sample were reweighed.

A second injection of microspheres was given approximately one hour later, either under control conditions (8 experiments) or during an infusion of phentolamine (7 experiments, see chapter 4). After the second injection and withdrawals had been completed, the ewe was killed with sodium pentobarbitone (Expiral, Abbott) or with a humane killer. The uterus was removed and its wall removed from the conceptus, cutting the endometrium from around the circumferences of the placental cotyledons. The fetus was removed from the amnion and the umbilical cord tied and cut. The wool was dried with a blow dryer as amniotic fluid was found to add ~10% to the body weight.

Following the division determined by Peeters (1978), the skin was removed in two pieces, one supplied from the brachiocephalic artery and one from the descending aorta, along a line running from the fourth thoracic vertebra at the back to the eighth rib at the sternum. The brain, heart, kidneys, adrenals, spleen, liver and gastro-intestinal tract were removed, weighed and placed in counting vials (the gut was emptied before weighing). The carcass was divided into anterior and posterior portions along a line running from the third thoracic vertebra to

the sixth rib at the front; the sternum was included in the anterior portion. The placental cotyledons were separated from the chorion at their bases; in the case of multiple pregnancies cotyledons were allocated to each fetus as determined by Mellor (1969). Shared cotyledons and the chorioallantois were allocated in favour of the experimental fetus. It would have been impractical to count the whole of the carcass, skin, cotyledons and membranes, so these organs were minced and an aliquot of the well mixed homogenate taken for counting. Both portions of skin were cut into small pieces as they were too tough for mincing.

2.2.4 Isotope counting procedure

A Packard gamma scintillation spectrometer (type 5320) was used to measure the activity in the samples, the windows used to count ^{46}Sc and ^{85}Sr are given in table 2.2, along with their counting efficiencies. Strontium counts for all tissue samples were corrected for scandium activity appearing in the strontium channel using separate correction factors for homogenous and settled samples measured on each counting run; 17 to 20 % of the scandium count was observed in the strontium window.

The gamma counter was set up using solutions of both isotopes so that the count rate varied less than 1.5% over sample heights up to 30mm. However, suspensions of microspheres gave substantially different counts when spheres were homogenously distributed than when settled, apparently due to collection of spheres at the sides and

Isotope	Counting Window keV	Counting Efficiency %
^{85}Sr	450 - 560	26.4
^{46}Sc	773 - 1255	21.8

Table 2.2

Counting windows and efficiencies for microsphere isotopes on Packard gamma counter (type 5320)

centre of the vials. Depending on the brand of vials used, this difference varied from 2 to 8%. All samples in which the microspheres were settled (blood, injectate, etc.) were corrected for this.

At high count rates the response of scintillation counters becomes non-linear; the range of linearity of the Packard counter was checked using samples of known activity. For the windows used, the counter was linear up to a sample activity of ~0.6 μCi (~3.5×10^5 cpm) for ^{85}Sr and ~0.25μCi (~1.2×10^5 cpm) for ^{46}Sc. All samples were divided such that the cpm per vial was less than 1×10^5. Samples were counted for 2 minutes, when sample activities were low the whole set of vials was recounted to keep counting errors to an acceptable level. As well as printed output from the counter, the data was automatically punched on paper tape and read into a computer for calculation of results. Vials containing known numbers of spheres were prepared by making a streak of the sphere suspension on a small strip of graph paper and counting the spheres under a microscope. These standards were included in each counting run to allow calculation of sample sphere numbers.

2.2.5 Calculations

A program to calculate the results of the microsphere experiments was written in FORTRAN IV on a CTL 8050 computer, a flow diagram showing the major steps is given in appendix 3(a). After correction of sample counts for background, geometry and crossover, flow was calculated

according to equation 2.1 (see section 2.1.1.5). The skin and carcass flows were calculated as the sum of their anterior and posterior portions. For pulmonary flow only the portion derived from the IVC could be calculated, as described by Rudolph and Heymann (1967). For this calculation, the total activity injected was taken as the mean of the total activity counted and the total estimated from activity of the weighed sample of injectate. Combined ventricular output (CVO) was taken as the sum of all organ and reference sample flows. Organ conductances were calculated according to the following formula

$$C = \frac{Q}{P_a - P_v} \qquad (2.3)$$

where C = conductance, Q = flow, P_a = arterial Pressure, P_v = venous pressure. Both absolute and specific (per 100g tissue) flows were calculated and the fraction of combined ventricular output. All stages of the calculation were checked for one experiment and for all others the manually entered data was included in the program printout and checked against the original recordings of the data.

To avoid as much manual handling of the data as possible, the results of the calculations were also stored on-line for subsequent analysis. A flow diagram indicating the various routes this could take is given in appendix 3(b). Most statistical analysis was performed using "Statistical Package for the Social Sciences" (SPSS)

(Nie, Hall, Jenkins, Steinbrenner and Bent, 1975). Both this package and a data retrieval program allowed display of any variables from experiments selected on the basis of values of any other variables, in this way individual values could be examined, aswell as statistics.

2.3 Results

Two aspects of the microsphere method were checked in these experiments; the reproducibility of the results and the effect of microspheres on the fetus were examined by comparing two consecutive sets of measurements; and the mixing of spheres was checked by considering the left:right distribution of spheres in paired organs.

2.3.1 Microspheres

The ranges, means and standard deviations of microsphere sizes are given in table 2.3 along with leaching data. Sphere sizes were approximately normally distributed in each batch and no spheres smaller than 12um were found in any batch. Occasionally, irregularly shaped or broken spheres were seen, however, these formed an insignificant fraction of the total. Less than 1% of the total suspension activity was found in the suspending solution.

2.3.2 Adequacy of mixing

This was assessed by comparing the numbers of spheres in left and right kidneys in relation to their weight. A similar comparison was made for the left and right cerebral

Isotope	Size µm Range µm	Mean µm	SD	Leaching %
	13 - 19	15.7 ±	1.2	0.72
^{85}Sr	12 - 16	14.0 ±	1.1	0.11
	12 - 16	14.1 ±	2.0	0.01
	13 - 20	15.7 ±	13.6	0.50
^{46}Sc	12 - 18	14.6 ±	1.3	0.00
	13 - 19	15.3 ±	1.3	0.84

Table 2.3

Sizes and leaching (% of suspension activity in solution) for the six batches of microspheres used

hemispheres; this also acted as a check on the effect of tying the left carotid artery on the left : right distribution of blood in the brain.

A modification of the relationship described by Buckberg et al (1971) was used. If the flow per unit weight to paired organs is assumed to be equal, then for a given total number of spheres in the pair, a distribution in proportion to their weights would be expected. Where, W is the weight, X is the number of microspheres and the subscripts r,l and t , right left and total (r+l) and $f=W_r/W_t$; the expected number of microspheres in the right-hand organ is fX_t and

$$z = \frac{X_r - fX_t}{X_t f(1-f)} \qquad (2.4)$$

z is a normal deviate, i.e it should have a mean of zero and a standard deviation of one. z was calculated for the kidneys for sixteen injections in eight control fetuses, four were made during phentolamine infusions but this should not affect the left:right comparison. The mean of z was 5.84 $\pm$ 4.09 (SE), not significantly different from 0; its standard deviation was 16.34. The distribution of z is shown in figure 2.5(a); the variability of z is greater than that expected from a random distribution of spheres.

The frequency distribution of thirty values of z obtained in fifteen lambs for the cerebral hemispheres is shown in figure 2.5(b). The mean is 0.23 $\pm$ 1.74 (SE) and the standard deviation 9.52. Though the mean is not

Figure 2.3

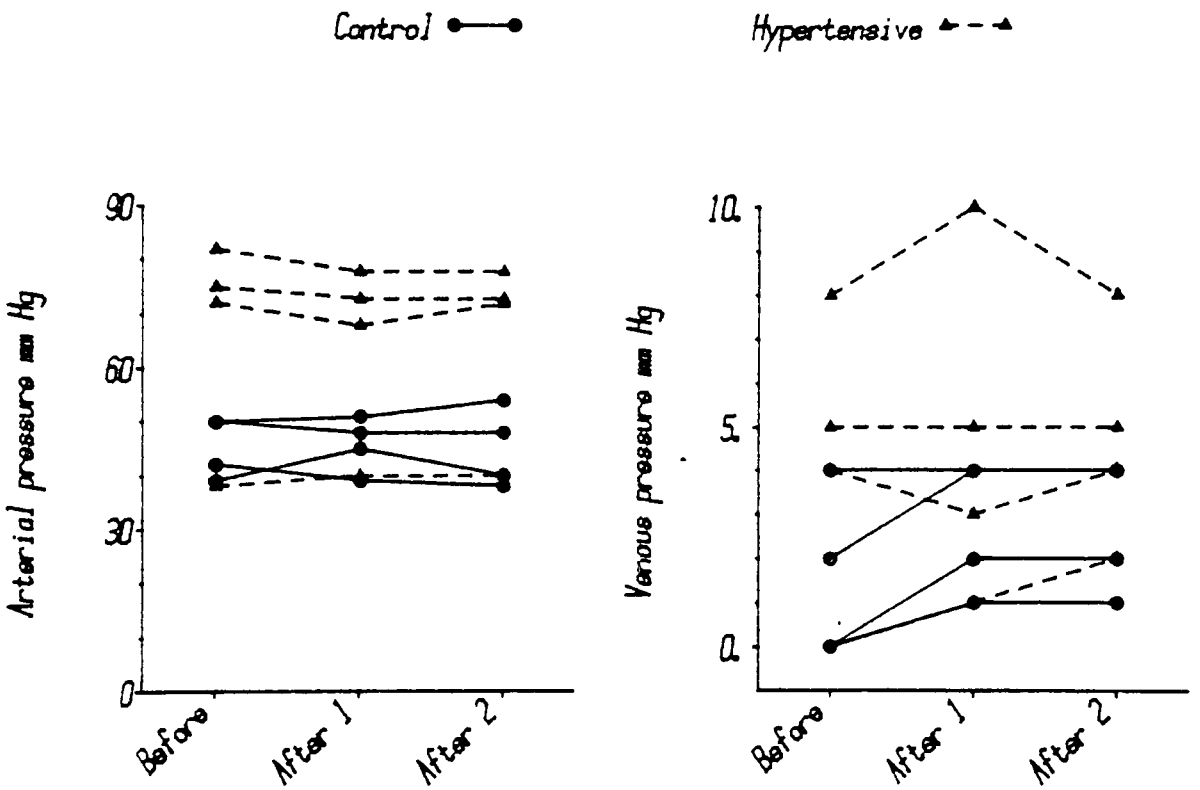
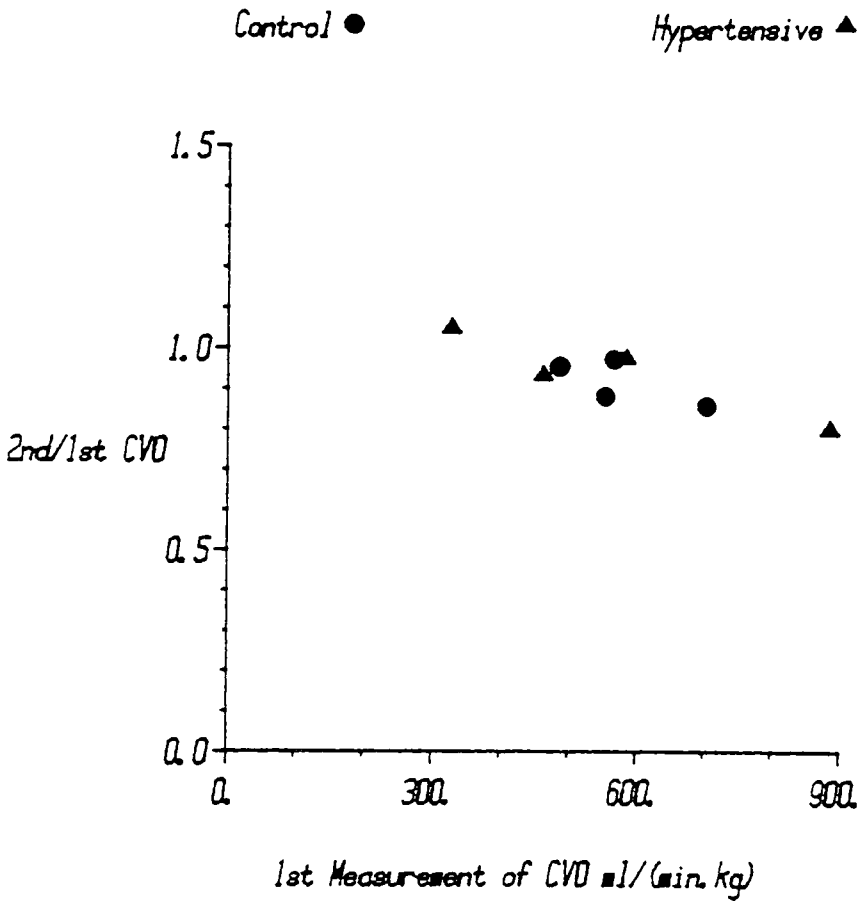


Figure 2.3. Mean arterial and venous pressures before and after each of two microsphere injections.

Figure 2.4



Ratio of 2nd:1st measurements of combined ventricular output (CVO) plotted against the first measurement for 4 control and 4 nephrectomized fetuses. (correlation coefficient, r = -0.88, P= 0.04).

Figure 2.5

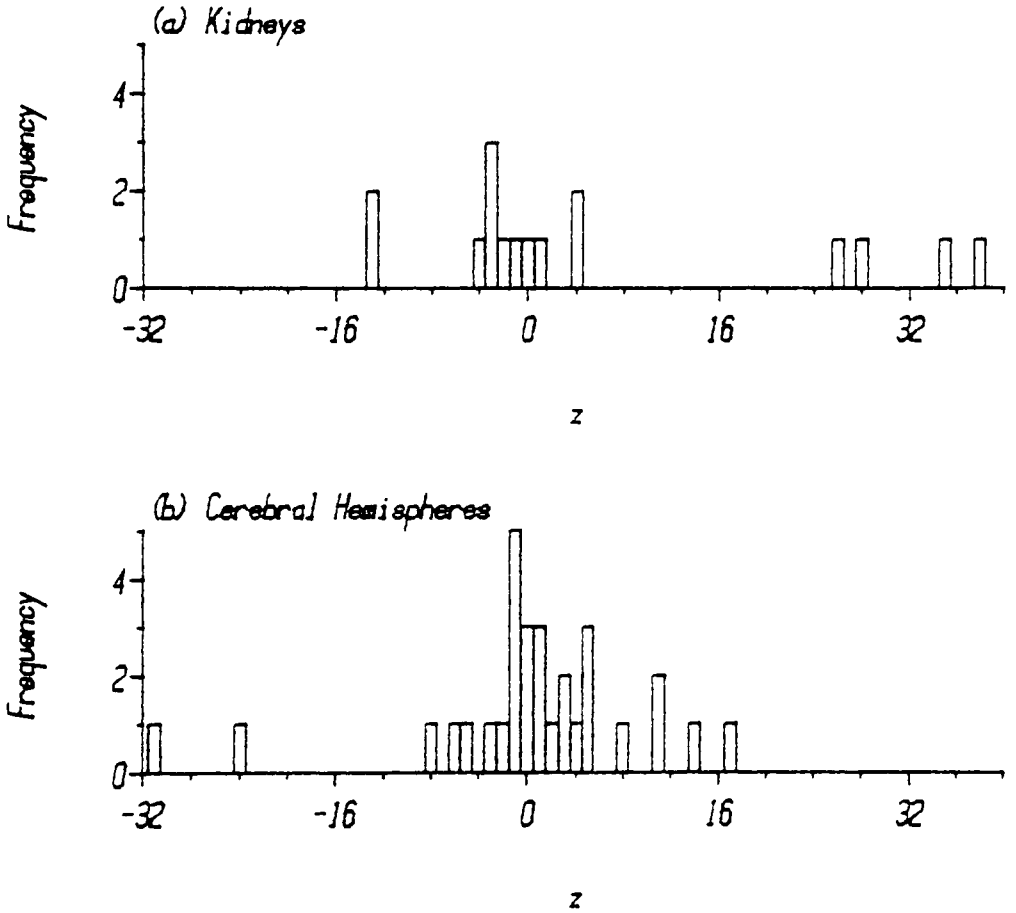


Figure 2.5. Frequency distributions of z (see text) for
(a) right and left kidneys (16 measurements)
(b) right and left cerebral hemispheres (30 measurements)

significantly different from 0, the distribution shows a slight predominance of positive values of z suggestive of lower flow in the left hemisphere than expected. The mean ratio (±SE) of left:right specific flow was 1.013 ± 0.021, and the standard deviation 0.113.

Figure 2.6 shows the differences between the left and right microsphere concentrations for the kidneys and brain. The theoretical 95% limits are also shown. The mean difference is not a suitable statistic to summarize the absolute percentage differences as they are not normally distributed. For the cerebral hemispheres, most injections show a concentration difference of less than 7%. Though there are more outlying points for the kidneys, the concentration differences are mainly clustered below 7%.

2.3.3 Reproducibility

In eight fetuses two microsphere injections were made under control conditions approximately one hour apart. This group consisted of four control fetuses, three nephrectomized and one with tied ureters. There were no significant changes in blood gas tensions, pH or haematocrit following one or two microsphere injections (table 2.4). Arterial and venous pressures were also unchanged as shown in figure 2.3 and table 2.4.

Table 2.5 shows the percent changes from first to second injections of combined ventricular output and organ blood flows. The second measurement of CVO is significantly lower than the first, the mean % change being

Figure 2.6

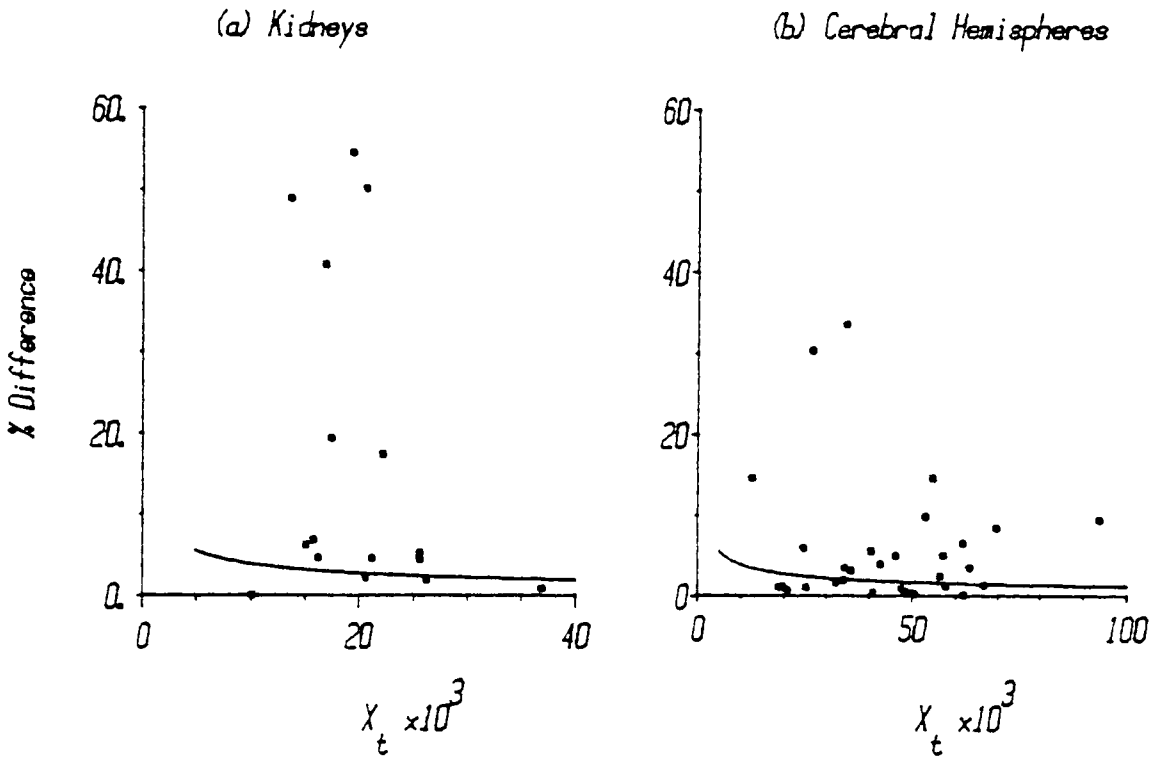


Figure 2.6. Percentage differences in microsphere concentrations in (a) right and left kidneys, (b) right and left cerebral hemispheres, plotted against the total number of spheres in the pair X_t. The curves are the upper 95% confidence limits predicted by Buckberg et al (1971).

	Change following one injection	Change following two injections
PO_2 mmHg	0.49 ± 0.78	0.03 ± 0.99 (n=6)
PCO_2 mmHg	-2.38 ± 1.46	-0.44 ± 3.19 (n=5)
pH	0.018 ± 0.008	-0.01 ± 0.012 (n=6)
Haematocrit %	-0.6 ± 0.51 (n=5)	-1.8 ± 1.3 (n=3)
Arterial pressure mmHg	-0.75 ± 1.24	-0.63 ± 1.02
Venous pressure mmHg	0.88 ± 0.40	0.88 ± 0.35
Heart rate beats.min^{-1}	-6.9 ± 3.8	-

Table 2.4

Mean changes (± SE) in carotid arterial blood gas tensions, pH, haematocrit, blood pressures and heart rate following one and two microsphere injections. None is significantly different from 0 (paired t-test). n=8 unless otherwise stated

	Mean % difference in flow (± SE)	Mean % difference in conductance (± SE)
CVO and TPC	-7.1 ± 2.8*	-4.8 ± 3.5
Brain	-13.5 ± 6.4	-11.7 ± 6.1
Heart	-15.5 ± 5.1*	-13.8 ± 4.5*
Adrenals	-3.4 ± 9.7	-1.6 ± 9.3
Spleen	33.2 ± 45.3	37.8 ± 47.8
Liver	-18.9 ± 3.6**	-16.6 ± 4.9*
Gut	-10.9 ± 5.7	-8.5 ± 6.7
Membranes	-14.0 ± 4.3*	-11.6 ± 5.3
Cotyledons	-4.3 ± 3.6	-1.9 ± 4.3
Carcass	-6.6 ± 4.2	-4.0 ± 5.3
Skin	-10.9 ± 4.2*	-8.6 ± 5.0
Lungs	55.9 ± 41.9	59.6 ± 43.7

Table 2.5

Mean percentage differences (± SE) between first and second measurements of combined ventricular output (CVO), total peripheral conductance (TPC) and organ blood flows and conductances

* - $p < 0.05$; ** - $p < 0.01$;

n = 8 for t-test on difference from 0

-7.1 ± 2.8 ($P<0.05$). There are also significant decreases in the flows to the heart, liver, membranes and skin. A plot of the ratios of second to first measurements of CVO against the first measurement (fig. 2.4) shows a highly significant negative correlation ($r= -0.88$, $P= 0.04$), in other words the higher the flow the greater the percentage fall in measured flow. The highest CVO was measured in a nephrectomized fetus which did not develop hypertension, the second measurement was 20% lower than the first. This fetus was excluded from the analysis of the changes associated with the hypertension for reasons stated later; if it is excluded from the calculation of the mean percentage change a value of 5.2 ± 2.4% is obtained, which is not significantly different from 0,despite an increase in the sensitivity of the test because of a lower standard deviation. However, the corresponding plots of second:first organ flows against first flow, do not show a similar relationship, and exclusion of the above fetus still gives significant decreases in coronary, hepatic, and membrane flows. The percentage changes in total and organ conductances are given in table 2.5. The mean percentage change in total peripheral conductance (TPC) was not significantly different from 0. There are significant decreases in the conductance of the heart (-13.8 ± 4.5 %) and liver (-16.6 ± 4.9%).

2.4 Discussion

2.4.1 Recirculation

The microspheres used were close to their nominal diameter of 15 μm, with none smaller than 12μm; so as indicated in the introduction, their shunting should have been minimal.

Though Hales (1973) found ~13% shunting of 15 μm spheres in heat-stressed adult sheep, it is unlikely that the fetus is in a comparable thermoregulatory state despite its high environmental temperature, as its body temperature is in the normal adult range. Nevertheless, as the thermoregulatory state of the fetus is uncertain, the possibility of sphere shunting should be borne in mind.

2.4.2 Mixing and streaming

The variation in numbers of spheres found in the kidneys and cerebral hemispheres is greater than predicted. It is likely that part of the extra variation is due to the invalidity of the assumption of equal flows per unit weight in paired organs. There does not appear to be any overall systematic bias in the dist ribution of spheres between the two kidneys. However, similar values of z were often found for the two measurements in each animal, which suggests either a consistent difference in flow between the kidneys, or a systematic streaming error; both specific to the individual.

Incomplete mixing of spheres in the ventricles would

also contribute to the variability of z; though this would be expected to show up as variability of z within an individual.

These points suggest that the distribution of spheres is subject to sources of variation other than the theoretical distribution errors. This was also observed by Buckberg et al (1971) though it is seldom recognized (e.g. Heymann et al, 1977).

The distribution of z for the cerebral hemispheres shows slight signs of bias towards the right hemisphere, probably as a result of a slightly lower specific flow in the left hemisphere, presumably caused by tying the left carotid artery. The sheep has a complete circle of Willis and so should largely be able to compensate for the occlusion of one carotid artery. The differences in flow to the two hemispheres were slight, as indicated by the mean ratio of left : right specific flow.

The difference in concentrations of microspheres between left and right kidneys and cerebral hemispheres was generally less than 7%. The 95% limit predicted for the numbers of spheres in these organs is 2-3%. The random error decreases with increasing numbers of spheres and it is likely that the additional error would similarly be affected, though without extensive empirical information it is difficult to put a figure on this.

Other factors, including anatomy, probably affect these errors; Makowski et al (1972) found a mean concentration

difference of 8.6% between blood samples withdrawn from the brachiocephalic artery, while Makowski et al (1968) found a difference of only 1.7% between umbilical arteries.

2.4.3 Effects of microspheres

No changes in blood pressures, heart rate, blood gases or haematocrit were observed following one or two injections, each of ~1.2 x 10^6 spheres. A lower cardiac output was obtained from the second injection than the first, and there is some evidence that this occured mainly at higher levels of cardiac output. The second injection also gave lower flows in the heart, liver and membranes. Though there was no significant fall in total peripheral conductance, the indications are that it was lower for the second injection. Though Heymann et al (1977) stated that considerably greater numbers of spheres than injected in this study do not affect the fetus, no experimental details or results were given. The mean number (±SD) of spheres injected in this study was 0.33 ± 0.03 x10^6 per kg bodyweight for the first injection, and 0.34 ± 0.04 x 10^6 for the second. Approximately 40% of the spheres would have gone to the placenta, so about 0.2 x 10^6 spheres/kg would be delivered to the fetal body. No physiological effects were seen following similar doses in dogs or lambs (see introduction).

Functional differences may affect the relative susceptibilities of the fetus and adult to microspheres; the fetal heart normally works towards the upper limit of its function curve (see section 3.1.3.2) Most tissues have

a higher blood flow in the fetus than in the adult and therefore may have a lower reserve capacity to compensate for blocking of vessels. Most fetal organs appear to have some reserve vascular capacity; cerebral and cardiac conductances are increased during hypoxia (Cohn et al, 1974); skeletal muscle conductance can also increase (Dawes, Lewis, Milligan, Roach and Talner, 1968; Iwamoto and Rudolph, 1979).

The changes in cardiac output, flows and conductances described are small, and the fact that blood gas tensions, pH and blood pressures are unchanged suggests that the effects on the fetus are not gross.

Where two control injections of spheres were made under control conditions only the results from the first injection have been used. In dealing with the effects of alpha-adrenergic blockade, where a second injection was made during the infusion of phentolamine, the inherent differences between first and second injection measurements will have to be considered.

2.4.4 Accuracy

The largest part of the variability of the method is due to the non-uniform mixing of spheres and blood. The factors influencing this are not properly understood and its measurement is difficult. However, for organs of moderate size, a tissue concentration difference of up to 7% is not unusual (fig. 2.6). Calculation of organ flows will involve the error in both the organ and the reference

sample, so a total error of up to 10% could be expected.

The larger organs are probably less susceptible to mixing errors, however, dissection errors are more significant in these organs, particularly for the cotyledons and membranes. These errors are estimated to be ~5% and so a total error of ~7% is therefore a reasonable estimate.

Cardiac output will mainly be susceptible to errors in the reference sample activity and its error will be about 7%. Approximately one third of pulmonary flow is not measured by this version of the method, however, this leads to only a small error in cardiac output.

The measurements of pressures are accurate to about 5%. This will mean that errors in conductances are slightly greater than the corresponding flows; about 9% for total conductance and 11% for organs like the brain and heart. Errors in counting the sample activities were usually under 1%; this is insignificant compared to the mixing errors.

CHAPTER 3

Haemodynamics during fetal renoprival hypertension

3.1 Introduction

3.1.1 Pressure, cardiac output and peripheral conductance

Fetal arterial pressure (P_a) is determined by the following relationship

$$P_a = \frac{CVO}{TPC} + P_{at} \qquad (3.1)$$

where P_{at} = atrial pressure, CVO = combined ventricular output and TPC = total peripheral conductance. Increased arterial pressure may thus be associated with increased cardiac output, decreased peripheral conductance, or a combination of the two. Changes in atrial pressure are generally small and of limited importance. Cardiac output may be influenced by changes in heart rate and stroke volume. The conductance (C) of a blood vessel length (l) and radius (r) is described by the following relationship derived from Poiseuille's equation.

$$C = \frac{\pi r^4}{8l\eta} \qquad (3.2)$$

3.1.2 Haemodynamic changes in hypertension in the adult

The relative contributions of cardiac output and conductance changes to any of the fetal hypertensions so

far described (see chapter 1) have not been investigated. Ferrario and Page (1978) have recently reviewed the role of cardiac output in the development of various experimental hypertensions in the adult. Generally, it seems that most established hypertensions, experimental and clinical, are due to increased peripheral resistance rather than increased cardiac output. However, during the development of hypertension, transient increases in cardiac output have been found which are later replaced by increases in peripheral resistance as the cause of the hypertension. It has been suggested that this increase in cardiac output is an important part of the pathogenesis of some types of hypertension. It is supposed that following the initial increase in cardiac output, with a parallel increase in organ blood flows, autoregulatory mechanisms take over to restore organ blood flows, peripheral resistance increases maintaining a high arterial pressure. Autoregulation here is taken to mean any control by organs and tissues of their own blood flow, by local mechanisms, to match their metabolic demands.

There is much apparently conflicting evidence for and against such a pattern of changes, based on sequential or continuous measurements of cardiac output and arterial pressure during the development of hypertension. Coleman, Samar and Murphy (1979) and Guyton (1980, Ch.39) have suggested some of the pitfalls in interpreting such experiments as evidence for or against autoregulation as the cause of hypertension. Whether a change in cardiac output is seen or not will depend on the relative speed and

magnitudes of the hypertensive stimulus and of autoregulation. Guyton (1980, Ch.39) describes how a slight increase in cardiac output during volume-loading hypertension when the stimulus was slow became larger when it was more abrupt. The difficulties are exacerbated by the variability of cardiac output, in that changes may be hidden by the noise arising from behavioural changes. In addition, many of the experimental hypertensions involve interference with the kidneys and as they receive 20% of adult cardiac output, consequent changes in renal blood flow may substantially affect cardiac output.

3.1.2.1 Neurogenic hypertension

The haemodynamics of experimental neurogenic hypertension in dogs, produced by denervation of the carotid sinus and aortic baroreceptors were investigated at 24 hours and 3 weeks by Ferrario, McCubbin and Page (1969). Both high cardiac output and high peripheral resistance hypertension were found at 24 hours; and 3 weeks later each dog tended to show the same cause of hypertension except for two in which a high cardiac output returned to normal and was replaced by high TPR.

3.1.2.2 Spontaneous hypertension

One of the difficulties in assessing the haemodynamic changes in spontaneously hypertensive rats (SHR) is in picking suitable control animals with which to compare them. Pfeffer and Frolich (1973) compared the Okamoto-Aoki strain of SHR with Wistar rats and found a higher cardiac output in the SHR at 9-12 weeks and 18-33

weeks when expressed in terms of body weight whereas at 62-97 weeks it was similar to the normotensive strain. However, Wistar-Kyoto rats had a similar cardiac output at 8-12 weeks to the SHR (Pfeffer, Frohlich, Pfeffer and Weiss, 1974), and these are generally accepted as a more appropriate control forthe Okamoto-Aoki strain so it would not appear that cardiac output is elevated during the development of this type of hypertension.

3.1.2.3 Mineralocorticoid hypertension

Two studies on the haemodynamics of mineralocorticoid hypertension found a range of variation in the responses of individual animals (Bravo, Tarazi amd Dustan, 1977; Miller, Bohr, Schork and Terris, 1979). Bravo et al (1977) found the hypertension caused by metyrapone in dogs was in some cases associated with increased cardiac output throughout the study period (8 weeks), while in others it was associated with increased peripheral resistance. A third group showed a transient increase in cardiac output at two weeks, as found in some other hypertensions, followed by a fall to control levels at 4-8 weeks. Some of the variability in response described by Miller et al (1979) for deoxycorticosterone acetate (DOCA) induced hypertension in pigs may be due to the state of the animals during the measurements. The pigs were tied on their flanks and some were said to have kicked and squealed before settling down when the measurements were made.

Conway and Hatton (1978) found slight increases in both CO and TPR contributing to hypertension produced by

DOCA and saline in dogs with one kidney. The hypertension still developed when the increase in cardiac output was prevented by beta-adrenergic blockade. Onoyama, Bravo and Tarazi (1979) gradually repleted previously sodium depleted dogs during administration of metyrapone, and found increased cardiac output in both these and control dogs undergoing sodium repletion. The hypertension that developed in the metyrapone treated dogs was thus attributed to increased peripheral resistance.

3.1.2.4 Renovascular hypertension

A transient increase in cardiac output was found by Ledingham and Cohen (1964) during the development of hypertension following the constriction of one renal artery in rats, the opposite kidney having been removed. Cardiac output, measured with an electromagnetic flowmeter, was increased at 1-5 and 5-10 days but had returned to normal by 10-15 days. Total peripheral resistance could not be calculated, as simultaneous pressure measurements were not made. In a later study (Ledingham and Pelling, 1967) this was achieved, but the changes in cardiac output were different and variable; it usually fell initially, with some increases later. For the group as a whole, cardiac output was higher than in normotensive rats with a wide clip on the remaining renal artery, and remained so for as long as it was measured (25 days).

Cardiac output was measured in the equivalent preparation in dogs by Olmsted and Page (1965) and Conway (1968); both found no increase in cardiac output during

the development of hypertension. Olmsted and Page (1965) found a decrease in CO over the first four days of hypertension after which it returned to control levels, while Conway (1968) found no change at 4 or 15 days after constriction. In the latter experiments, nephrectomy and clipping were performed under general anaesthesia, necessitating starvation, which disturbed the sodium balance and possibly affected the haemodynamic response.

Contrasting results were found in measurements made up to day 7 post-constriction of one renal artery in dogs with one kidney, Bianchi, Tenconi and Lucca (1970); and two kidneys, Bianchi, Baldoli, Lucca and Barbin (1972). The results in both preparations were similar, a very short term (at 40 and 120 minutes) increase in TPR was followed by an increase in cardiac output on day 1. In the one kidney dogs, cardiac output remained elevated at day 7 though it had returned to normal by this stage in those with two kidneys.

A longer lasting increase in cardiac output was observed by Ferrario (1974) following renal artery constriction in dogs with one kidney. The experiments were technically well executed, with daily measurements averaged for each week. During the first two weeks, cardiac output was about 28% higher, TPR was unchanged but started to increase during the third week, gradually taking over as the haemodynamic cause of the hypertension.

Maxwell, Lupu, Viskoper, Aravena and Waks (1977) found CO and TPR both contributed to the hypertension in dogs

with one of two renal arteries constricted, over the first three days, but on days 4 and 5 cardiac output had returned to normal levels. No increase in CO was found during the development of renovascular hypertension in sodium-depleted dogs (Stephens, Davis, Freeman, DeForrest and Early, 1979) though it increased on sodium repletion without any further increase in arterial pressure.

3.1.2.5 Renal wrapping hypertension

An increase in cardiac output has also been found in hypertension induced by cellophane wrapping of one kidney, followed by removal of the second, in dogs. In this case, TPR was also increased and this pattern sustained for three weeks before CO returned to normal during weeks 4-6, as TPR increased further (Ferrario, Page and McCubbin, 1970).

Though Fletcher, Korner, Angus and Oliver (1976) observed an increase in CO in rabbits following wrapping of both kidneys with cellophane, similar post-operative changes were observed in sham-operated control rabbits which remained normotensive, so the hypertension was attributed to increased peripheral resistance.

3.1.2.6 Renoprival and volume loading hypertensions

One of the most striking examples of a transient increase in the development of hypertension comes from Coleman and Guyton (1969). Following a 70% reduction in renal mass, volume loading was produced by saline in lieu of drinking water, or by saline infusions. Over the first three days this resulted in hypertension due to increased

cardiac output, after which CO fell towards control levels and TPR gradually increased.

Grollman, Turner, Levitch and Hill (1951) found no increase in cardiac output (measured by the Fick method) from 8-60 days following bilateral nephrectomy in dogs kept alive by peritoneal dialysis. Arterial blood pressures of 140-200 mm Hg were developed. Similarly, the development of hypertension over the four days following nephrectomy in dogs also given parenteral sodium was entirely due to increased peripheral resistance (Muirhead, Kosinski and Brooks, 1965).

In contrast, Ledingham and Pelling (1970) found that renoprival hypertension in rats was due to increased cardiac output, at least during the first three days post-nephrectomy. Sodium loading (via the drinking water) was required for the hypertension to develop. Technically, these experiments were superior to those of Grollman et al (1951) and Muirhead et al (1965). Although all three groups used trained animals, both of the latter used direct vessel puncture to obtain blood pressures and samples. Ledingham and Pelling (1970) used animals with chronically implanted catheters and an electromagnetic flowmeter.

Though none of the above hypertensions seems to be exclusively dependent on an overt increase in cardiac output, it is a contributory factor in many instances. In addition, the absence of a detectable cardiac output increase does not preclude autoregulation as a mechanism

behind the hypertension.

3.1.3 Regulation of cardiac output

Cardiac output in the fetus will be affected, as in the adult, by factors which change heart rate or stroke volume. The latter is dependent on, among other things, the intrinsic properties of cardiac muscle, which are best studied in isolated heart or muscle preparations.

3.1.3.1 Studies on isolated cardiac muscle

The standard length-tension relationship, with active muscle tension increasing to a maximum as muscle length is increased, is present in near term fetal lamb cardiac muscle (Friedman, 1973). This provides the mechanical basis for the Frank-Starling relationship describing the increase in stroke volume which occurs when ventricular filling is increased. However, the tension developed at a given fraction of L_{max} (the length at which maximal tension is developed) is less than in the adult. In addition, the velocity and extent of shortening for a given load are lower in the fetus. These differences appear to arise from a lower percentage contractile mass of 30% in the fetus compared with 60% in the adult; the properties of the individual musclefibresbeing similar (Friedman, 1973).

3.1.3.2 Studies in intact animals

3.1.3.2.1 The Frank-Starling relationship

There is no doubt that the fetal heart is capable of responding to changes in end-diastolic size with changes in

muscle shortening; what is less clear is where in the range of response the fetal heart normally functions and what degree of adjustment is possible in response to various stimuli.

Brinkman, Johnson and Assali (1972) found that stroke volume was increased during bradycardia produced by vagal stimulation (in the absence of arrhythmia) such that cardiac output fell only slightly. However, these results were obtained in acute preparations with three electromagnetic flowmeters placed on the great vessels; cardiac output was low (<200 $ml.min^{-1}.kg^{-1}$) suggesting the heart was working far below its normal level.

Rudolph and Heymann (1973) concluded that the fetal heart had little capacity to increase its stroke volume above normal working levels, as large infusions of saline into the jugular vein produced only small increases in right ventricular output despite a large increase in right atrial pressure from 12 to 30 mm Hg. Heart rate was maintained constant by pacing or atropine. With such a large increase in right atrial pressure these changes could represent a shift to the descending portion of the ventricular function curve.

This inability to increase stroke volume was confirmed by examining the changes in ventricular outputs measured by electromagnetic flowmeters over varying heart rates and changes produced by vagal stimulation or pacing (Rudolph and Heymann, 1976). Both ventricular outputs varied in parallel with the spontaneous heart rate changes, stroke

volume being unchanged; when spontaneous changes are studied it cannot be certain what changes in contractility accompany the heart rate changes. Right ventricular output also fell when bradycardia was produced by cervical vagal stimulation, though this would be expected to alter contractility as well as heart rate. During pacing of either atrium, the opposite ventricular output rose as heart rate was increased, reaching a maximum and then falling with further increases. Pacing of the ipsilateral atrium usually caused left ventricular output to fall and only small increases in right ventricular output. From these three approaches it was concluded that heart rate was the principle determinant of fetal cardiac output, the fetus having little capacity to alter stroke volume.

Pitlick, Kirkpatrick and Friedman (1976) suggested the effects of atrial pacing were artefactual. Pacing of the left or right atrium did not produce any change in combined ventricular output in chronically prepared fetal lambs, but altered the contributions of the two ventricles, that of the ventricle opposite to the paced atrium being increased. The reason for this was supposedly changes in the right-left atrial pressure difference produced by pacing.

This group maintained that the Frank-Starling mechanism was an active control mechanism in the fetus on the basis of chronic experiments in which left ventricular internal diameter was continuously measured using small sonomicrometer crystals (Kirkpatrick, Pitlick, Naliboff and Friedman, 1976). Decreases in left ventricular

end-diastolic diameter were produced by balloon inflation in the superior vena cava, and increases by infusion of blood into the left atrium. Strong positive relationships were found to exist both between left ventricular end-diastolic diameter and end-diastolic pressure and the extent of shortening of diameter. From control values obtained in the same preparation (Kirkpatrick, Covell and Friedman, 1973) it would seem that this mechanism is active over the normal range of cardiac function. The techniques used involve extensive interference with the heart, the left ventricular wall being punctured in three places to implant a micromanometer and the two sonomicrometer crystals. Though two weeks were allowed for recovery, it could be that under these circumstances cardiac function is depressed below normal but the capacity to increase stroke volume is retained. However, the absolute left ventricular outputs of 900-1000 $ml.min^{-1}$ measured by dye dilution in these preparations, at gestational ages of 131-141 days, are not low by most standards.

Gilbert (1977 and 1980) has described the fetal venous return and cardiac function curves and their role in the determination of cardiac output. As in the adult, the venous return is determined by the difference in right atrial and mean systemic pressure (the latter describes the degree of vascular filling) and the resistance to venous return (Gilbert, 1977). An increase in blood volume shifted the venous return curve upwards, a higher venous return being seen at a particular right atrial pressure. The cardiac function curve was obtained using only three

points, each being a mean from the same group of animals (Gilbert, 1980). Ten percent increases and decreases produced 20-24% changes in mean systemic pressure and 65% increases and 22% decreases in right atrial pressure. Cardiac output fell by 20% during decreased blood volume but was not significantly increased following increased blood volume, though stroke volume was increased. This suggests that the Frank-Starling mechanism is functional in the fetus, but that the heart is normally operating near the top of its function curve and cardiac output is nearly maximal.

3.1.3.2.2 Myocardial contractility

Increased myocardial contractility would shift the cardiac function curve upwards, increasing maximal stroke volume and increasing it at a given atrial pressure. Evidence indicating the potential for increased contractility in the fetal heart is sparse. Friedman (1973) demonstrated a beta-adrenergic mediated increase in contractility in isolated fetal lamb cardiac muscle, but the relationship of this to the resting state in the intact fetus is uncertain. Rudolph and Heymann (1973) presented limited evidence for a beta-adrenergic positive inotropic action, in that the right ventricular output of a chronically prepared fetus during isoprenaline stimulation was higher than at the same heart rate when produced by pacing. A 17% increase in fetal cardiac output was found during angiotensin II infusion by Iwamoto and Rudolph (1981) in chronically prepared fetal lambs. This was

attributed to a direct chronotropic action of angiotensin II (the effects of blockers suggested it was not an autonomic effect), though there was possibly a slight inotropic component involved.

Another of the few experimental manipulations which have been found to increase fetal cardiac output is 4-5 days of tri-iodothyronine infusion; during which combined ventricular output was 22% higher than in control fetuses (Lorijn, Nelson and Longo, 1980). However, this increase was entirely attributable to increased heart rate.

The experiments described in this chapter were designed to determine the changes in cardiac output and peripheral resistance associated with fetal renoprival hypertension, along with the changes in organ blood flows and resistances, in an attempt to uncover something of the more fundamental mechanisms behind the hypertension.

3.2 Methods

Sixteen fetuses were used in this part of the study, eight were catheterized controls, seven were nephrectomized and one had both ureters tied. Operative procedures and measurements were performed as described in chapter 2. Only the results from the first microsphere injection in each fetus are described here.

3.3 Results

3.3.1 Daily measurements

3.3.1.1 Selection of the hypertensive group The daily measurements of arterial pressure over the first eight days post-operation are plotted in figure 3.1; measurements were only taken when appropriate calibrations were available, as a result figures are not available for every fetus on every day. Generally, the arterial pressure in the anuric fetuses was increased above control levels after two or three days.

The main objective of these experiments was to analyse the haemodynamic causes of the hypertension, so two fetuses which did not develop hypertension were excluded from the comparison with the control fetuses. One nephrectomized fetus (no 140) maintained an arterial pressure well within the normotensive range; another (no 68) was hypertensive immediately post-operation but its arterial pressure fell to 55-60 mm Hg later on (fig.3.1). Although this is above the normotensive range its arterial pressure at the time of the first microsphere injection was 55 mm Hg, within two standard deviations of the control mean. This fetus was exceptionally heavy (7.84 kg body weight) and this was a further reason for excluding it from the hypertensive group, as its organ weights and absolute flows were also high, leading to skewed distributions and large standard deviations. The results from both these fetuses are described separately.

Figure 3.1

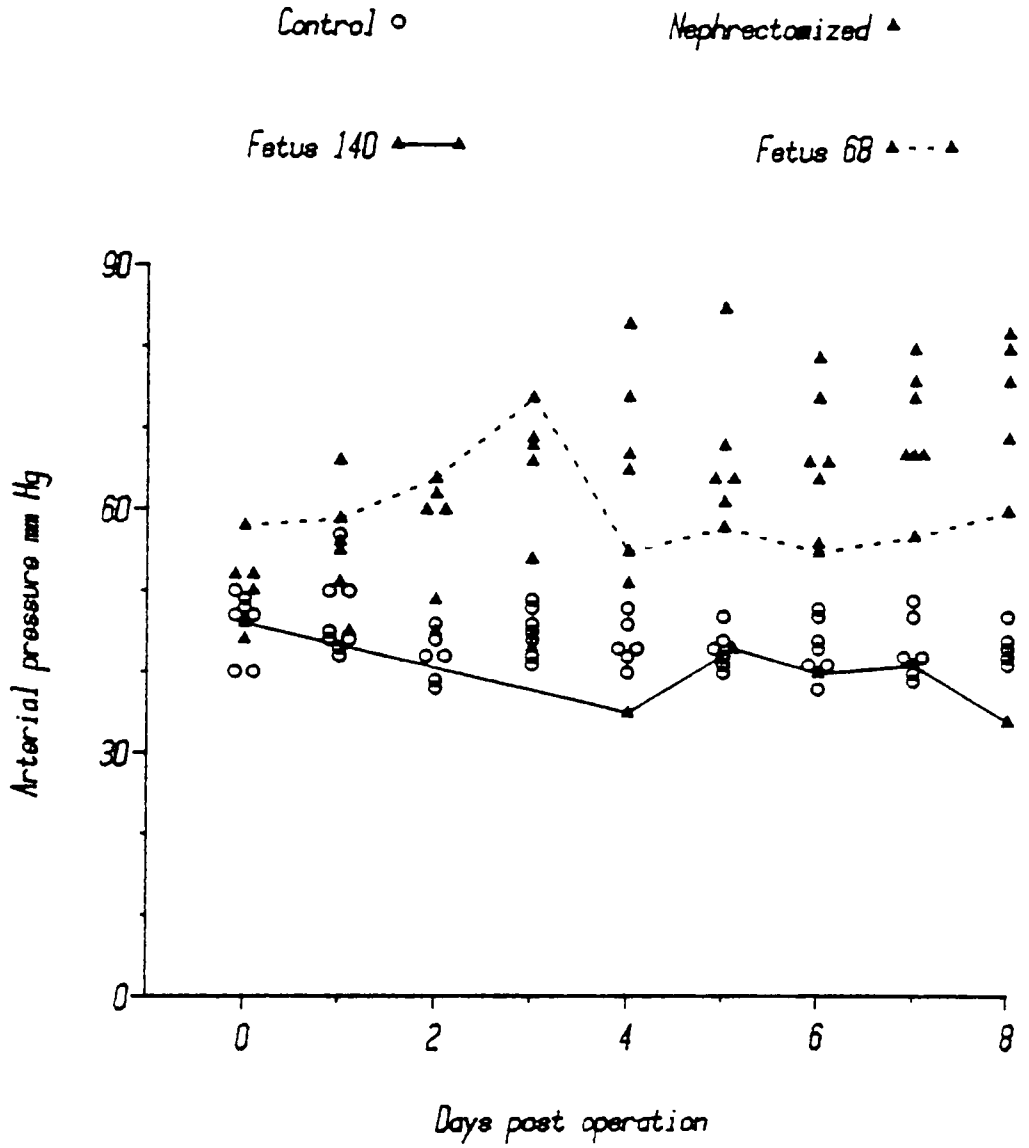


Figure 3.1. Daily measurements of mean arterial pressure after a control operation (8 fetuses) or bilateral nephrectomy (8 fetuses).

There are therefore, six fetuses in the hypertensive group and eight in the control group. Comparisons were made between the two using the unpaired t-test, taking $P<0.05$ as the level of statistical significance.

3.3.1.2 Pressures and heart rates

Figure 3.2(a) shows the mean daily measurements of arterial pressure for the fetuses considered hypertensive and for the control group. Considering the groups as a whole shows that the hypertension begins to develop by the first day post-operation, the difference on day 1 is statistically significant ($P=0.05$). The arterial pressure continues to rise, and by day four the hypertension is well established but continues to increase slightly. The changes in inferior vena caval pressure during the development of hypertension (fig 3.2(b)) are more variable, owing to the difficulty of accurately measuring venous pressure. It shows signs of rising by day 2 though is not substantially increased until days 7-8. Heart rate (fig. 3.2(c)) is slightly higher in the hypertensive group throughout, but not significantly so, except on day 3.

3.3.1.3 Blood gases and pH

The femoral arterial PO_2 of both the control and hypertensive fetuses fell after the operation (fig.3.3(a)) though there were no significant differences between the groups on any day until day 8, when there were only four fetuses in each group. The mean decrease in PO_2 between days 1 and 6 was 4.9 mm Hg in the controls ($P<0.05$, paired

Figure 3.2

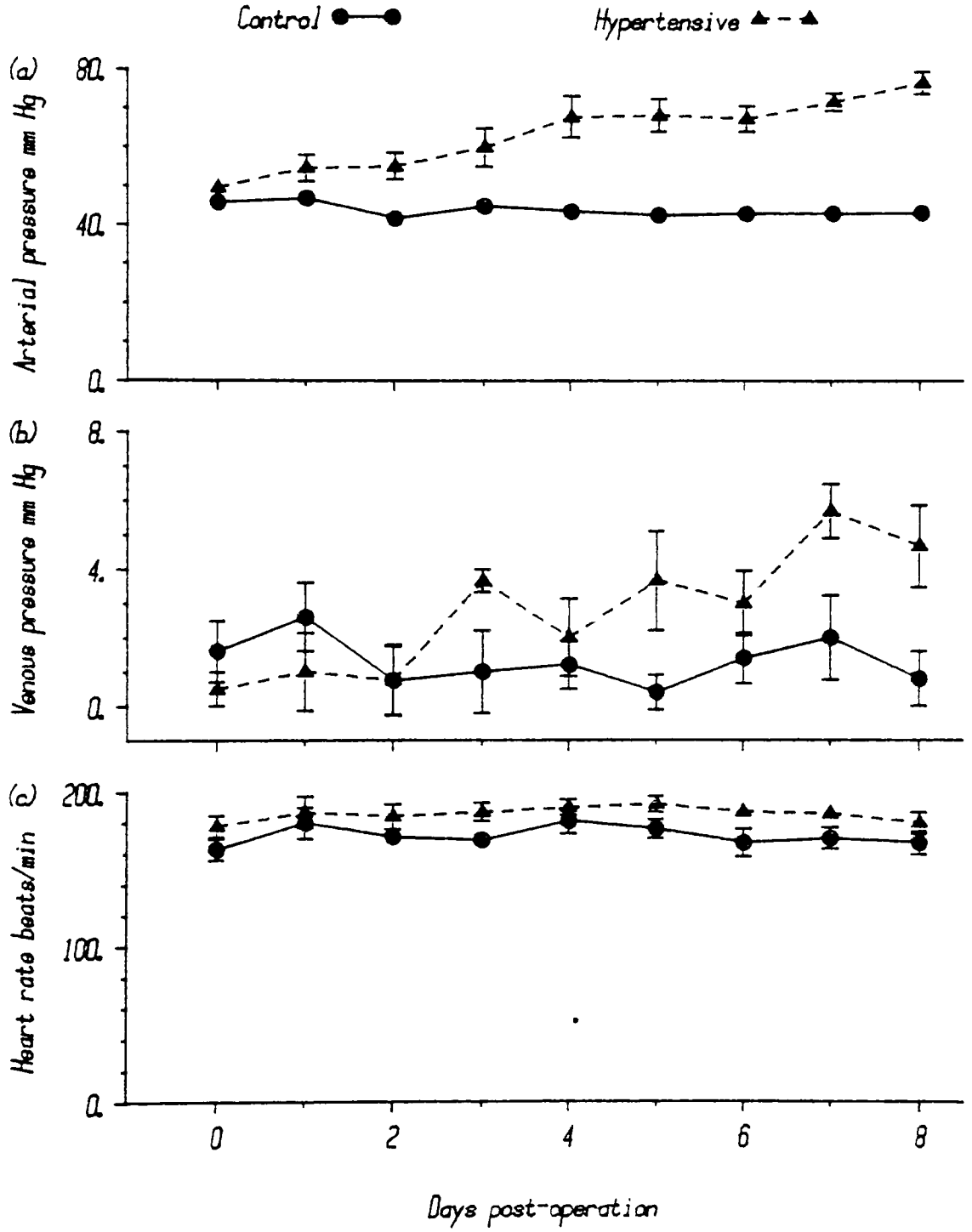


Figure 3.2. (a) Mean arterial pressure (b) venous pressure and (c) heart rate over the first eight days post-operation in control (n=8) and hypertensive (n=6) fetuses. All points are mean values ± SE.

Figure 3.3. Mean values (±SE) over the first 8 days post-operation in control (n=8) and hypertensive fetuses (n=6) for (a) femoral arterial PO_2, (b) PCO_2, (c) pH and (d) haematocrit.

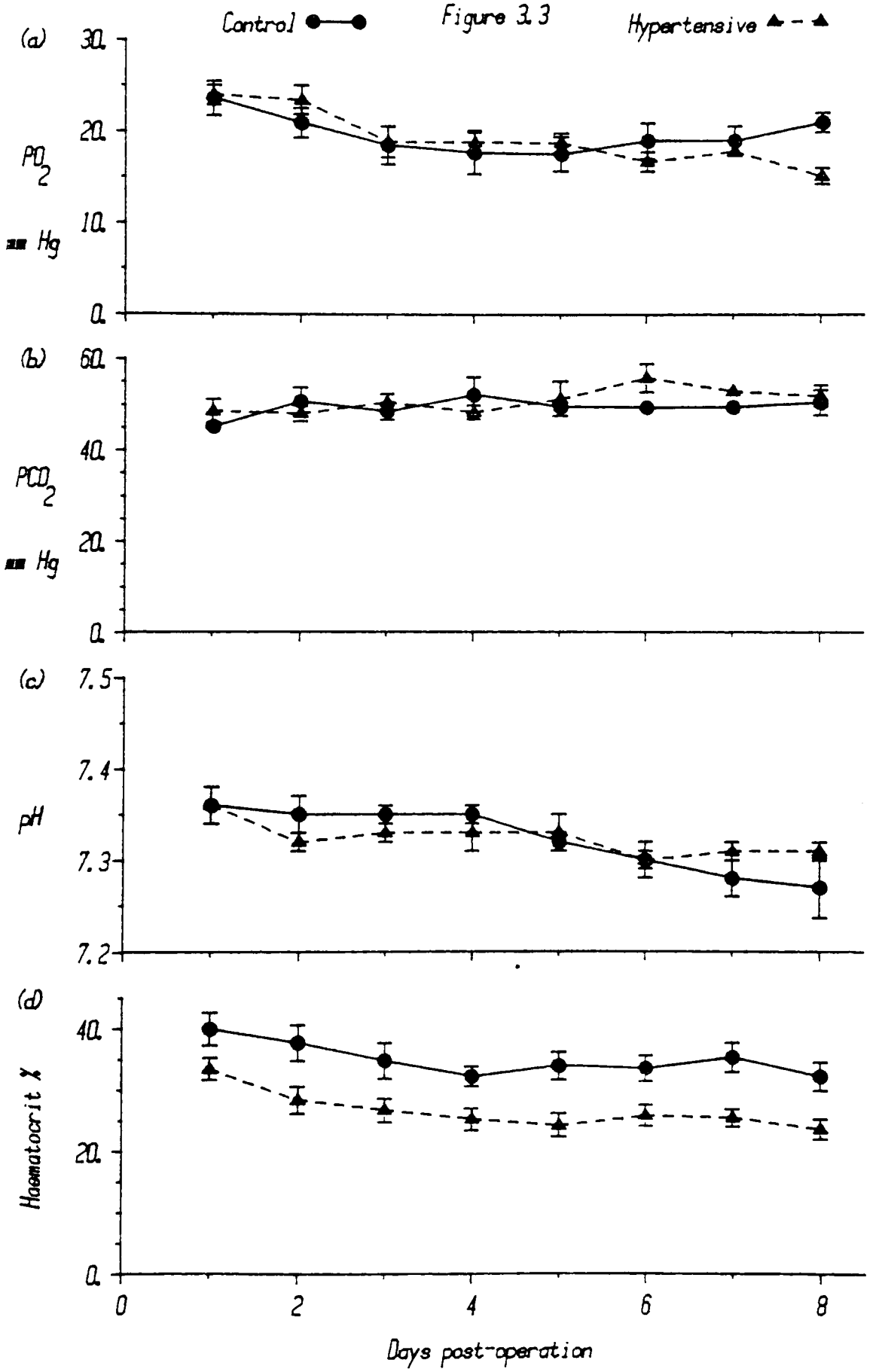

Control
Hypertensive
(a)
30
20
10
0
PO_2
mm Hg
(b)
60
40
20
0
PCO_2
mm Hg
(c)
7.5
7.4
7.3
7.2
pH
(d)
40
20
0
Haematocrit %
0
2
4
6
8
Days post-operation

Figure 3.3

t-test, n=7) and 7.2 mm Hg in the hypertensives ($P<0.05$, n=6). Arterial PCO_2 (fig. 3.3(b)) was not significantly different on each of days 1-8 in the two groups. There was a 5.5 mm Hg increase in PCO_2 between days 1 and 6 in the control group ($P=0.01$, n=7) but the 7.2 mm Hg increase in the controls just failed to reach statistical significance ($P=0.06$). There was no difference in arterial pH (fig. 3.3(c)) between the two groups on any of days 1-8, though both groups showed a 0.06 pH unit fall between days 1 and 6 ($P<0.05$ for both groups).

3.3.1.4 Haematocrit

Both groups also showed a significant post-operative fall in femoral arterial haematocrit (fig. 3.3(d)). The differences between day 1 and day 6 were 6.5% ($P<0.01$) in the controls, and 7.8% ($P<0.01$) in the hypertensives. There was also a consistent difference between the haematocrits of the two groups, of approximately 7-10% between days 2 and 8 ($P<0.05$, except day 7 when $P<0.01$). The 6.5% difference on day 1 was not statistically significant but indicates the fall is under way at this early stage.

The possibility that changes in red cell volume may have occured due to osmotic effects was investigated by comparing the mean cellular haemoglobin concentration in the two groups. This is calculated by dividing the haemoglobin concentration by the haematocrit. Serial measurements were available for three control and five nephrectomized fetuses and as no post-operative changes

were apparent, the mean value for each fetus was taken. The overall means for the groups were 4.51 ± 0.18 mM for the controls and 4.61 ± 0.08 mM for the nephrectomized group, not significantly different.

3.3.2 Microsphere experiments

The mean blood gas tensions, pH, haematocrit and calculated oxygen contents for the control and hypertensive groups at the time of microsphere injections are given in table 3.1. No significant differences were found in carotid PO_2, PCO_2, or pH. Oxygen content was significantly lower in the hypertensive group at 1.99 ± 0.16 mM compared with 3.12 ± 0.21mM. This can largely be accounted for by the lower haematocrit of the hypertensive group.

3.3.2.1 Pressures, CVO and TPC

Figure 3.4 shows the mean arterial pressures, combined ventricular outputs (CVO) and total peripheral conductances of the two groups. Mean arterial pressure was 70% higher in the hypertensive group, though there was no significant difference in CVO corrected for body weight. Unsurprisingly, therefore, calculated TPC was 44% lower in the hypertensive group. The numerical values for these variables, along with figures for venous pressure, perfusion pressure, heart rate, stroke volume, stroke work and cardiac power are given in table 3.2. Central venous pressure was higher in the hypertensive group, by nearly 5 mm Hg, making perfusion pressure 60% higher. Heart rate

	Controls (n=8)	Hypertensives (n=6)	
PO_2 mmHg	21.5 ± 2.2	17.8 ± 1.1	
$[O_2]$ mM	3.12 ± 0.21	1.99 ± 0.16	p=0.02
PCO_2 mmHg	47.7 ± 2.8	47.6 ± 1.6	
pH	7.31 ± 0.02	7.31 ± 0.01	
Haematocrit %	33.4 ± 2.1	24.6 ± 1.5	p=0.008

Table 3.1

Mean (± SE) values for carotid arterial blood gas tensions, oxygen content $[O_2]$, pH and haematocrit in control and hypertensive fetuses. P values are for an unpaired t-test

Figure 3.4

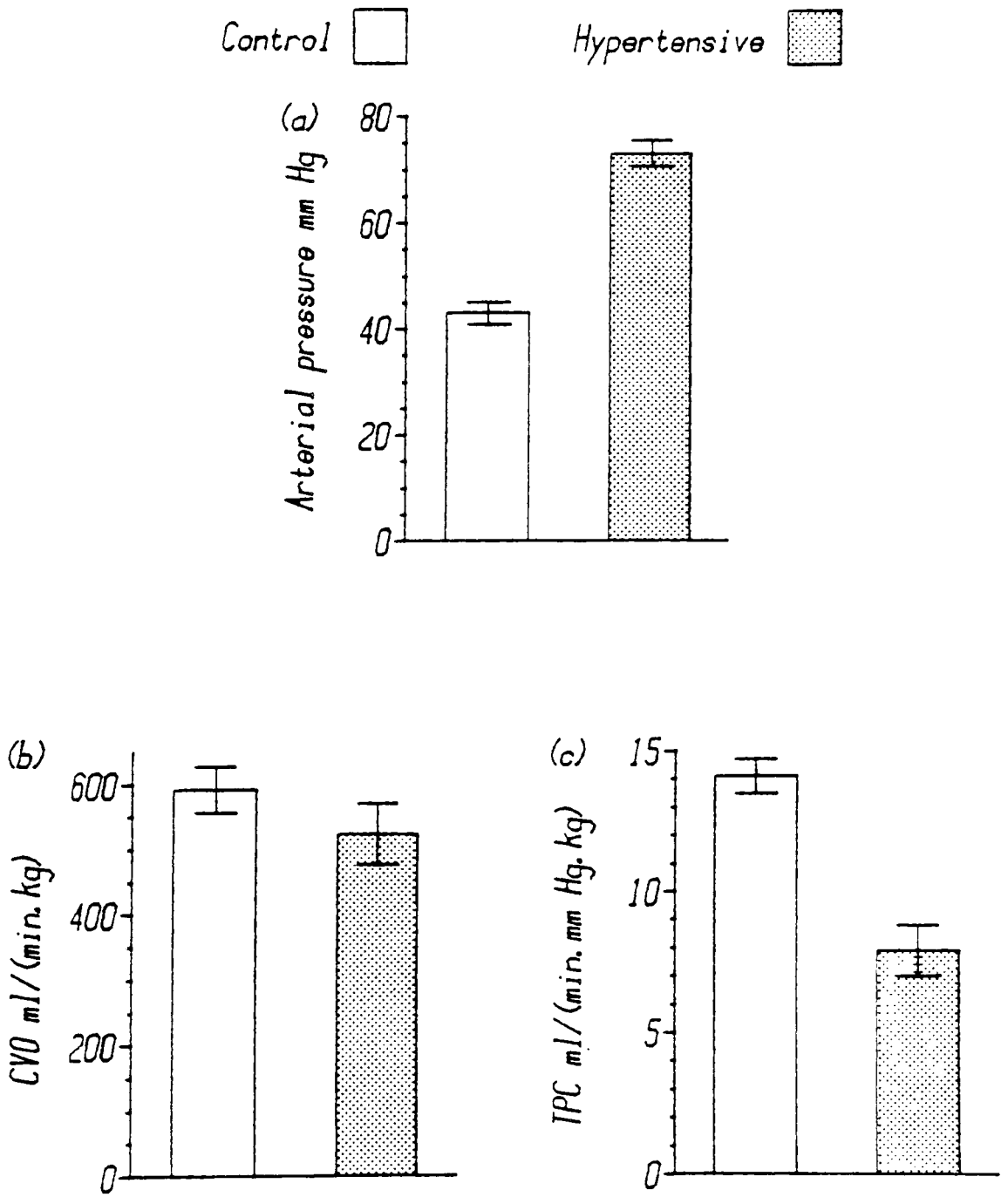


Figure 3.4. (a) Mean arterial pressure, (b) combined ventricular output (CVO) and (c) total peripheral conductance (TPC) in control (n=8) and hypertensive (n=6) fetuses. All points are means ± SE.

	Control (n=8)	Hypertensive (n=6)	
Body weight kg	3.28 ± 0.18	3.75 ± 0.11	
Arterial pressure mmHg	43.1 ± 2.1	73.2 ± 2.4	$p < 0.001$
Venous pressure mmHg	1.1 ± 0.5	5.8 ± 1.5	$p = 0.007$
Perfusion pressure mmHg	42.0 ± 2.0	67.2 ± 2.9	$p < 0.001$
Heart rate beats.min^{-1}	180 ± 10	182 ± 5	
CVO ml.min^{-1}.kg^{-1}	592 ± 35	524 ± 47	
TPC ml.min^{-1}.mmHg^{-1}.kg^{-1}	14.1 ± 0.6	7.9 ± 0.9	$p < 0.001$
Stroke volume ml.kg^{-1}	3.33 ± 0.23	2.90 ± 0.29	
Stroke work joules.kg^{-1}	0.194 ± 0.002	0.280 ± 0.002	$p = 0.02$
Cardiac power watts.kg^{-1}	0.0572 ± 0.006	0.0842 ± 0.006	$p = 0.007$

Table 3.2

Mean (± SE) values for haemodynamic variables in control and hypertensive fetuses. CVO, combined ventricular output. TPC, total peripheral conductance. Standardization of CVO, TPC etc. is for body weight. P values are for an unpaired t-test.

was similar in both groups, stroke volume too, was no different. The external work of the heart per beat, stroke work, calculated as the CVO multiplied by the arterial pressure and converted to joules, was 44% higher in the hypertensive group. The rate of work, cardiac power, was increased by a similar amount.

The relationship between perfusion pressure and CVO may not be completely described by total peripheral conductance, therefore these two variables have been plotted against one another in figure 3.5. Generally, for the control fetuses, the points lie along a line radiating from the origin, corresponding to a particular vascular conductance, whereas those for the hypertensive fetuses are more scattered. The coefficient of variation for the total peripheral conductance was 12% for the controls and 28% for the hypertensives.

A factor which may slightly confuse the comparison of control and hypertensive fetuses is the oedema found in the latter group, causing increased body and organ weights without any increase in the amount of metabolizing tissue. This potentially affects those variables normalised for body or organ weights. However the effect is unlikely to be of great importance here as the difference between the mean weights of the two groups was 14% of the control mean, and not statistically significant (see table 3.2). Where changes in organ weight seem important, they have been mentioned.

Figure 3.5

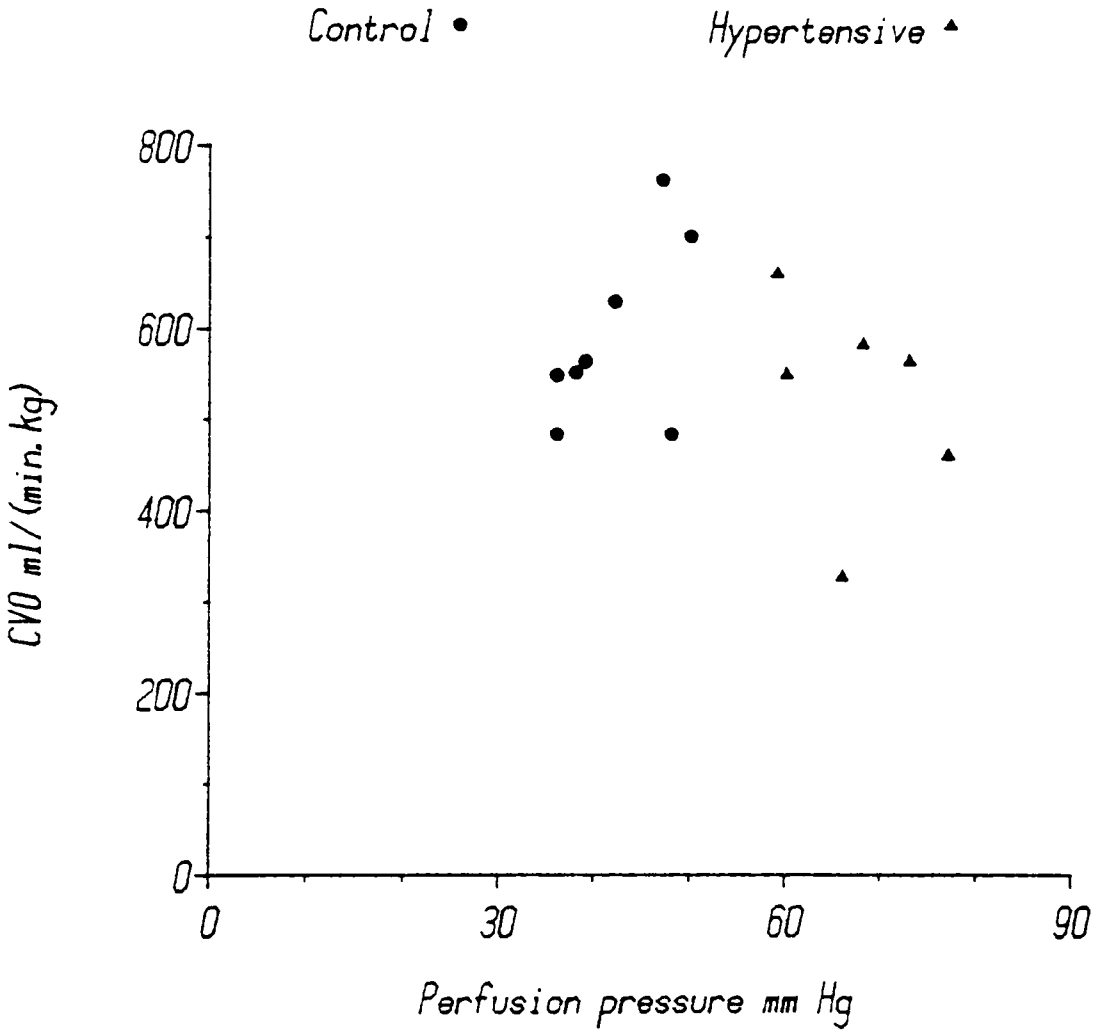


Figure 3.5. Pressure-flow diagram for combined ventricular output (CVO) and systemic perfusion pressure in 8 control and 6 hypertensive fetal lambs.

3.3.2.2 Distribution of cardiac output

Which organs take part in the decrease in conductance is indicated by the changes in the distribution of CVO (figure 3.6). There are only slight changes; the shares flowing to the carcass, skin and gut, which represent the bulk of the body, are unchanged. The fractions of CVO received by the adrenal glands, spleen and fetal membranes were also unchanged. The brain received a slightly greater proportion of CVO in the hypertensive group, 5.5 $\pm$ 0.3% compared with 4.3 $\pm$ 0.4%, P= 0.03. The share received by the hepatic arterial circulation was substantially increased from 0.37 $\pm$ 0.07% to 0.93 $\pm$ 0.24% (P=0.025) though this makes little demand on CVO as a whole.

The most striking change in the distribution of CVO in the hypertensive group was the increase in the share flowing to the heart; 9.7 $\pm$ 1.0% compared with 3.1 $\pm$ 0.4% in the control group (P<0.001). This blood was redirected from several sources. The kidneys received 2.1 $\pm$ 0.1% of CVO in the controls and were absent in the hypertensive fetuses (except one with tied ureters). The placental cotyledons received 40.0 $\pm$ 1.7% of CVO in the control group and 34.3 $\pm$ 2.7% in the hypertensive group, though this difference just failed to reach statistical significance (P=0.09). There was also a substantial decrease in the fraction received by the lungs; 2.6 $\pm$ 0.5% in the controls and 0.8 $\pm$ 0.2% in the hypertensive fetuses; though this result should be treated with some caution for the

Figure 3.6

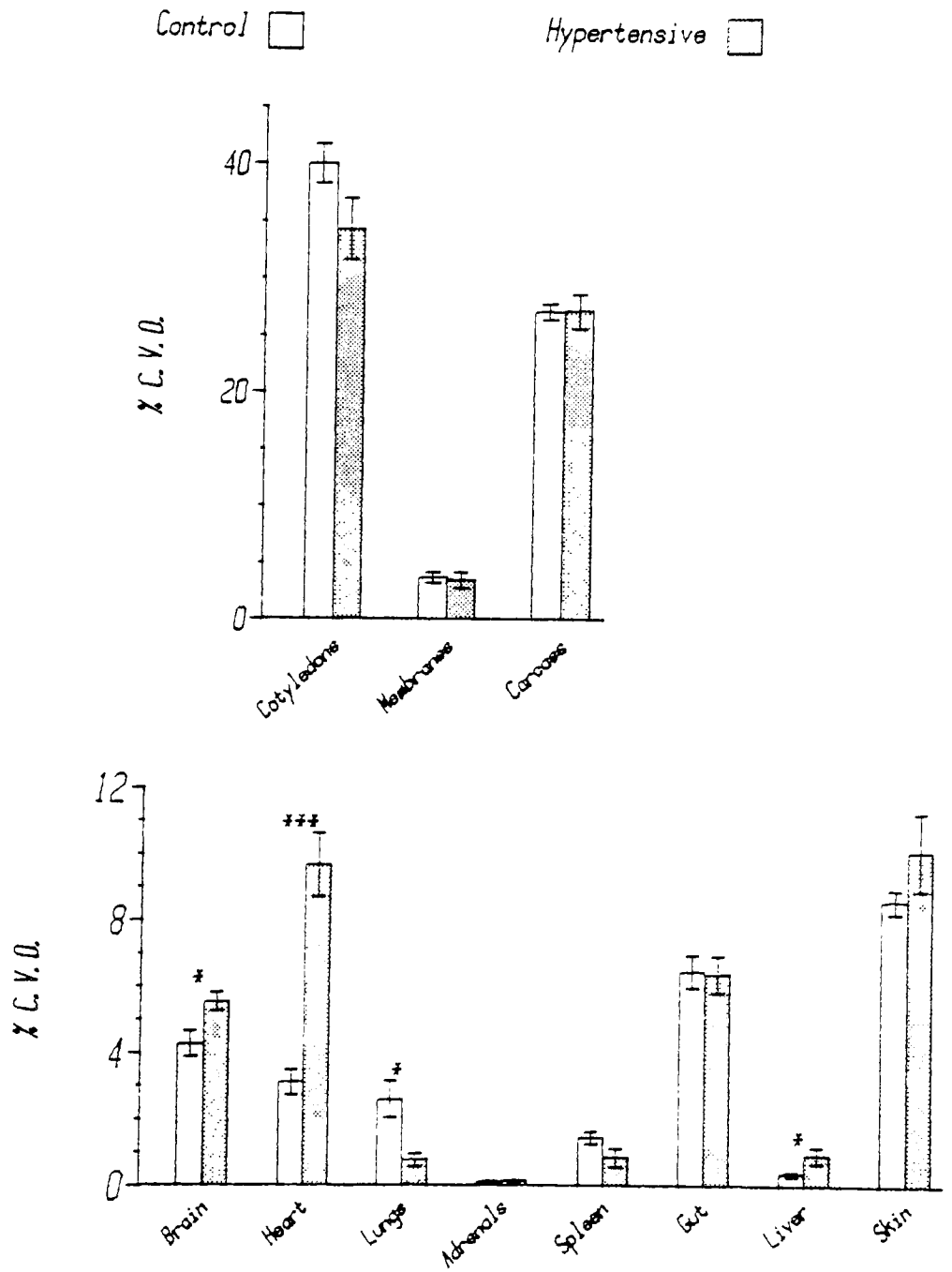


Figure 3.6. Distribution of combined ventricular output (CVO) to the organs of control (n=8) and hypertensive (n=6) fetal lambs. All values are means ±SE. *, P<0.05; ***, P<0.001; unpaired t-test.

methodological reasons stated in chapter 2.

3.3.2.3 Cotyledonary circulation

The changes in placental cotyledonary flows and conductances are considered in figure 3.7, separately from the other organs, and are expressed in terms of body weight rather than organ weight. This emphasizes their quantitative importance to the fetus as a whole. Cotyledonary flow was 176 $\pm$ 13 $ml.min^{-1}.kg^{-1}$ in the hypertensive group and 240 $\pm$ 24 $ml.min^{-1}.kg^{-1}$ in the controls, this difference was not significantly significant ($P=0.06$). There was a 53% lower cotyledonary conductance in the hypertensive group, 2.65 $\pm$ 0.26 $ml.min^{-1}.mm\ Hg^{-1}.kg^{-1}$ compared with 5.68 $\pm$ 0.42 ($P<0.001$). A pressure flow diagram for the cotyledons, fig.3.8, shows the hypertensive fetuses lie apart from the pressure-flow relationship of the control animals, in which flow tended to increase with perfusion pressure.

3.3.2.4 Blood flows

The mean organ blood flows of the two groups are illustrated in figure 3.9. As suggested by the distribution figures, flows to the carcass, skin, gastro-intestinal tract, spleen, and adrenal glands do not differ in control and hypertensive lambs. There was a substantial decrease in membrane blood flow but this can largely be attributed to a higher membrane weight in the hypertensive fetuses, as shown by figure 3.10(a). The membranes of these fetuses were often covered in a thick

Figure 3.7

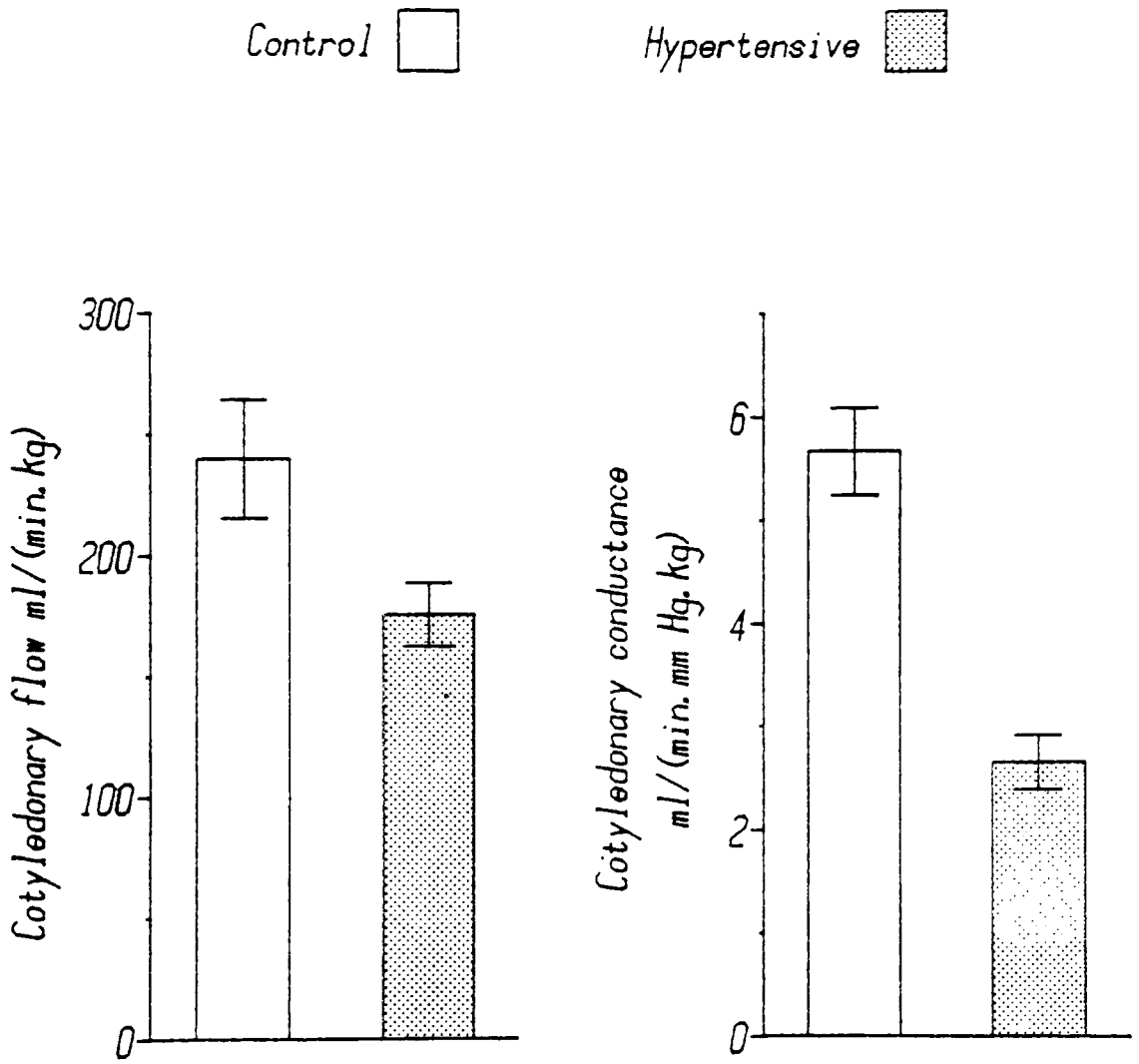


Figure 3.7. Mean (±SE) values for blood flow through the placental cotyledons and their vascular conductance, in control (n=8) and hypertensive (n=6) fetuses. P= 0.06 for flow and < 0.001 for conductance, unpaired t-test.

Figure 3.8

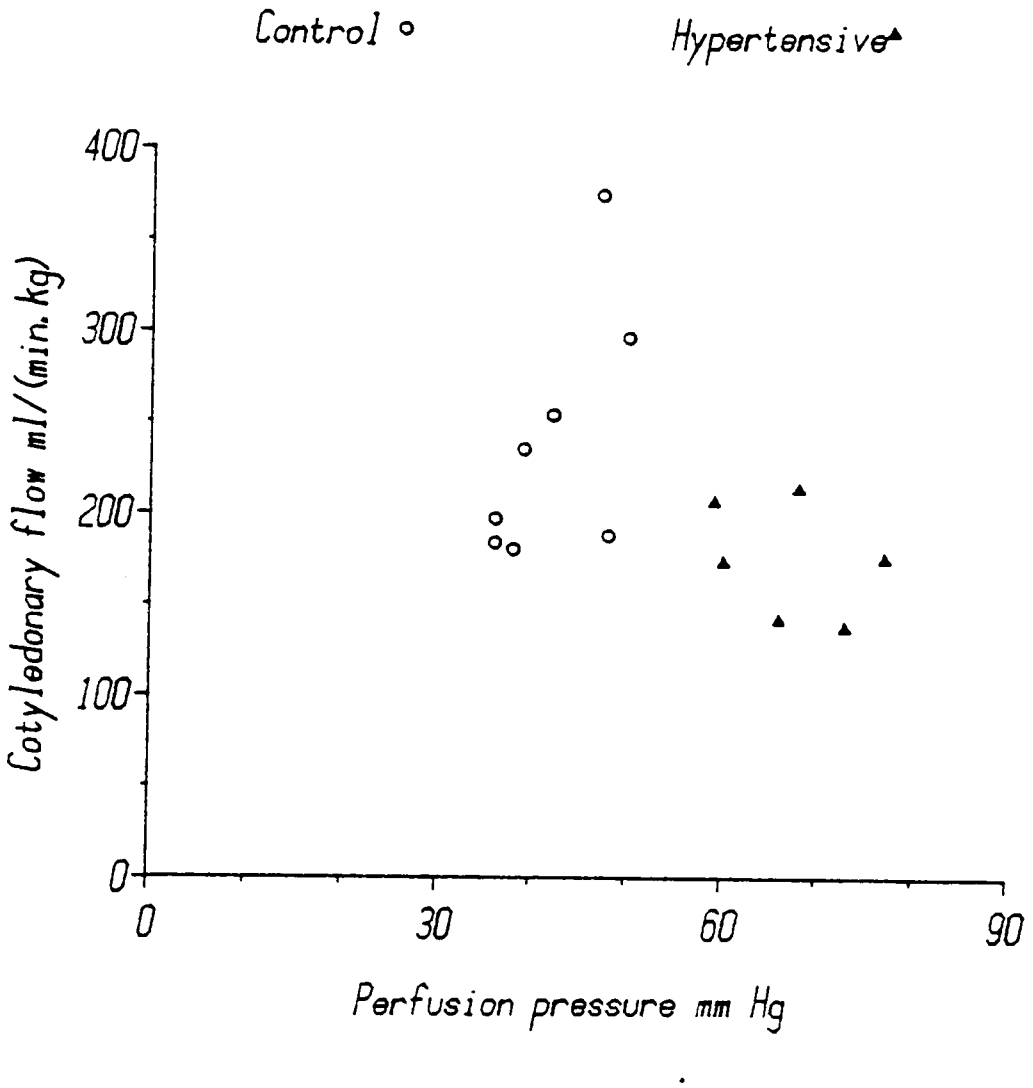


Figure 3.8. Pressure-flow diagram for the placental cotyledons in 8 control and 6 hypertensive fetuses

Figure 3.9

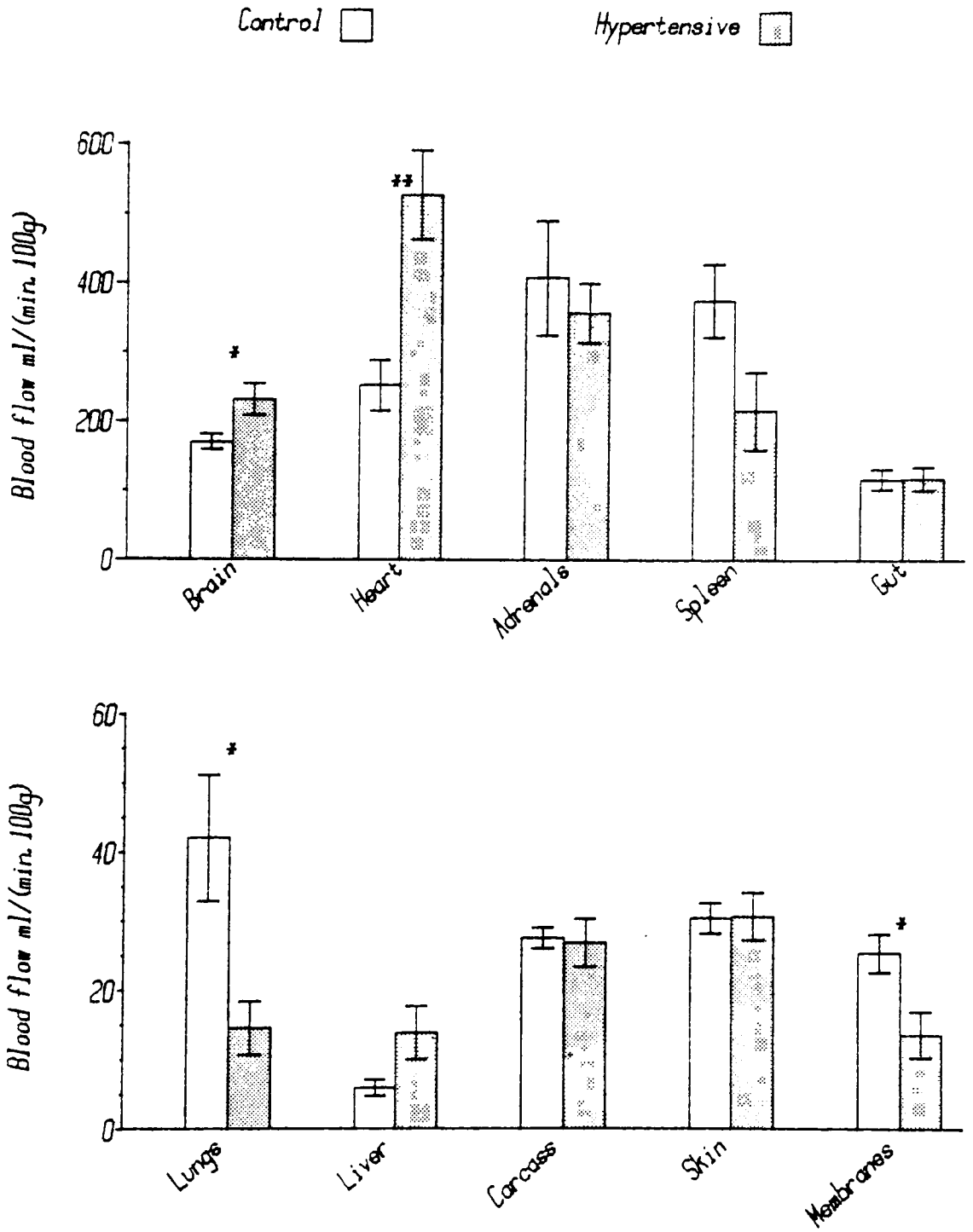


Figure 3.9. Mean (±SE) organ blood flows per unit weight in control and hypertensive fetuses (n= 8 & 6 respectively) *, $P < 0.05$ and **, $P < 0.01$, unpaired t-test.

Figure 3.10

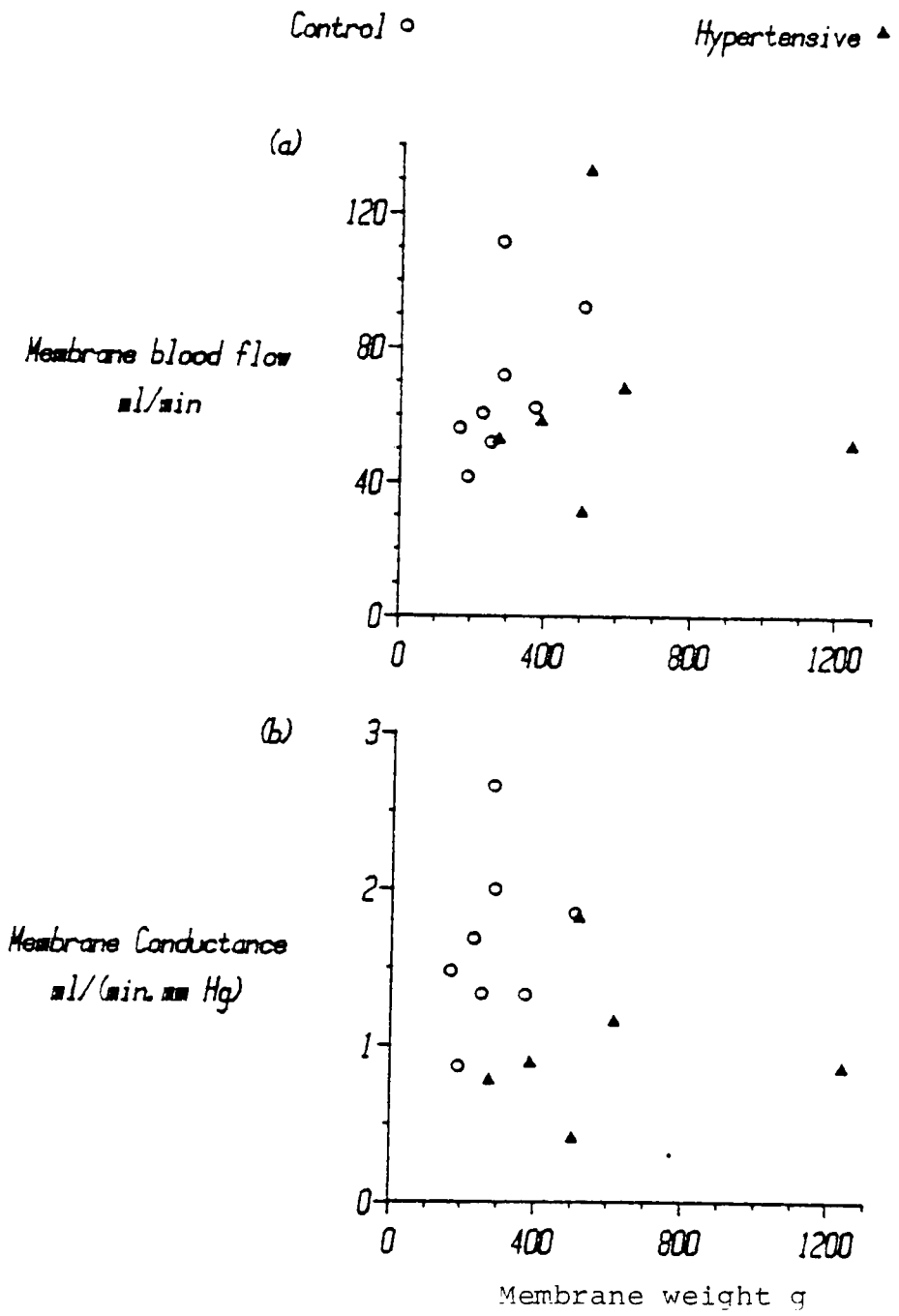


Figure 3.10. (a) Absolute membrane blood flow and (b) conductance, plotted against membrane weight for control (n=8) and hypertensive (n=6) fetuses.

gelatinous material which was difficult to remove, leading to an artificially high membrane weight. The mean membrane weight for the controls was 284 ± 38g, approximately half that found for the hypertensives, 589 ± 139g (P=0.03).

The blood flow to the brain was higher in the hypertensive group at 232 ± 23 $ml.min^{-1}.100g^{-1}$; the mean control value being 170 ± 11 (P=0.02). It is possible that this could be due to the lower oxygen content of the hypertensive fetuses' blood. Brain blood flow is shown plotted against carotid oxygen content in figure 3.11, with curves expressing the relationship between cerebral blood flow and oxygen content found by Peeters, Sheldon, Jones, Makowski and Meschia (1979). This figure suggests that the increased brain blood flow in the hypertensive fetuses is largely due to decreased carotid arterial oxygen content.

Hepatic arterial flow was also significantly increased in the hypertensive fetuses, from 5.86 $ml.min^{-1}.100g^{-1}$ in the controls to 13.8 ± 3.8 (P=0.045).

The threefold increase in the share of cardiac output received by the coronary circulation has to be weighed against the increased mass of the heart in the hypertensive fetuses. The hearts of the hypertensive fetuses (mean 35.9 ± 1.9g) were 50% heavier than those of the control animals (24.0 ± 1.2g, P<0.001). However, coronary flow was increased by a greater proportion and was doubled when expressed in terms of myocardial mass; control 253 ± 38

Figure 3.11

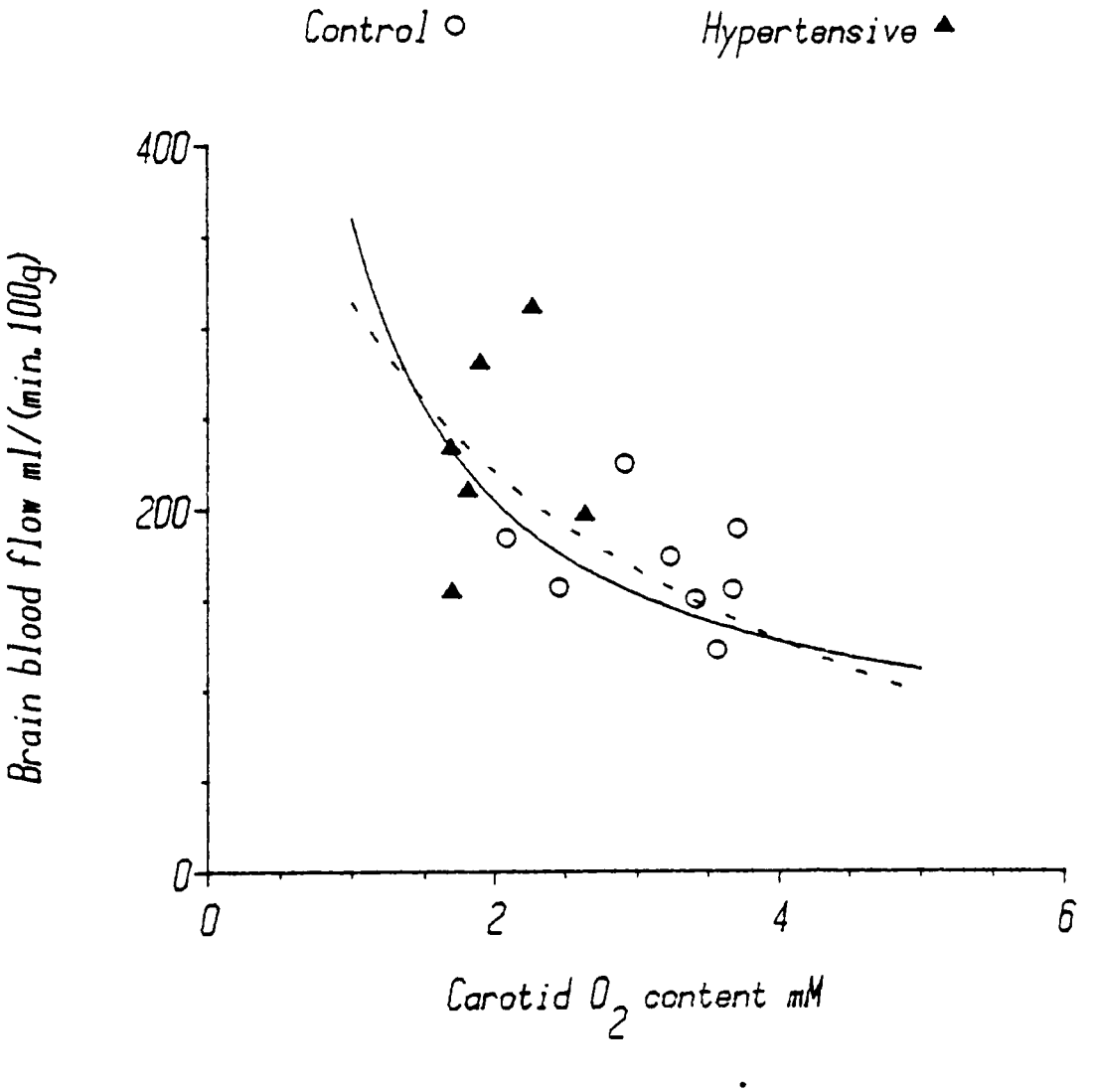


Figure 3.11. The relationship between brain blood flow and carotid arterial oxygen content in 8 control and 6 hypertensive fetuses. The curves are those described for cerebral blood flow by Peeters, Sheldon, Jones, Makowski and Meschia (1979).

$ml.min^{-1}.100g^{-1}$, hypertensive 530 $\pm$ 64, P=0.002. The relationship between coronary flow and oxygen content is shown in fig 3.12(a), again with curves calculated by Peeters et al (1979) to express the relationship between these two variables. Part of the increased coronary flow can be explained by the lower oxygen content in the hypertensive fetuses, but not all of it, as five out of the six lie above the curves. When coronary flow is plotted against the rate of work (power, see 3.3.2.1 for calculation) of the heart, expressed per unit weight (fig.3.12(b)) it becomes apparent that the higher coronary flow can also be partially attributed to increased work by the heart. The relationship between coronary oxygen delivery and carotid oxygen content (fig.3.12(c)) suggests thst the increased coronary flow is more than enough to maintain the normal level of oxygen supply to the heart. However, there is no significant difference between the coronary oxygen deliveries of the two groups; control 0.758 $\pm$ 0.093, hypertensive 1.068 $\pm$ 0.146 $mM.min^{-1}.100g^{-1}$.

Other studies have shown that the adrenal blood flow increases during hypoxaemia, so the relationship between adrenal blood flow and femoral arterial oxygen content was plotted in figure 3.13 in comparison to that found by Peeters et al (1979). The adrenal glands of the hypertensive fetuses have low blood flows for the prevailing oxygen contents. The control fetuses however, have flows distributed on either side of the lines fitted by Peeters et al (1979) to their data. Adrenal blood flow is therefore lower than expected in the hypertensive

Figure 3.12

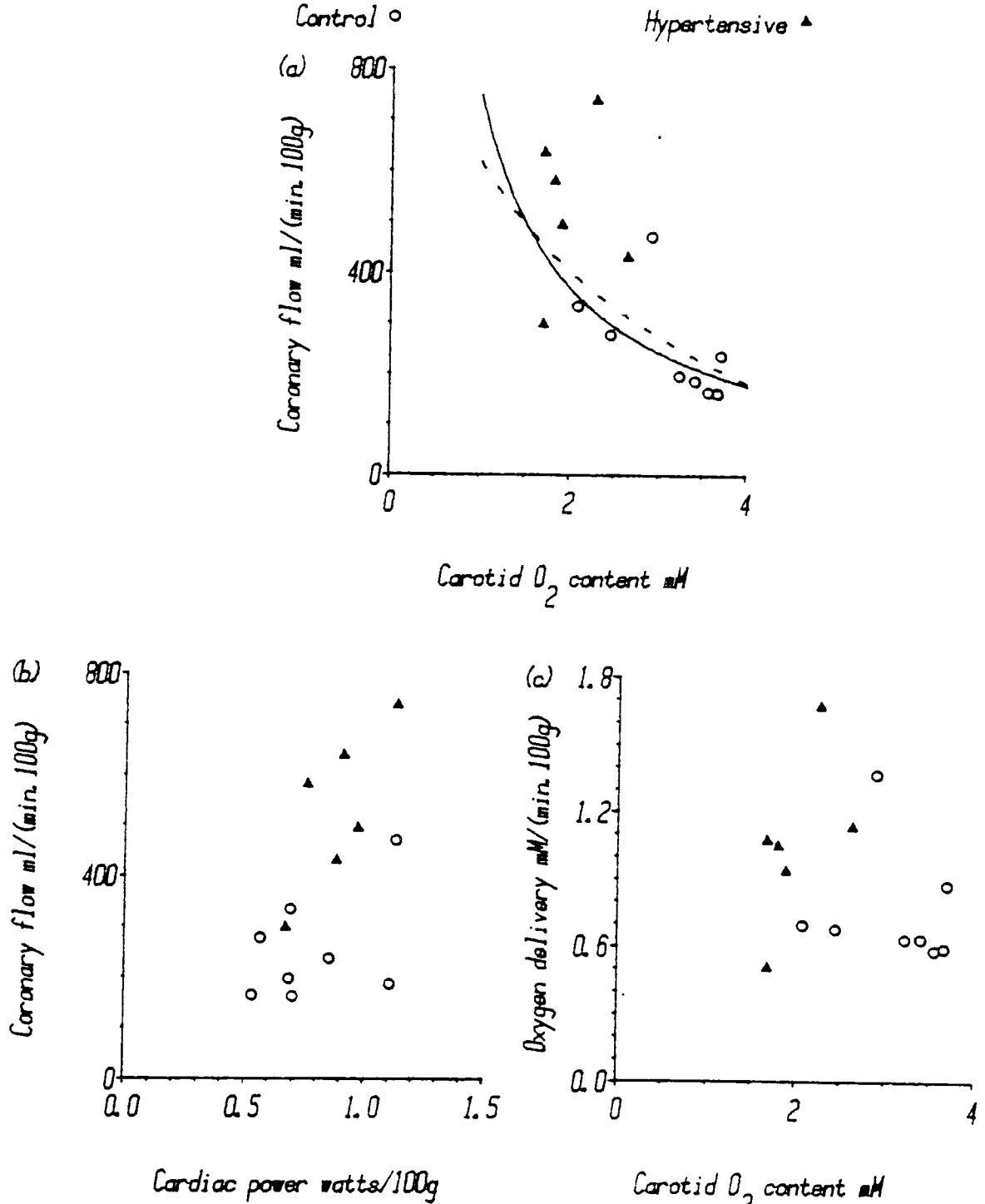


Figure 3.12. Relationships between coronary blood flow and (a) carotid arterial oxygen content and (b) cardiac power output; and between coronary oxygen delivery and carotid arterial oxygen content (c). The curves in (a) are those described by Peeters et al (1979).

Figure 3.13

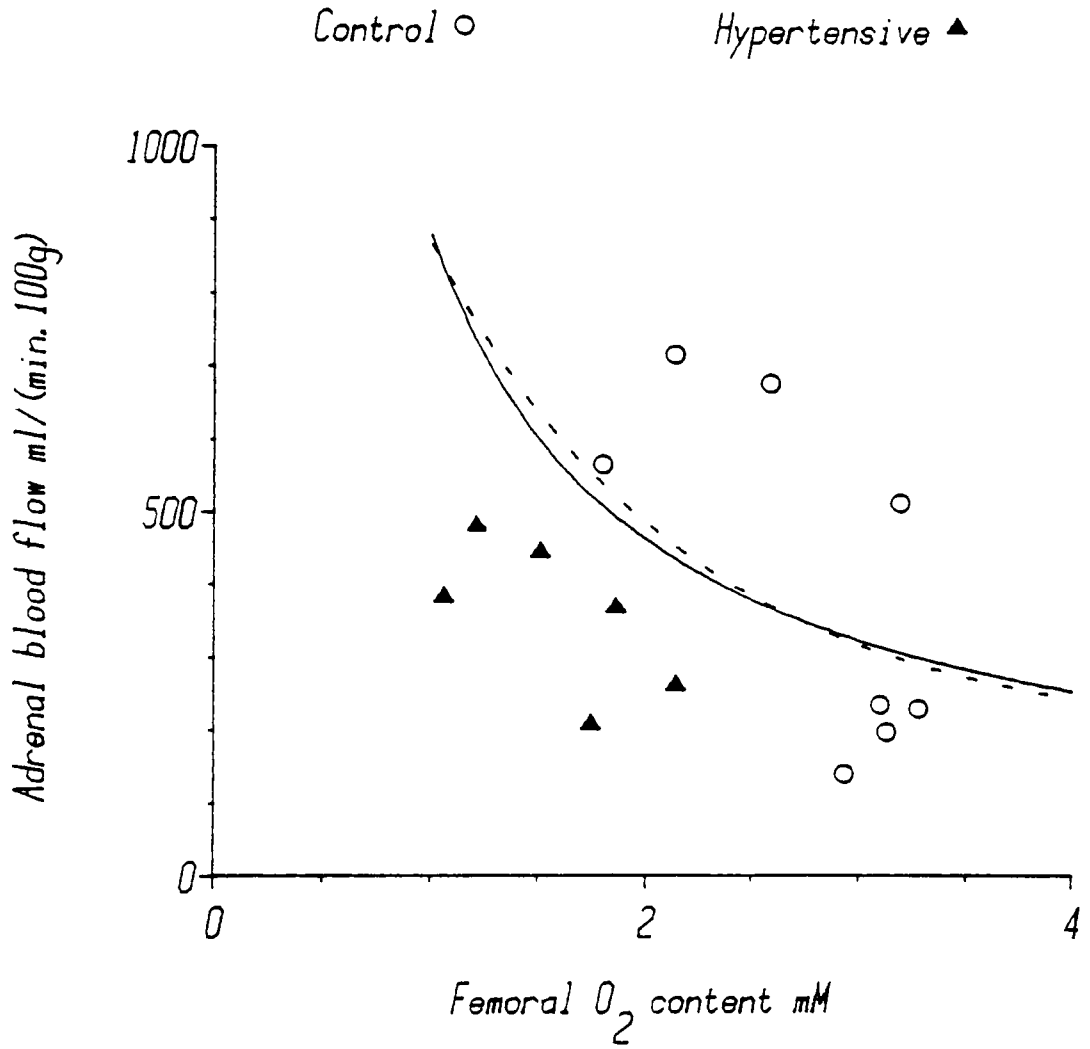


Figure 3.13. The relationship between adrenal blood flow and femoral arterial oxygen content in control and hypertensive fetuses. The curves are those described by Peeters et al (1979).

fetuses. Total adrenal weight was higher in the hypertensive fetuses at 679 ± 37mg compared with 487 ± 32mg (P=0.002).

3.3.2.5 Vascular conductances

Changes in organ conductances are much as would be expected from the changes in distribution and flows (fig. 3.14). There were 38% and 36% lower conductances in the carcass and skin respectively, of the hypertensive fetuses. A similar difference was found for the gastro-intestinal circulation, however, this was not statistically significant (P=0.08). The vascular conductance of the fetal membranes was also lowered, though some of this can be attributed to the increased weight of the membranes (fig. 3.10(b)). Absolute membrane conductance was also lower in the hypertensive group at 0.98 ± 0.19 $ml.min^{-1}.mm\ Hg^{-1}$ compared with 1.65 ± 0.19 (P=0.03).

There was no statistically significant difference in adrenal conductance between control and hypertensive groups, despite a comparatively large difference between the mean values.

The conductance of the hepatic arterial circulation was similar in both groups. The brain's vascular conductance was also maintained in the hypertensive fetuses and that of the heart, though showing some signs of being higher in the hypertensive animals, was also unchanged.

Despite the reservations concerning the measurement of pulmonary flow, the changes are worthy of note. The flow

Figure 3.14

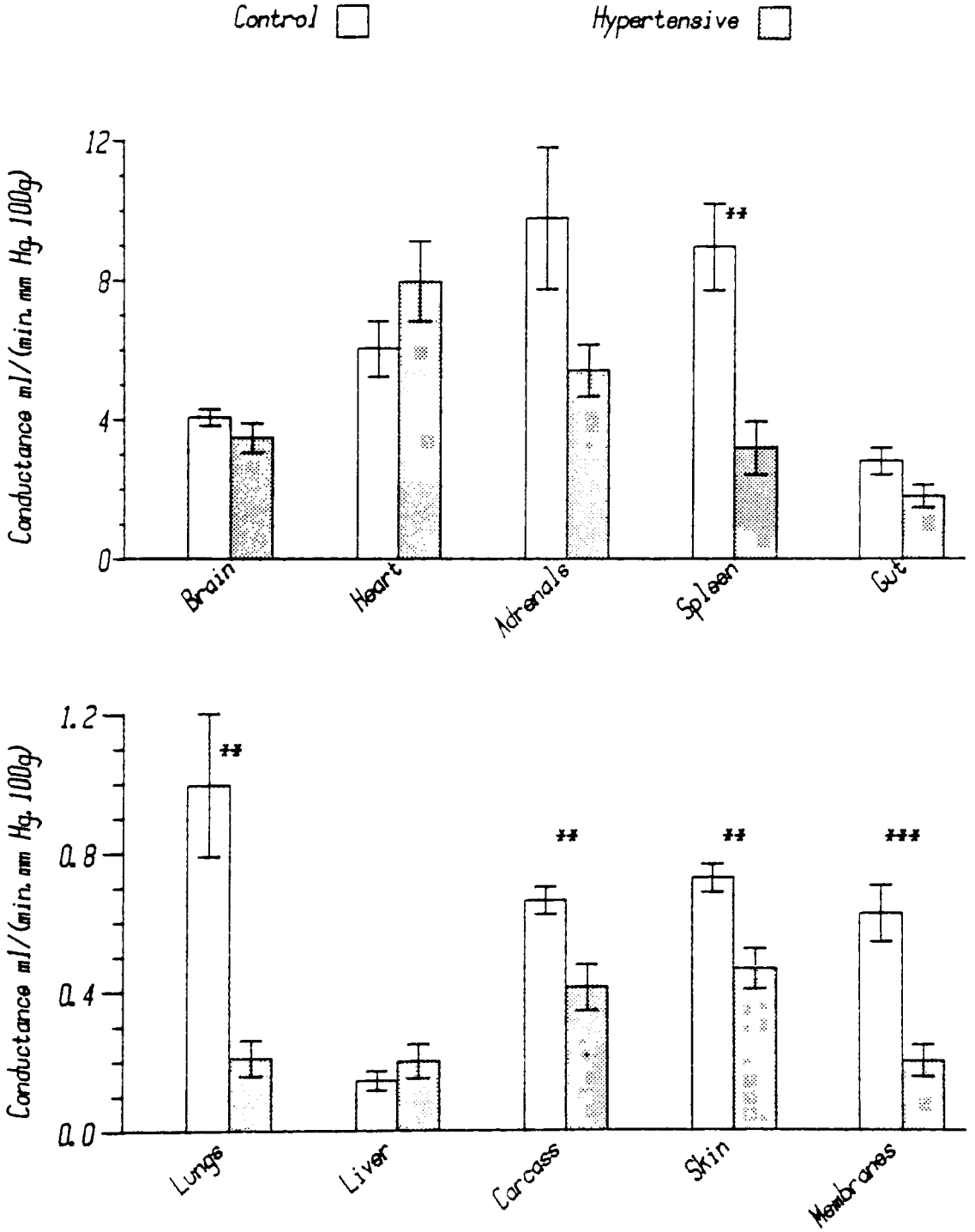


Figure 3.14. Mean (±SE) organ conductances per unit weight in control (n=8) and hypertensive (n=6) fetuses.
**, $P < 0.01$ and ***, $P < 0.001$, unpaired t-test.

to the lungs derived from the inferior vena cava was 65% lower and the corresponding conductance 79% lower.

3.3.3 Atypical fetuses

Fetus no 68, though nephrectomized, did not develop as marked a hypertension as the other nephrectomized fetuses, in other respects too it was intermediate between the control and hypertensive groups. This fetus had an arterial pressure of 55 mm Hg at the time of the microsphere experiment. The blood flows and conductances observed in this fetus are shown in table 3.3. It had a combined ventricular output of 632 $ml.min^{-1}.kg^{-1}$, towards the upper end of the hypertensive range. Its total peripheral conductance was higher than that of the hypertensive fetuses, but not as high as that of the controls. Generally, fetus 68's cardiac output was distributed similarly to that of the hypertensive fetuses. However, only 2.6% of CVO was received by the brain, which represented a smaller proportion of body weight than in the other animals; brain blood flow was appropriate for the prevailing carotid oxygen content of 1.31 mM (cf. fig.3.11). The heart received 13.8% of CVO, high even compared with the hypertensive fetuses. Coronary flow was 879 $ml.min^{-1}.100g^{-1}$ also very high, even when the level of oxygenation and the cardiac power output of 0.779 $watts.100g^{-1}$ are taken into account (cf. fig.3.12).

The share of cardiac output received by the placenta, 24.5%, was at the bottom end of the hypertensive range, while the cotyledonary flow, 155 $ml.min^{-1}.kg^{-1}$ body weight

Arterial pressure	55	mmHg	Carotid PO_2	15.1 mmHg
Venous pressure	6	mmHg	Carotid PCO_2	51.8 mmHg
Heart rate	130	$bts.min^{-1}$	Carotid pH	7.25
CVO	632	$ml.min^{-1}kg^{-1}$	Carotid $[O_2]$	1.31 mM
TPC	12.9	$ml.min^{-1}mmHg^{-1}kg^{-1}$	Haematocrit	21.0%
Placental flow	155	$ml.min^{-1}kg^{-1}$	Body weight	7.84 kg
Placental conductance	3.2	$ml.min^{-1}mmHg^{-1}kg^{-1}$	Placental weight	2.79 kg

	%CVO	Organ flow $ml.min^{-1}.100g^{-1}$	Organ conductance $ml.min^{-1}.mmHg^{-1}.100g^{-1}$
Brain	2.6	249	5.1
Heart	13.8	879	18.0
Adrenals	0.11	1060	21.6
Spleen	1.5	710	14.5
Liver	1.4	19.8	0.403
Gut	3.4	86.8	1.77
Membranes	4.3	12.1	0.247
Carcass	36.2	44.7	0.911
Skin	10.0	36.6	0.747
Lungs	2.2	56.2	1.15

Table 3.3

Measurements of haemodynamic and associated variables in fetus 68. CVO, combined ventricular output, TPC, total peripheral conductance

was average for the hypertensive group. The carcass, on the other hand, received a proportion closer to that received by the carcass of the control animals at 36%. The blood flow to the carcass was 44.7 $ml.min^{-1}.100g^{-1}$, also high.

Another organ receiving a higher than normal share of cardiac output was the lungs, taking 2.2% at a flow of 109 $ml.min^{-1}.100g^{-1}$.

The other exceptional nephrectomized fetus was no 140 which showed no signs of developing hypertension, and could even be considered hypotensive with an arterial pressure of 38 mm Hg. The results of the microsphere experiment on this fetus are given in table 3.4. CVO was exceptionally high, 885 $ml.min^{-1}.kg^{-1}$, as was its total peripheral conductance, 23.3 $ml.min^{-1}.mm\ Hg^{-1}.kg^{-1}$. Stroke volume was also high at 5.06 ml as heart rate was not increased. The distribution of cardiac output in this fetus was similar to that of the hypertensive fetuses, including the share received by the heart, of 9.0%. The power output of the heart was 0.954 $watts.100g^{-1}$ and the coronary flow was 1016 $ml.min^{-1}.100g^{-1}$, high for this work rate and the coronary oxygen content of 1.83 mM. The heart weighed 33.9 g, close to the mean value of the enlarged hearts of the hypertensive fetuses.

The individual organ flows and conductances were generally higher than those found in both control and hypertensive fetuses. The hepatic arterial circulation was an exception to this, with a flow and conductance of

Arterial pressure	38 mmHg	Carotid PO_2	19.1 mmHg
Venous pressure	0 mmHg	Carotid PCO_2	49.5 mmHg
Heart rate	175 bts.min^{-1}	Carotid pH	7.32
CVO	885 ml.min^{-1}.kg^{-1}	Carotid [O_2]	1.83 mM
TPC	23.3 ml.min^{-1}.mmHg^{-1}.kg^{-1}	Haematocrit	20.0%
Placental flow	276 ml.min^{-1}.kg^{-1}	Body weight	4.34 kg
Placental conductance	7.3 ml.min^{-1}.mmHg^{-1}.kg^{-1}	Placental weight	1.14 kg

	%CVO	Organ flow ml.min^{-1}.100g^{-1}	Organ conductance ml.min^{-1}.mmHg^{-1}.100g^{-1}
Brain	5.5	497	13.1
Heart	9.0	1016	26.7
Adrenals	0.12	579	15.2
Spleen	1.3	442	11.6
Liver	0.26	5.3	0.139
Gut	8.8	263	6.92
Membranes	2.3	16.1	0.423
Carcass	27.9	47.9	1.26
Skin	11.8	67.0	1.76
Lungs	1.7	58.1	1.53

Table 3.4

Measurements of haemodynamic and associated variables in fetus 140. CVO, combined ventricular output, TPC, total peripheral conductance

around control levels. The flow to the fetal membranes, and their vascular conductance, were intermediate between those of the controls and hypertensives.

3.4 Discussion

The control and hypertensive fetuses were comparable in most respects with those described by Dutton, Mott and Valdez-Cruz (1978) and Mott (1980, this paper includes some of the fetuses described here). There are a few exceptions to this; the difference between the mean body weights of the groups was less, and there was no significant difference in heart rate. As described by Mott (1980), not all the nephrectomized fetuses developed hypertension, one out of the eight remaining normotensive, and another developing only mild hypertension. Possible explanations for this will be considered below in discussing the results from these two fetuses.

The development of the hypertension is under way by the first day after nephrectomy and after four days has reached a reasonably steady level of around 70 mm Hg. To ensure that all the fetuses were being observed at a comparable stage in the development of hypertension, the microsphere experiments were carried out 7-9 days post-nephrectomy. The control experiments were performed 5-15 days post-operation at comparable gestational ages to the hypertensive fetuses.

3.4.1 Blood gases and pH

The changes in PO_2, PCO_2 and pH indicate there is some deterioration of the preparations after operation. These changes appear to occur in both control and hypertensive fetuses.

3.4.2 Haematocrit

The difference in haematocrit between the groups sets in early (day 1) and could be due either to increased plasma and blood volumes or to decreased red cell volume caused by haemorrhage. To account for the decrease on the basis of haemorrhage, followed by the restoration of blood volume by increased plasma volume, would require a blood loss of about 100ml, about 25% of the blood volume of a 3kg fetal lamb (Creasy, Drost, Green and Morris, 1960). Though blood clots were often found in the renal fat of nephrectomized fetuses at post-mortem examination, nothing of this magnitude was seen.

In the adult erythropoietin is produced by the kidneys and stimulates erythropoiesis. The absence of the kidneys is unlikely to explain the decreased haematocrit here, as nephrectomy does not affect the increase in erythropoietin seen following haemorrhage in the goat fetus (Zanjani, Gidari, Peterson, Gordon and Wasserman, 1973). It has now been established that the liver is the main site of erythropoietin formation in the sheep fetus at 95-115 days (Zanjani, Poster, Burlington, Mann and Wasserman, 1977).

If the differences in haematocrit between control and

hypertensive groups were solely due to plasma volume changes, then the increases in plasma volume required to account for the observed differences on days 2-8 range from 41-61% of control. The corresponding blood volume differences are 28-40% of control.

The daily blood sampling probably contributed to the post-operative falls in haematocrit, but only to a small degree as the sampling protocol did not exceed 5-6 ml per day.

The possibility that the difference in haematocrit between control and hypertensive groups, or the post-operative falls, are due to altered red cell volume caused by their swelling or contraction is removed by the absence of any differences or changes in cellular haemoglobin concentration.

The blood gas, pH and haematocrit differences at the time of microsphere experiments reflect the differences described for the daily changes. In addition, a lower oxygen content was found in the hypertensive group partly due to a slightly lower oxygen tension but largely attributable to the fall in haematocrit. With a carotid oxygen content of 2 mM, the hypertensive group should be considered hypoxaemic. Longo, Wyatt, Hewitt, and Gilbert (1978) found a mean control oxygen content of 4.05 mM, and 2.81 mM and 2.93 mM during hypoxic hypoxia and carbon monoxide hypoxia respectively.

3.4.3 Comparisons with other studies

The control values of arterial pressure, heart rate and CVO are similar to those found in the wide range of similar experiments in chronically prepared fetal lambs (see table 1.1), though the mean CVO is towards the high end of the range. This may in part be due to the drying of the wool in the present study, as even after wiping down, the wool holds about 300 ml of amniotic fluid at this stage of gestation. It is not clear whether most other workers have taken this precaution. The distribution of cardiac output was also similar to that found by other groups (Cohn et al, 1974; Longo et al, 1978; Sheldon et al,1979).

Both brain (170 $ml.min^{-1}.100g^{-1}$) and heart (253 $ml.min^{-1}.100g^{-1}$) blood flows were high compared with those found by Cohn et al (1974) (96 and 179 $ml.min^{-1}.100g^{-1}$) and Longo et al (1978) (140 and 196 $ml.min^{-1}.100g^{-1}$) though not in relation to those of Peeters et al (1979) at the appropriate levels of oxygenation (see figs. 3.11 and 3.12). The blood flow to the gut was also high at 118 $ml.min^{-1}.100g^{-1}$ compared with about 70 $ml.min^{-1}.100g^{-1}$ found by both Cohn et al (1974) and Longo et al (1978); it would appear that these workers weighed and counted the G.I. tract with its contents.

Other workers have generally considered the body and skin together as carcass, whereas here they have been considered separately, though the flow to both is similar in terms of body weight. To compare this study with others, the combined flow to the carcass and skin was

calculated, the mean value being 28.1 $ml.min^{-1}.100g^{-1}$, slightly high compared with the 20-23 $ml.min^{-1}.100g^{-1}$ found by Cohn et al (1974) and Longo et al (1978).

From the sums of their distribution figures it would appear that many investigators have included the fetal membranes as part of the placenta, whereas here they have been considered separately from the cotyledons, which receive a much higher flow. The total umbilical flow in these experiments was 261 $ml.min^{-1}.kg^{-1}$ body weight, which is high compared with the 191 $ml.min^{-1}.kg^{-1}$ obtained by Cohn et al (1974) and 185 $ml.min^{-1}.kg^{-1}$ by Longo et al (1978).

Both these groups found blood flows of less than 300 $ml.min^{-1}.100g^{-1}$ to the spleen, whereas here a figure of 377 $ml.min^{-1}.100g^{-1}$ was obtained. The splenic flow is particularly labile and this may explain the difference.

Apart from those already mentioned, there are no methodological differences between the present study and other studies using microspheres which would account for the differences in flows described above. It seems reasonable to attribute these discrepancies to variations between animals and breeds.

3.4.4 Haemodynamic changes in renoprival hypertension

Seven to nine days after nephrectomy, mean arterial pressure was 73.2 $\pm$ 2.4 mm Hg, 30 mm Hg higher than in control fetuses. Central venous pressure was also increased, by about 5 mm Hg, to 5.8 mm Hg, which partly

explains the increased arterial pressure. Atrial pressure would have been a preferable measurement, though the difference from inferior vena caval pressure would have been slight and would not have justified the risks associated with a chronic atrial catheter.

In the main, the arterial hypertension is due to a 44% lower total peripheral conductance; cardiac output, stroke volume and heart rate were no different. As mentioned in the introduction to this chapter, it has generally been found in the adult, that established hypertension is due to decreased peripheral conductance. However, transient increases in cardiac output have been found during the development of renovascular, cellophane wrapping, volume loading and mineralocorticoid hypertensions. Though in many of these hypertensions the transient increase in cardiac output is in progress at 7-9 days, the absence of such an increase here does not exclude an increase of shorter duration in fetal renovascular hypertension. In fact, transient increases in cardiac output which were over by day 7 were found by Bianchi et al (1972) and Maxwell et al (1977) in renovascular hypertension; and by Coleman and Guyton (1969) in volume loading hypertension.

3.4.4.1 Cardiac output

If cardiac output is to increase in the fetus it must be achieved by an increase in heart rate or an increase in myocardial contractility, as the fetus is normally operating near the top of its ventricular function curve (Gilbert, 1980). Though there was a slight difference between the

heart rates of the control and hypertensive groups during the development of hypertension, it was not significant apart from on day 3.

As reviewed in section 3.1.3.2.2 the potential for increased contractility in the fetal heart is slight.

Two points from the present study add support to the suggestion by Heymann and Rudolph (1973a) that the fetus is like the adult in heart failure. Firstly, the increased central venous pressure without an increase in CVO suggests that the fetal heart is pumping nearly as much blood as it can. Secondly, the results described here do not include a number of nephrectomized fetuses which died in utero, apparently from congestive heart failure, central venous pressure being dramatically increased.

It therefore seems unlikely that a transient increase in cardiac output would occur during the development of fetal renoprival hypertension. A suitable approach to resolving this point would be to use the four-way thermodilution method described by Gilbert et al (1980) to obtain repeated serial measurements of CVO. If smaller numbers of spheres were injected, the microsphere method could also be used for repetitive measurements with the advantage of providing at least large scale distribution figures.

3.4.4.2 Peripheral conductance

The principal variable determinants of peripheral vascular conductance are vessel diameter and blood

viscosity. The latter factor is not of any great importance here as the change in haematocrit will result in a fall rather than an increase in viscosity, and that will be small. The decrease in conductance is therefore due to some form of vasoconstriction, either brought about by contraction of the vascular smooth muscle or due to structural narrowing of the vessel lumens. Some clues as to the cause of the vasoconstriction may be obtained from examining the pattern of regional changes in blood flow and conductance.

Most of the fetal organs are involved to a similar degree in the decrease in conductance, with possibly a slightly greater proportionate fall in the conductance of the cotyledons. The brain, heart and hepatic arterial circulation do not take part in the decrease, and these organs received increased shares of cardiac output.

Decreases in conductance throughout much of the body have been found in several types of experimental hypertension (Flohr, Breull, Dahners, Redel, Conradi and Stoepel, 1976; Bralet, Wepierre and Bralet, 1973; and Alexander and DeQuattro, 1974) and spontaneous hypertension in rats (Nishiyama, Nishiyama and Frohlich, 1976). The fact that conductance changes are usually spread throughout the body has been taken to suggest that the mechanism is autoregulation, as most vasoconstricting agents have varying effects in different regions. As the fetus appears to have little capacity to increase its cardiac output, it seems unlikely that an autoregulatory decrease

in conductance could occur.

3.4.4.3 Effects of hypoxaemia

The comparison between control and hypertensive groups is slightly complicated by the hypoxaemia in the hypertensive group indicated by the difference in calculated blood oxygen content. The changes in distribution of cardiac output and organ blood flows during hypoxaemia are well documented so it is possible to gain some idea of what part of the changes might be due to hypoxaemia. However, it is difficult to make comparisons between studies as the degree of hypoxaemia varies. Cohn et al (1974) used a severe hypoxaemia, carotid arterial PO_2 decreasing from 21 mm Hg to 12 mm Hg. The fetuses fell into two groups, those which developed a simultaneous acidaemia and those which did not. Longo et al (1978) attempted to produce a hypoxic hypoxia in which both oxygen content and tension were decreased, and carbon monoxide hypoxia in which only oxygen content was decreased. In practice both were similar mild forms of hypoxaemia, ascending aortic PO_2 being 24.2 mm Hg in the controls and 19.0 and 21.6 in the hypoxic hypoxia and CO hypoxia groups respectively. The oxygen contents were 2.81 mM and 2.93 mM compared with 4.05 mM in the controls. Sheldon et al (1979) and Peeters et al (1979) studied a continuum of oxygen contents over the 1-6 mM range. All four studies used microspheres to follow the blood flow changes.

All found that brain, heart and adrenal blood flows were increased during hypoxaemia, this being achieved by

the redistribution of an unchanged cardiac output. The relationships between brain, heart and adrenal blood flows and oxygen content described by Peeters et al (1979) and Jones et al (1978) indicate that oxygen supply to these tissues is maintained over the 1-6 mM range of O_2 content.

The increased blood flow and share of cardiac output to the brain in the hypertensive fetuses appear to follow the relationship described by Peeters et al (1979) for the cerebrum. The brain is spared from the general decrease in conductance such that its oxygen supply is maintained. The heart, however, exhibits a different flow-oxygen content relationship in the hypertensive fetuses, blood flow being much higher than expected. Nevertheless, the increased coronary flow (from 250 $ml.min^{-1}.100g^{-1}$ to 530 $ml.min^{-1}.100g^{-1}$) can in part be attributed to the lower oxygen content, which alone would be expected to increase it to about 350 $ml.min^{-1}.100g^{-1}$.

The adrenal blood flow in the hypertensive fetuses was low compared with that found for comparable oxygen contents by Peeters et al (1979). This may in part be due to the increased adrenal weight, if this is caused by oedema.

Both Peeters et al (1979) and Longo et al (1978) found pulmonary blood flow fell with decreasing oxygen content, and this may in part explain the fall in pulmonary flow in the hypertensive fetuses. The relationship found by Peeters et al (1979) showed no decrease in pulmonary flow below an oxygen content of 4 mM, which is higher than the contents found in both the control and hypertensive

fetuses. Longo et al (1978), however, found that relative pulmonary flow continued to fall over the range of $[O_2]$ in the present study.

Other than the above, little change in organ flows or shares of cardiac output would be expected over the fall in oxygen content in the hypertensive fetuses. Peeters et al (1979) demonstrated that carcass flow changes little over the 1.5 to 3.5 mM range, and that to the G.I. tract is fairly constant from 2 to 6 mM. They also found umbilical blood flow was unchanged across the whole of the range studied. Longo et al (1978) also found little change in carcass, liver, spleen, G.I. tract and umbilical blood flows during hypoxaemia.

3.4.4.4 Cotyledonary circulation

The changes in the placental circulation of the hypertensive lambs are particularly striking. The placental cotyledons received 34% of cardiac output in the hypertensive fetuses compared with 40% in the controls, though this difference was not statistically significant. There was also a nearly significant decrease in cotyledonary flow and cotyledonary conductance was decreased by 53%.

The change in cotyledonary conductance may partly occur in the ductus venosus and liver aswell as the cotyledons themselves, as the pressure difference used to calculate conductance is that between the femoral artery and inferior vena cava. However, it seems likely that the

bulk of the decrease occurs in the cotyledons as a disproportionately large decrease in conductance is not seen in the fetal membranes, which also return their blood via the ductus venosus and liver.

Though the placental vasculature is not innervated, it is responsive to many vasoactive compounds, and umbilical conductance has been found to change during a number of experimental manipulations. Iwamoto and Rudolph (1981) found that umbilical blood flow passively followed the increased blood pressure produced by infusion of angiotensin II, umbilical conductance being unchanged. The decrease in umbilical flow produced by angiotensin II blockade was also a passive change following arterial pressure. Berman, Goodlin, Heymann and Rudolph (1978) however, found that angiotensin II did increase umbilical vascular resistance (UVR). Increases in UVR have also been found in response to vasopressin (Iwamoto, Rudolph, Keil and Heymann, 1979) and possibly following haemmorhage (Toubas, Silverman, Heymann and Rudolph, 1981).

The prostaglandins, or more particularly PGE_2, have been found to be potent umbilical vasoconstrictors. Novy, Piasecki and Jackson (1974) found UVR was increased about 11% by 100-300 $\mu g.kg^{-1}$ of $PGF_{2\alpha}$; though Berman et al (1978) observed a 10% increase at the much lower total dose of 15 ug injected directly into the umbilical circulation. PGE_2 (20-50 $\mu g.kg^{-1}$) caused a maximum increase in umbilical resistance of about 80%, which fell to about 40% after 10 minutes (Novy et al, 1974). Rankin and Phernetton (1976)

found a 200% increase in UVR produced by 10 $\mu g.kg^{-1}$ of systemically injected PGE_2. Local arterial injection of 5 μg PGE_2 resulted in a 360% increase in UVR. The same dose of PGE_1 produced only a 44% increase in UVR.

Two other compounds found to be umbilcal vasoconstrictors by Berman et al (1978) were bradykinin, 5μg of which produced a 35% increase in UVR; and 5-hydroxytryptamine (5-HT), which on a weight basis was the most potent vasoconstrictor found, a 1 μg dose producing a 180% increase in UVR.

Noradrenaline, in a systemic dose of 1-2 $\mu g.kg^{-1}$ was found not to change UVR by Novy et al (1974). Berman et al (1978) also found no response to a locally injected dose of 2μg. These workers also found no response to acetylcholine, isoprenaline, dopamine, histamine or tolazoline at doses which exerted systemic effects.

The possible involvement of these vasoactive substances in the decreased conductance described here is considered below in conjunction with the other organs.

3.4.4.5 Coronary flow

The contribution of the hypoxaemia to the increase in coronary flow has already been considered, the power output of the heart also plays an important part in the increase. The relationship between coronary flow and cardiac power has not previously been described in the fetus, though there are instances in which an increase in cardiac power output may be assumed to have occured, which are associated with

increases in coronary flow. The 22% higher cardiac output in fetuses receiving chronic infusions of tri-iodothyronine (T_3) was associated with a 29% higher coronary flow (Lorijn et al, 1980). Despite an increase in arterial pressure, no change was found in coronary flow during vasopressin infusion (Iwamoto et al, 1979). Angiotensin II infusion produced increases in both CVO and arterial pressure (Iwamoto and Rudolph, 1981) but although coronary flow was increased from 250 to 340 $ml.min^{-1}.100g^{-1}$ this change was not statistically significant.

3.4.5 Possible mechanisms of the vasoconstriction

One of the problems in looking for the agent which causes the decrease in peripheral conductance is that the change is chronic whereas studies on the effects of vasoactive substances have been acute. Over a period of time, adaptations may occur in the response to a particular stimulus so that comparisons of the observed changes with the known effects of vasoconstrictors is confused.

3.4.5.1 Circulating agents

3.4.5.1.1 Angiotensin II This can be excluded as a candidate even without consideration of its regional haemodynamic actions, as it is absent from the plasma of nephrectomized fetuses (Broughton-Pipkin et al, 1974).

3.4.5.1.2 Prostaglandins The powerful vasoconstrictor action of prostaglandin E_2 in the umbilical vascular bed has already been noted, however its effects on the fetal systemic circulation are less well documented. Rankin and

Phernetton (1976) found PGE_2 increased fetal renal vascular resistance. In the adult PGE_2 is generally a vasodilator and may be so in the fetal systemic circulation. The transient decrease in arterial pressure produced by PGE_2 before an increase associated with umbilical vasoconstriction set in, was taken as indicating a systemic vasodilatation by Novy et al (1974).

Prostaglandins are both produced and destroyed by the fetal kidney (Mitchell), and Walker and Mitchell (1978) found high levels in fetal urine. Whether the latter represents a major route of clearance of prostaglandins in the fetus is uncertain.

The milder vasoconstrictive actions of PGE_1 and $PGF_{2\alpha}$ on the umbilical circulation were mentioned above. As with PGE_2 the systemic effects of these compounds are unknown in the fetus.

3.4.5.1.3 Vasopressin Vasopressin is a more likely cause of the vasoconstriction. Infusions of vasopressin which resulted in a 13-fold increase in plasma vasopressin concentration resulted in a 9 mm Hg increase in arterial blood pressure due to a 36% increase in total peripheral resistance, cardiac output being unchanged (Iwamoto et al 1979). However the resistance increase predominantly occured in the fetal body, the umbilical share of cardiac output being increased from 41% to 50%, though umbilical vascular resistance was also increased. The vascular resistance of the carcass was more than doubled, it received 12% less of CVO. The resistance of the gut was

similarly increased. The brain was spared from the increase in resistance, receiving an increased share of CVO but not a significantly greater blood flow. Even though this does not fit the required pattern in a number of respects, it shows that vasopressin is capable of causing widespread increases in vascular resistance in the fetus.

Alexander, Bashore, Britton, and Forsling (1976) have demonstrated increased vasopressin secretion following infusion of hyperosmolar NaCl in the fetus. The hypertensive fetuses have been shown to be hypernatraemic (Dutton and Mott,1979); there is, therefore, a potential mechanism for increased vasopressin secretion. The sites of vasopressin clearance from the fetal circulation have not been fully established, though it seems the placenta is involved to some degree (Jones and Rurak, 1976). In the adult, the kidneys are responsible for about 40% of the total vasopressin clearance (Lauson, 1974). If the renal extraction in the fetus were similar to that in the adult, Rurak (1975) estimated that renal clearance could account for about 18% of the total fetal clearance. The effects of nephrectomy on fetal vasopressin clearance would depend on the ability of the placenta and other organs to cope with an increased load of this magnitude.

3.4.5.1.4 Catecholamines Noradrenaline does not fit the pattern of changes very well. A transient pressure increase was observed by Lorijn and Longo (1980) to a 0.4-1.1 $ug.kg^{-1}.min^{-1}$ infusion. Coronary and pulmonary flows were increased though cardiac output was unchanged.

The biggest discrepancy with the hypertensive changes is the 27% increase in umbilical flow from 177 to 226 $ml.min^{-1}.kg^{-1}$. As mentioned above, other workers have failed to show an effect of noradrenaline on the umbilical vascular bed.

Despite the lack of response of the umbilical vascular bed, alpha-adrenergic stimulation with methoxamine has been shown to produce a general vasoconstriction in the fetus (Barrett, Heymann and Rudolph, 1972). Arterial pressure was increased, though less so in mature fetuses, and cardiac output and umbilical flow were decreased, indicating increased vascular resistances. Distribution of cardiac output was similar except for a decrease in the share flowing to the kidneys and an increase in that to the lungs.

3.4.5.1.5 Other vasoconstrictors The fetal systemic effects of 5-HT, which causes a substantial increase in in umbilical vascular resistance is, also unknown but its unimportance as a vasoconstrictor in the adult argues against a general systemic role.

Assali, Johnson, Brinkman and Huntsman (1971) found bradykinin dilated the pulmonary and systemic vascular beds, and lowered arterial pressure. Umbilical flow fell passively following the pressure change. This contrasts with the findings of Berman et al (1978) for the umbilical vascular bed which have already been mentioned. Bradykinin does not seem a likely candidate for the vasoconstriction.

3.4.5.2 Vessel wall thickening

An alternative to a smooth muscle mediated vasoconstriction as the cause of the decreased conductance is a narrowing of the vascular lumen caused by increased vessel wall thickness. The theoretical effects of this were outlined by Folkow, Grimby and Thulesius (1958). Not only would resistance be increased at any particular level of vascular tone but the effects of vasoconstrictors would be potentiated, a given degree of muscle shortening causing a greater increase in vascular resistance. The current state of knowledge of this phenomenon has been reviewed by Folkow (1978). Such changes have been found in spontaneously hypertensive rats, with little change in vascular tone. The stimulus to increase wall thickness is supposedly the load against which the smooth muscle works, i.e. the tension in the vessel wall, and may be intermittent or continuous. Reversal of the structural changes in hindlimb vessels of hypertensive rats has been achieved by aortic obstruction which lowered the regional arterial pressure. Increased pressure therefore has been taken as a suitable stimulus in itself to increased wall thickness, which once established would maintain hypertension. This mechanism still requires an initial triggering stimulus to increase the load on the peripheral vessels to set in train the structural changes. However, this would not necessarily have to produce a vasoconstriction of the pattern finally seen, if vessels in all regions responded to the ensuing pressure increase. In addition vessels with thickened walls retain the

capacity to vary their tone in response to the usual stimuli and would allow changes consequent on metabolic demand such as those seen in the brain and heart.

It would appear that changes of this nature occur within a week in renal hypertensive rats (Folkow, 1980). The growing fetus should well be able to produce the necessary hyperplasia or hypertrophy within 7-9 days. The capacity of the fetus to make rapid structural adaptations in response to hypertension is indicated by the 50% higher cardiac weight in the hypertensive fetuses.

Increased medial width and decreased external diameter were found in fifth generation arterioles (resistance vessels) in the pulmonary vascular beds of fetuses with arterial pressures of 70 to 100 mm Hg (Levin, Hyman, Heymann and Rudolph, 1978). This implies narrowing of the vascular lumen, but this was not actually measured. The studies were carried out 8-20 days after renal artery constriction (4 fetuses), accidental umbilical constriction (1 fetus) or ductus arteriosus occlusion (1 fetus). The lungs were fixed for histological examination by perfusion at pressures found in vivo. Ruiz, Piasecki, Balagh, Polansky and Jackson (1972) found increased amounts of collagen in the pulmonary vessel walls and adventitia 9-36 days following ligation of the ductus arteriosus; vessel sizes were not measured.

3.4.6 Atypical fetuses

Fetus 68 can be regarded as partially developing the

changes seen more fully established in the hypertensive fetuses. It is not clear why this is so. Fetus 140 is more exceptional; it cannot be categorically stated that the high flows and low conductances in this fetus were a consequence of nephrectomy, as the situation before nephrectomy is unknown. The heart weight of this fetus is comparable to that of the hypertensive fetuses, as is the haematocrit; the latter perhaps indicating increased plasma and blood volumes. The high cardiac output and stroke volume combined with the low venous pressure suggest that the heart was able to pump the increased venous return consequent on an increased blood volume. This would imply an elevated ventricular function curve in this fetus, the cause of which is uncertain.

3.4.7 Summary

Increased vessel wall thickness is an attractive proposition for the cause of the fall in conductance and is worth investigating. A triggering pressor stimulus is also required, however, and vasopressin is a promising candidate, but plasma vasopressin was found to be normal in two nephrectomized fetuses (Mott, 1980). Measurements of circulating prostaglandins E_2, $F_{2\alpha}$, and F metabolite were above normal in two lambs (Mott, 1980) which may explain the increased cotyledonary resistance. Though not producing the right pattern of conductance changes, the catecholamines seemed a strong possibility as plasma catecholamine levels were found to be elevated in preliminary experiments, and phentolamine infusion in some

instances produced profound falls in arterial pressure. It was therefore decided to investigate the effect of alpha-adrenergic blockade with phentolamine on the hypertension and increased conductance.

CHAPTER 4

Cardiovascular effects of alpha-adrenergic blockade in control and hypertensive fetuses

4.1 Introduction

The development and role of autonomic regulation of the fetal lamb's cardiovascular system

4.1.1 Histochemical and biochemical studies

4.1.1.1 Sympathetic Sympathetic innervation of the fetal lamb heart was shown by Lebowitz, Novick and Rudolph (1972) to be present from 100 days of gestation. The developing adrenergic plexus becomes more extensive as gestation proceeds, becoming associated with all the contractile and vascular elements of the heart. The innervation of the sino-atrial pacemaker necessary for any direct chronotropic sympathetic influence was not investigated. Though Friedman (1973) concluded from both biochemical and histochemical evidence that the sympathetic innervation of the fetal lamb heart was not fully developed at birth, Pappano (1977) considered that in comparison with mammals less mature at birth, it was well developed.

4.1.1.2 Parasympathetic Friedman (1973) stated that the density of cardiac cholinergic innervation was similar in the near-term lamb and the adult sheep.

4.1.2 Responses of isolated tissues

4.1.2.1 Sympathetic Studies of the chronotropic responses of isolated fetal sheep hearts do not appear to be

available. Wildenthal (1973) found an increasing positive chronotropic response in the fetal mouse heart to noradrenaline and isoprenaline (which suggests a beta receptor mediated effect) from 13 days gestation (term = 22 days). This occurs before the sympathetic innervation develops, which is confirmed by the failure of tyramine (which releases endogenous noradrenaline) to increase heart rate until 21-22 days.

The positive inotropic action of noradrenaline was found to be more sensitive in the term fetal than the adult isolated sheep heart, though this was attributed to lower neuronal uptake in the fetus; beta-receptor sensitivity (as indicated by the response to isoprenaline) being similar in fetus and adult.

These studies indicate the potential influence of circulating catecholamines on the fetal heart, but do not say anything about effective neuroeffector transmission, which requires the pre-synaptic elements as well.

Some impression of the development of the responsiveness of the peripheral vasculature may be gained from the work of Wyse, Van Petten and Harris (1977) who found noradrenaline caused contraction of helical strips from fetal lamb ear arteries. The effect increased dramatically from 110-115 days gestation to 133-137 days, and further post-natally. Electrical transmural nerve stimulation (such as not to stimulate smooth muscle directly) exerted an increasingly greater influence from being non-existent at 110-115 days. Receptor sensitivity

appeared unchanged during pre- and postnatal development, changes in response thus being attributed to changes in innervation and effector mechanisms (Wyse et al, 1977).

Contractile responses of isolated fetal blood vessels to noradrenaline and 5-hydroxytryptamine (5HT) are present, though comparatively slight, from 53-90 days (Su, Pegram, Bevan, Assali and Brinkman, 1978). The responses of the carotid artery were greater at 115-130 days but did not increase much further after this. In contrast, the development of the contractile response to transmural nerve stimulation occured more gradually, increasing at both 115-130 days and after 140 days. This response was always less than that elicited by the vasoactive agents, particularly at 115-130 days. However, neuronal uptake of noradrenaline was comparable at all stages, suggesting the low responsiveness to neural stimulation was due to some immaturity of the neuro-effector relationship rather than lack of innervation. Other vessels also showed contractile responses, though those of the great vessels were small. All vessels studied were low in the vascular tree, the responsiveness of the precapillary arterioles which account for most of the vascular resistance are not known.

4.1.2.2 Parasympathetic In the isolated chick embryo heart a neurally based negative chronotropic cholinergic mechanism is present from day 12, preceding the development of the sympathetic mechanism (Pappano, 1977). The rate of beating of isolated fetal mouse atria was decreased by

acetylcholine from the earliest stage (13 days) investigated by Wildenthal (1973) though the effect increased with age. Isolated human fetal atria (8-9.9 mm stage) exhibit a negative chronotropic response apparently due to cholinergic nerves acting at muscarinic receptor sites (Pappano, 1977).

Studies of cholinergic mechanisms in the isolated fetal lamb heart have been confined to inotropic responses (Friedman, 1973). During the last two weeks of gestation a negative inotropic action of exogenous acetylcholine was found, of similar sensitivity to that in the adult.

Generally, as in the sympathetic system, the responsiveness of the post-synaptic receptor and effector mechanisms develop before the pre-synaptic neural elements (Pappano, 1977).

Su et al (1978) found isolated fetal sheep vessels contracted in response to acetylcholine, though not consistently at 53-90 days, the effect increasing with gestational age.

4.1.3 Studies in the whole animal

4.1.3.1 Nerve stimulation and section

The presence of functional neural regulation can only fully be tested by nerve stimulation and section, however this type of experiment is rare in the fetus. Born, Dawes and Mott (1956) showed slowing of fetal lamb heart rate by vagal stimulation was present but slight (about 10%) at 60

days gestation, increasing to about 20-50% between 80 and 90 days. The effectiveness of cardiac and splanchnic nerve stimulation increased after 80 days (Born et al, 1956).

4.1.3.2 Pharmacological effects

4.1.3.2.1 Sympathetic There have been many more studies of the effects of exogenous autonomic transmitters. Dawes, Mott and Rennick (1956) found that heart rate was increased by adrenaline in immature fetal lambs (69-98 days gestation) under pentobarbitone anaesthesia. The absolute pressure changes are not clear, but the percentage increase in heart rate for a given increase in blood pressure was larger in immature than mature fetuses (>132 days). A vagally mediated bradycardia in response to the pressure increase produced by a large dose of adrenaline was found in a 90 day old fetus. Adrenaline and noradrenaline increased heart rate and blood pressure in mature fetal lambs, though large doses which produced a large increase in pressure were associated with a bradycardia abolished by vagal section. Umbilical blood flow increased roughly in proportion to the increase in arterial pressure, suggesting minimal effects of catecholamines on the umbilical vasculature; though adrenaline caused a slightly lower increase in flow for a given increase in pressure than noradrenaline. Acetylcholine produced falls in heart rate and blood pressure in mature fetuses, and in heart rate in immature fetuses.

The beta-adrenergic responses of fetal lambs were

investigated by Van Petten and Willes (1970) in chronic preparations from 100-120 days onwards. Isoprenaline increased heart rate and decreased arterial pressure, a maximum response being elicited by about 1 $\mu g.kg^{-1}$. Blockade by propanolol confirmed these were beta-adrenergic responses.

Joelsson, Barton, Daniel, James and Adamsons (1972) found no changes in alpha and beta adrenergic responses from 95-136 days in chronically prepared fetal lambs. Adrenaline produced an increase in blood pressure and a decrease in heart rate supposedly mediated by the baroreceptors. It is surprising that blockade (with phenoxybenzamine) of the alpha receptor mediated pressor response, did not uncover a tachycardia in response to adrenaline. Noradrenaline produced similar changes and also small increases in heart rate following alpha blockade. A beta-adrenergic tachycardia was observed in response to isoprenaline, blood pressure being decreased. The conclusions drawn about the arterial baroreceptors in chapter 1 do not favour them as mediators of the fall in heart rate produced by adrenaline and noradrenaline as is often suggested.

Barrett, Heymann and Rudolph (1972) investigated the effects of alpha and beta receptor stimulation in acute experiments under maternal spinal anaesthesia, but used a small number of fetuses to cover a wide gestational age range. Again alpha stimulation (with methoxamine) in fetuses from 105-150 days gestation increased arterial

pressure, with the effect falling off slightly towards term. Heart rate was unchanged. Isoprenaline infused into fetuses from 60-145 days gestation increased heart rate, though the tendency for arterial pressure to fall was greater in younger fetuses. Cardiac output and umbilical blood flow were decreased following methoxamine administration by roughly equal proportions as was flow to most fetal organs. This indicates similar increases in the resistances of each organ, though renal resistance was increased by a greater proportion. Pulmonary blood flow, however, was increased during methoxamine infusion. Umbilical blood flow was increased by isoprenaline though changes in cardiac output were variable. The heart received an increased proportion of CVO during isoprenaline infusion.

Nuwayhid, Brinkman, Su, Bevan and Assali (1975a) found noradrenaline (at doses comparable to those used in other studies) increased heart rate along with arterial pressure and ascending aortic and pulmonary flows. The responses were present in fetal lambs from 60 days until term though they increased, as percentage changes, with maturity. Blocking experiments with propanolol and phenoxybenzamine showed the heart rate and flow increases were beta effects, whereas the pressure increase was abolished by alpha blockade. The sensitivity of the receptor mechanisms (as measured by threshold and dose required to elicit half maximal effect, ED_{50}) were unchanged from immature to mature fetuses, indicating the increase in response was due to development of the effector mechanisms.

A decrease in heart rate associated with increased arterial pressure following alpha-adrenergic stimulation with phenylephrine was found in the chronically prepared fetal lamb from 100 days (Harris and Van Petten, 1978). This bradycardia was not completely abolished by atropine. Ephedrine, which has direct alpha and beta effects as well as causing release of endogenous noradrenaline, showed similar effects, though a tachycardia was revealed following cholinergic blockade with atropine. The possibility of central actions of ephedrine (Crossland, 1980) slightly obscures the implications of these results. All effects tended to increase as gestation proceeded.

Jones and Ritchie (1978) carried out an extensive series of experiments in chronic preparations of 115-141 days gestation on the responses of heart rate and blood pressure during hour long infusions of adrenergic agonists and antagonists.

The bradycardia produced by adrenaline was found to be transient and blocked by the alpha antagonist phentolamine, along with the pressor response. It was followed by a beta-receptor mediated bradycardia. No transient bradycardia was produced by noradrenaline, a slowly developing tachycardia blocked by propanolol was found, with an alpha mediated pressor response. Though isoprenaline produced the expected beta effects of a tachycardia and a fall in arterial pressure, the pure alpha agonist methoxamine had no effects on either variable.

Noradrenaline was also administered by infusion of

similar doses by Lorijn and Longo (1980) though the pressor response appeared to be transitory and accompanied by a bradycardia. Fifty minutes after the infusion was started, a time for which no pressure measurement was given, cardiac output was unchanged though there were changes in its distribution. Coronary and pulmonary blood flows were increased by 40% and 160% respectively, and umbilical blood flow was increased by 27%. The extra blood required to supply these organs would appear to have been diverted from the carcass and gut.

4.1.3.2.2 Parasympathetic Studies of the effects of cholinergic agonists on the fetal lamb are rare. Nuwayhid et al (1975a) found acetylcholine decreased heart rate and arterial pressure, and increased ascending aortic and main pulmonary arterial flow, each effect increasing in magnitude from 60 days of age to maturity. Combined systemic and umbilical resistance was unchanged but pulmonary resistance was decreased. As with the adrenergic system, thresholds and ED_{50} values were similar at all ages, indicating progressive maturation of the effector systems.

4.1.3.3 Effects of blocking agents

Some idea of the influence exerted by the different arms of the autonomic nervous system can be gained by studying the effects of various autonomic blocking agents.

4.1.3.3.1 Sympathetic Joelsson et al (1972) found the effects of alpha blockade with phenoxybenzamine at 95-136

days gestation were slight, blood pressure fell and heart rate increased, both by about 5%. A 5% reduction in blood pressure was also produced by propanolol, though a larger decrease (15%) in heart rate was observed.

The development of autonomic control was investigated more systematically by Vapaavouri, Shinebourne, Williams, Heymann and Rudolph (1973) from 85 days until term in chronic preparations. Alpha-blockade caused larger absolute decreases in blood pressure as gestation proceeded. The percentage fall was about 5% from 85-100 days and about 10% from 101 days to term. Beta-blockade decreased heart rate at all ages, the effect increasing after 120 days.

Nuwayhid, Brinkman, Su, Bevan and Assali (1975b) found that the sympathetic nervous system exerted a tonic influence on arterial pressure in the fetal lamb from 60 days, the alpha component of which increased as gestation proceeded. Alpha-blockade with phenoxybenzamine produced a 10% fall in arterial pressure in 0.3-1.5 kg fetuses, 26% in those of 1.6-2.5 kg and 30% in 2.6-5.8 kg fetuses. Beta blockade with propanolol resulted in a 10% fall in heart rate at all levels of maturity.

The findings of Walker, Cannata, Dowling, Ritchie and Maloney (1978) concerning the sympathetic control of heart rate should ideally be considered alongside those for the parasympathetic influence. During combined beta and muscarinic blockade, Walker et al (1978) found the "intrinsic" heart rate showed little variation from 60 days

until term. The progressive fall in heart rate as gestation proceeds was attributed to increased vagal tone, as atropine produced progressively greater increases in heart rate. The decrease in heart rate elicited by propanolol was less in older lambs, but as there was no gestational change in the response to propanolol following atropine, it was concluded that the sympathetic influence did not change. The possibilty of changes secondary to blood pressure changes is slight as atropine produced only small changes in arterial pressure, and propanolol none.

Jones and Ritchie (1978) found tonic beta-adrenergic stimulation of heart rate, revealed by a 15% fall in heart rate during propanolol infusion at 115-141 days. Alpha-blockade with phentolamine decreased arterial pressure 12% and increased heart rate 5%. In contrast, Rankin and Phernetton (1978) found no significant difference in arterial pressure from controls during alpha-blockade with phenoxybenzamine in 120-130 day fetuses though there was a 16% lower umbilical resistance which was not significant.

4.1.3.3.2 Parasympathetic An increasing parasympathetic influence on heart rate was also found by Vapaavouri et al (1973), atropine causing a smaller increase in heart rate before 100 days. Vagal section however, did not cause any change in heart rate from 120-160 days gestation (Nuwayhid et al, 1975b). The latter group concluded that the parasympathetic system exerted an inhibitory influence on heart rate from about 130 days gestation as atropine had no

effect on heart rate before this stage.

4.1.3.4 Chemical sympathectomy

Chronic chemical sympathectomy with 6-hydroxydopamine did not result in any long-term changes in fetal heart rate or blood pressure, indicating the ability of other regulatory mechanisms to maintain arterial pressure (Tabsh, Nuwayhid, Murad, Ushioda, Erkkola, Brinkman and Assali, 1982).

Roebuck (1982) performed chemical sympathectomy with guanethidine, post-treatment survival was not good, but mean arterial blood pressure and heart rate were unchanged. The stability of the cardiovascular system was impaired by guanethidine treatment, frequent "cardiovascular crises" being seen (Roebuck,1982). These consisted of a fall in heart rate and blood pressure, followed by an increase in pressure and sometimes in heart rate. The basal plasma noradrenaline level was increased 2-3 fold by guanethidine treatment, probably reflecting increased secretion by the adrenal medulla, as plasma noradrenaline was not elevated by guanethidine in adrenal demedullated fetuses. Plasma arginine vasopressin concentrations were also increased following guanethidine treatment, suggesting an increased role of this substance in cardiovascular control in the absence of post-ganglionic sympathetic innervation (Roebuck, 1982).

4.1.4 The adrenal medulla

The release of catecholamines from the adrenal medulla can be elicited by splanchnic nerve stimulation from 125 days gestation in the fetal lamb (Comline and Silver, 1961), the effect increasing towards term. Prior to 125 days the fetal lamb adrenal releases predominantly noradrenaline, more adrenaline being secreted as gestation proceeds. Comline and Silver (1961) also demonstrated the release of noradrenaline in response to asphyxia before 125 days by a local rather than a neurally mediated mechanism. Whether this locally mediated release of noradrenaline can be elicited by other stimuli is uncertain.

Adrenal demedullation with formalin was followed by a fall in plasma adrenaline concentration but plasma noradrenaline and dopamine were unchanged (Roebuck, 1982). Resting arterial pressure and heart rate were also unaffected by this procedure.

4.1.5 Autonomic role in the response to asphyxia and hypoxia

The importance of the adrenergic system in the response to asphyxia produced by cord occlusion was demonstrated by Hyman, Haworth, Bowe, Daniel and James (1972). Combined alpha and beta adrenergic blockade resulted in a fall rather than a rise in arterial pressure during 5 minute cord occlusions. Arterial pressure and heart rate remained depressed on release of the cord, in comparison to the tachycardia and further increase in pressure seen in the controls. The fetuses under

adrenergic blockade died after one or two cord occlusions, the controls survived at least four.

Cohn, Piasecki and Jackson (1978) found that the bradycardia which occurs during fetal hypoxaemia could be abolished by atropine. They also found the increase in stroke volume, which normally allows the maintenance of cardiac output during hypoxaemia, was absent during parasympathetic blockade. In fact, stroke volume was halved and cardiac output fell by 44% during hypoxaemia under these circumstances. The fall in stroke volume is difficult to explain, but may in part be due to an increased afterload caused by increased vascular resistance. The bradycardia during hypoxaemia was even more pronounced and sustained following beta-receptor blockade with propanolol. Stroke volume was unchanged, perhaps indicating a beta-inotropic component in the usual increase, and as a result cardiac output fell. Both groups did not show the slight hypertension which normally accompanies hypoxaemia. It would thus appear that both arms of the autonomic nervous system participate in the fetal cardiovascular response to hypoxaemia (Cohn et al, 1978).

Walker, Cannata, Dowling, Ritchie and Maloney (1979) also found parasympathetic and sympathetic influence on the heart increased during hypoxia, though in this case only the chronotropic response of the heart was studied. Before 120 days the net effect was no change in heart rate; between 120 days and term the parasympathetic influence

predominated and a bradycardia occured. The intrinsic heart rate (during beta and muscarinic blockade) was unchanged during hypoxia.

Lewis, Donovan and Platzker (1980) also found the bradycardia during hypoxia was blocked by atropine, uncovering a tachycardia. The pressor response to hypoxia was found to be an alpha mediated effect (blocked by phentolamine), the bradycardia being unaffected, suggesting it was not mediated by the baroreceptors. Beta blockade with propanolol enhanced the bradycardia.

The role of the alpha-adrenergic system in the redistribution of cardiac output that occurs during hypoxia was demonstrated by Reuss, Parer, Harris and Krueger (1982). As found by others, alpha blockade (with phenoxybenzamine) reversed the pressor response, and in this case the bradycardia was turned to a tachycardia. Alpha blockade during hypoxia resulted in increased cardiac output. Flows to the gut, spleen, liver and lungs, which decrease during hypoxia, allowing redistribution to the brain, heart and adrenals and maintenance of flow to the placenta, were increased by phenoxybenzamine. Carcass blood flow also decreases during hypoxia but this effect was not completely reversed by alpha-blockade. The increases in the vascular resistances of these organs during hypoxia were generally reversed by phenoxybenzamine, as was that of the placenta. Coronary vascular resistance, already decreased during hypoxia, fell further during alpha-blockade.

The neural and local stimulation of adrenal catecholamine release by asphyxia described by Comline and Silver (1961) has already been described. Comline, Silver and Silver (1965) ascertained that it was the hypoxic element of asphyxia that caused both the local and neurally mediated effects.

The relative importance of adrenal medullary release of catecholamines and release from adrenergic nerve terminals in hypoxia was investigated by Roebuck (1982). Adrenal demedullated fetuses showed no pressor response to hypoxia though the bradycardia was still present. Peripheral adrenergic depletion (excluding the adrenal medulla) with guanethidine abolished both the pressor response and the bradycardia. This is particularly striking as increases in plasma catecholamines were still present during hypoxia, and the cardiovascular effects of adrenaline and noradrenaline were increased in adrenal demedullated and guanethidine treated fetuses. A possible explanation is a decreased responsiveness of the heart and peripheral vasculature during hypoxia.

The experiments described in this chapter were designed to investigate the role of alpha-adrenergic vasoconstriction in fetal renoprival hypertension. In addition the effects of alpha-adrenergic blockade on cardiac output and organ blood flows in intact fetuses were investigated as these have not previously been described.

4.2 Methods

The operative, microsphere and recording procedures were as described in chapter 2. Seven fetuses were used for the phentolamine experiments; four were controls and three were hypertensive. All were included in the results described in chapter 3.

After the first microsphere procedure was complete, a priming dose of 1mg phentolamine mesylate (Rogitine, CIBA) was given via the jugular vein catheter. This was followed by an infusion at 110 or 270 $ug.min^{-1}$ so as to maintain a fall in arterial pressure. Thirty to fifty minutes later the second microsphere injection was made.

For all statistical comparisons of variables before and during phentolamine infusion the paired t-test was used. The statistical significance of mean percentage changes was tested for difference from zero using a t-test.

4.3 Results

4.3.1 Progressive arterial pressure changes

Measurements of arterial pressure in individual fetuses at 10 minute intervals before and after the phentolamine priming dose and start of infusion are shown in figure 4.1. For each measurement from 10 to 50 minutes the mean change in pressure is given in table 4.1, the control value for each animal being the mean of the three pre-phentolamine measurements. In the control fetuses the mean fall was about 3 mm Hg at each 10 minute point, though

Figure 4.1

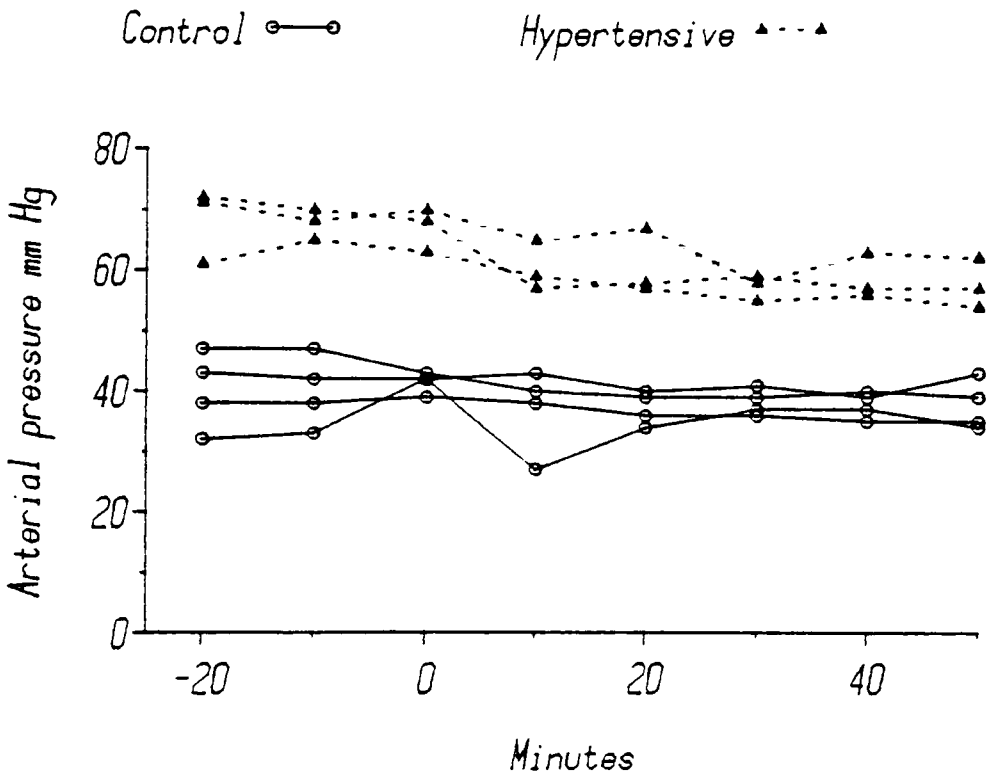


Figure 4.1. Individual measurements of mean arterial pressure at 10 minute intervals, before and during infusion of phentolamine to 4 control and 3 hypertensive fetal lambs.

Time mins	Controls (n=4)	Hypertensives (n=3)
10	-3.5 ± 2.2	-7.2 ± 2.9
20	-3.3 ± 1.2	-5.1 ± 4.3
30	-2.8 ± 1.6	-10.2 ± 1.1*
40	-2.8 ± 1.5	-8.9 ± 2.1*
50	-2.8 ± 1.6	-10.0 ± 1.5*

Table 4.1

Mean (± SE) changes in arterial pressure (in mmHg) at 10 minute intervals after the start of phentolamine administration.

*$p < 0.05$

Paired t-test for each group

none of these was statistically significant. By 30 minutes the fall in the arterial pressure of the hypertensive fetuses had reached statistical significance though all three showed falls at 10 minutes, and in two the initial fall did not develop much further. Over the 30-50 minute period the mean fall was 9-10 mm Hg.

4.3.2 Microsphere experiments

The blood gas values, pH and haematocrit of both control and hypertensive groups were unchanged by the infusion of phentolamine (see table 4.2). The individual changes in arterial pressure, CVO, TPC and heart rate during phentolamine infusion are shown in figure 4.2. Mean values for these and other haemodynamic variables are given in table 4.3.

4.3.2.1 Arterial pressure, CVO and TPC

Mean arterial pressure was unchanged by phentolamine in both groups, though substantial falls were seen in all three hypertensive fetuses, the mean fall being 10.3 mm Hg or -14.5 $\pm$ 3.7% as a percentage change. There were some signs of a slight increase in CVO in the hypertensive group, all three fetuses showing an increase, but the controls showed no change. Though two control fetuses showed substantial increases in total peripheral conductance, overall the change was slight. In the hypertensive fetuses, however, TPC increased by 31.0 $\pm$ 4.0% from 9.36 $\pm$ 1.00 $ml.min^{-1}.mm\ Hg^{-1}.kg^{-1}$ to 12.20 $\pm$ 2.84 (P=0.003). Heart rate was unaffected by phentolamine

	Controls (n=4)			Hypertensives (n=3)		
	Pre-infusion	Phentolamine	Change	Pre-infusion	Phentolamine	Change
PO_2 mmHg	24.9 ± 3.4	23.8 ± 3.1	-1.1 ± 0.7	18.1 ± 1.8	18.6 ± 2.1	+0.5 ± 1.1
$[O_2]$ mM	3.28 ± 0.28	3.06 ± 0.40	-0.21 ± 0.12	1.98 ± 0.14	1.98 ± 0.18	0.0 ± 0.2
PCO_2 mmHg	49.5 ± 5.2	43.8 ± 5.3	-5.7 ± 10.2	47.2 ± 3.3	48.4 ± 2.9	+1.1 ± 0.6
pH	7.29 ± 0.03	7.29 ± 0.04	0.00 ± 0.01	7.31 ± 0.01	7.29 ± 0.02	-0.02 ± 0.01
Haematocrit %	30.5 ± 5.5	29.5 ± 4.5	-1.0 ± 1.0	24.3 ± 2.6	23.8 ± 1.8	-0.5 ± 0.9

Table 4.2

Mean (± SE) carotid arterial blood gas, pH and haematocrit values before and during infusion of phentolamine and mean changes. None of the changes is statistically significant

Figure 4.2

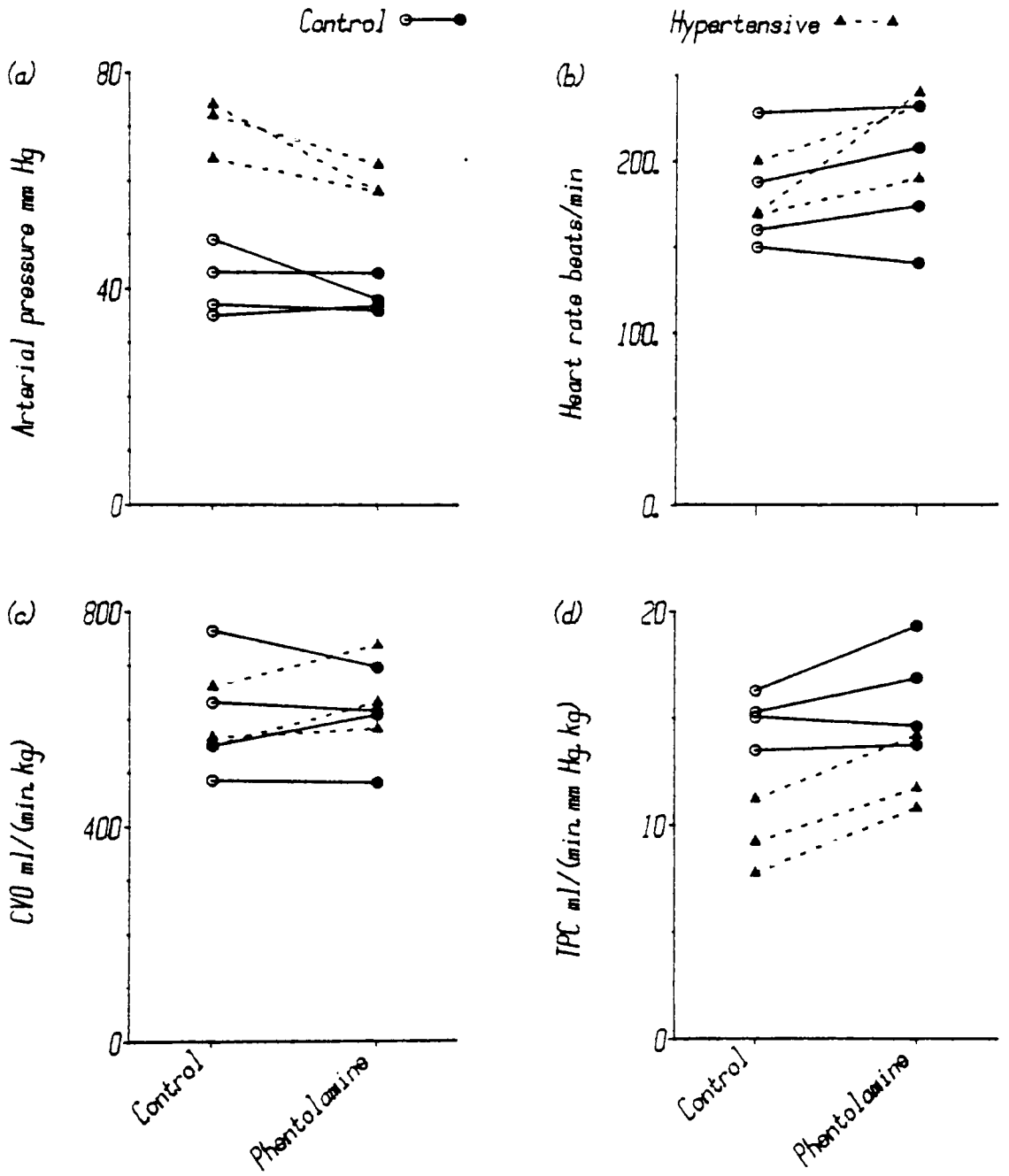


Figure 4.2. Individual changes in (a) arterial pressure, (b) heart rate, (c) combined ventricular output (CVO) and (d) total peripheral conductance (TPC) during infusion of phentolamine to 4 control and 3 hypertensive fetuses.

	Controls (n-4)			Hypertensives (n=3)		
	Pre-infusion	Phentolamine	Change	Pre-infusion	Phentolamine	Change
Arterial pressure mmHg	41.0 ± 3.2	38.5 ± 1.6	-2.5 ± 2.9	70.0 ± 3.1	59.7 ± 1.7	-10.3* ± 3.0
Venous pressure mmHg	0.8 ± 0.6	1.3 ± 0.3	0.5 ± 0.5	6.0 ± 3.2	6.0 ± 1.7	0.0 ± 1.5
Perfusion pressure mmHg	40.3 ± 2.7	37.3 ± 1.6	-3.0 ± 2.7	64.0 ± 4.5	53.3 ± 0.7	-10.7 ± 4.2
Heart rate	182 ± 17	189 ± 20	7 ± 6	179 ± 10	221 ± 16	41 ± 15
CVO $ml.min^{-1}.kg^{-1}$	607 ± 60	599 ± 44	-8.5 ± 26	592 ± 35	649 ± 46	57.8 ± 21.0
TPC $ml.min^{-1}.kg^{-1}$	15.0 ± 0.6	16.1 ± 1.3	1.1 ± 0.8	9.36 ± 1.00	12.2 ± 1.03	2.84** ± 0.17

Table 4.3

Means (± SE) for haemodynamic variables in control and hypertensive fetuses before and during infusion of phentolamine. * $p < 0.05$; ** $p < 0.01$; paired t-test for each group

infusion in the controls whereas the hypertensive fetuses all showed increased heart rate. The average increase of 41 $\pm$ 15 beats.min^{-1} in the hypertensives was not significant.

4.3.2.2 Phentolamine dosages

At the time of the experiments, the rate of phentolamine infusion was adjusted to maintain a fall in arterial pressure, though in retrospect this was not successful in the case of the control fetuses. Three out of the four controls received a dose of 270 μg.min^{-1}, while 110 μg.min^{-1} was given to the fourth. In two of the hypertensive fetuses 110 μg.min^{-1} produced a fall in arterial pressure but the third was given repeated bolus injections of phentolamine of up to 6 mg and an infusion of 530 μg.min^{-1} before a fall was elicited. The infusion was reduced to 266 μg.min^{-1} for 50 minutes before the microsphere injection was given, at which point there was a 6 mm Hg fall in arterial pressure. Doses have not been corrected for body weight as all the above fetuses were in the 2.6-3.8 kg range. These results indicate the hypertensive fetuses were more sensitive to phentolamine.

4.3.2.3 Cotyledonary circulation

The cotyledonary circulation was particularly affected by phentolamine, the individual changes in its share of CVO, flow and conductance are illustrated in figure 4.3. The mean values for these variables are given in table 4.4. The share of CVO received by the cotyledons was increased

Figure 4.3

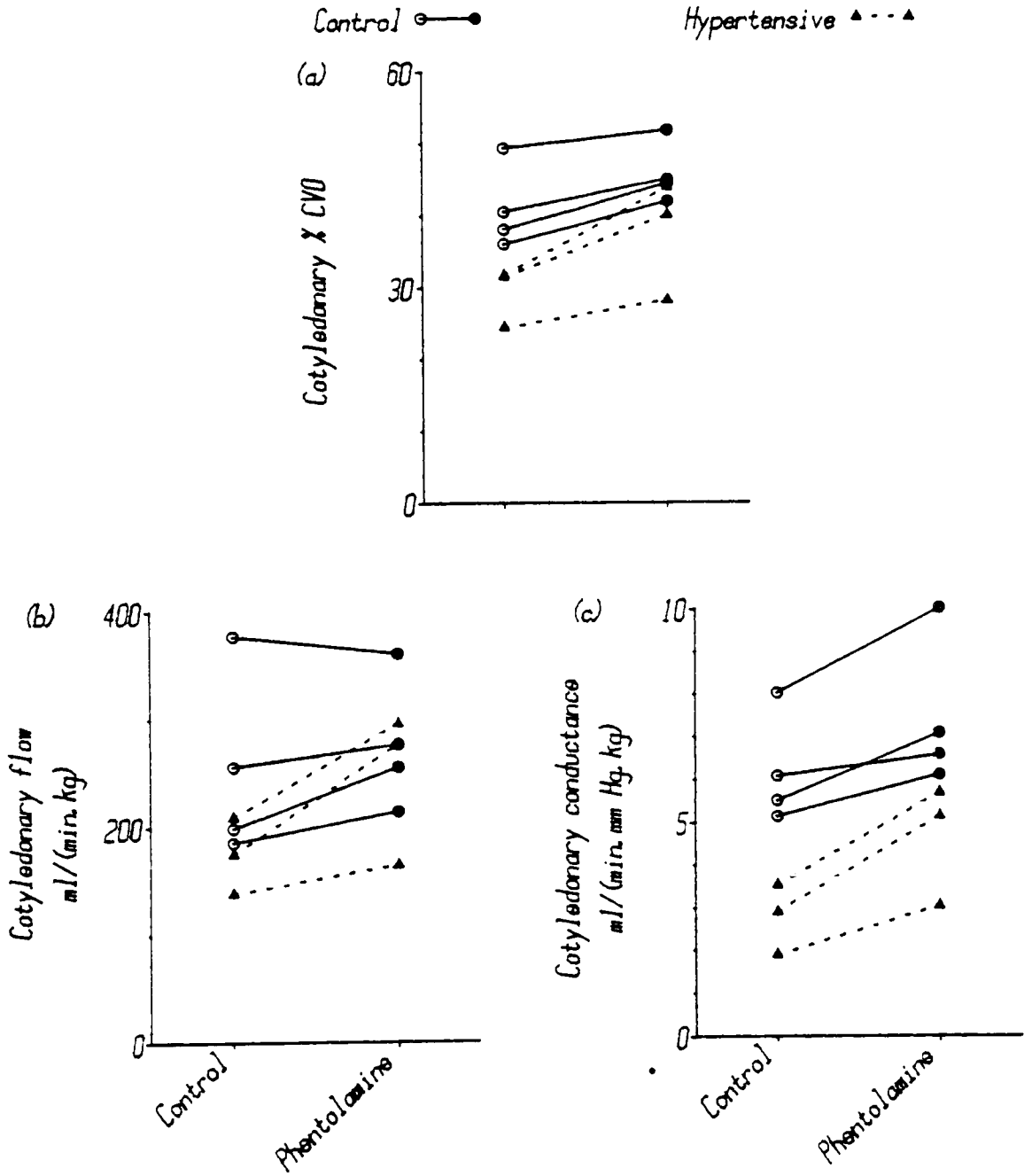


Figure 4.3. Individual changes in (a) the cotyledonary share of combined ventricular output (CVO), (b) blood flow and (c) vascular conductance during infusion of phentolamine to 4 control and 3 hypertensive fetal lambs.

	Controls (n=4)			Hypertensives (n=6)		
	Pre-infusion	Phentolamine	Change	Pre-infusion	Phentolamine	Change
CVO per cent	40.9 ± 2.9	45.8 ± 2.1	4.8 ± 0.9 *	29.2 ± 2.4	37.4 ± 4.7	8.2 ± 2.4
Flow $ml.min^{-1}.kg^{-1}$	254 ± 44	276 ± 31	22 ± 15	173 ± 20	245 ± 41	72 ± 23
Conductance $ml.min^{-1}.kg^{-1}$	6.18 ± 0.64	7.44 ± 0.88	1.26 ± 0.33 *	2.77 ± 0.48	4.61 ± 0.81	1.84 ± 0.35 **

Table 4.4

Mean (± SE) values for placental cotyledonary share of CVO, blood flow and vascular conductance, before and during phentolamine infusion in control and hypertensive fetuses

* $p < 0.05$; ** $p < 0.01$

paired t-test for each group

from a mean of 40.9% to 45.8% in the control fetuses, the mean percentage change being 12.4 ± 2.7% ($P<0.05$). Though the mean change in the hypertensive fetuses was greater, increasing from 29.2 ± 2.4 %CVO to 37.4 ± 4.7 %CVO, it was not statistically significant ($P=0.08$).

Cotyledonary blood flow was not greatly altered by phentolamine in either group, though all three hypertensive fetuses showed slight increases. The conductance of the cotyledons was, however, increased in both groups. In the control fetuses the increase was from 6.18 to 7.44 $ml.min^{-1}.mm\ Hg^{-1}.kg^{-1}$ ($P=0.03$), a mean percentage change of 20.1 ±4.5% ($P<0.05$). A greater increase was seen in the hypertensive fetuses; from 2.77 to 4.61 $ml.min^{-1}.mm\ Hg^{-1}.kg^{-1}$ ($P=0.03$), a 66.0 ± 5.2% increase ($P<0.01$).

An overall picture of the changes in the cotyledonary circulation can be gained from the pressure-flow diagram in figure 4.4; in which lines of equal conductance would radiate from the origin. The conductance increase in the hypertensive fetuses is associated with a fall in perfusion pressure and an increase in blood flow. Perfusion pressure is little changed in the controls, flow increasing with conductance.

4.3.2.4 Distribution of cardiac output

The changes in distribution of cardiac output to the fetal body (except the carcass) and membranes following phentolamine are shown in figure 4.5. In the control fetuses the carcass' share fell from 27.2 ± 1.1 %CVO to

Figure 4.4

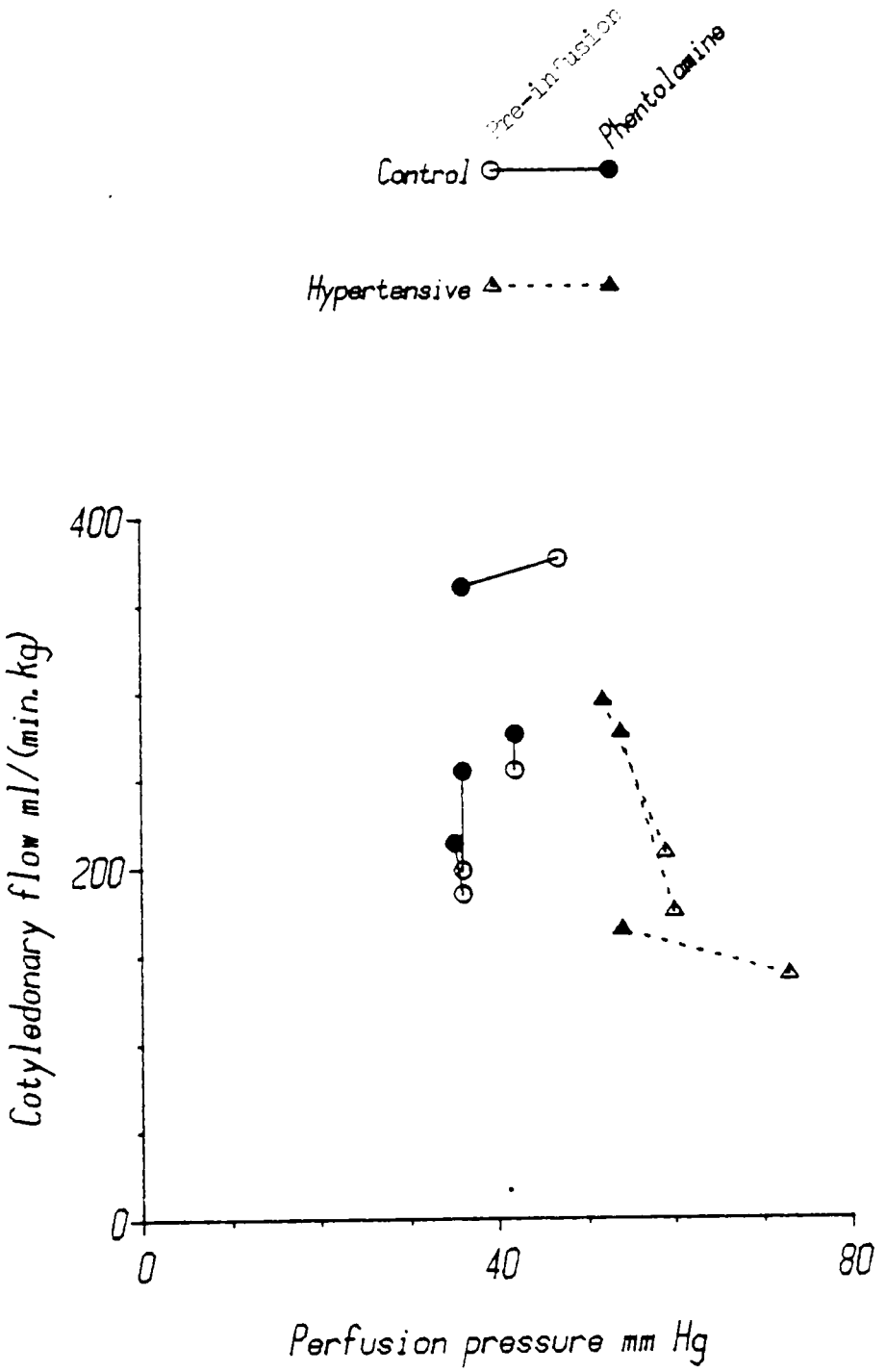


Figure 4.4. Pressure-flow diagram for the placental cotyledons showing the changes during phentolamine infusion (closed symbols; pre-infusion, open symbols) in control and hypertensive fetuses.

Figure 4.5

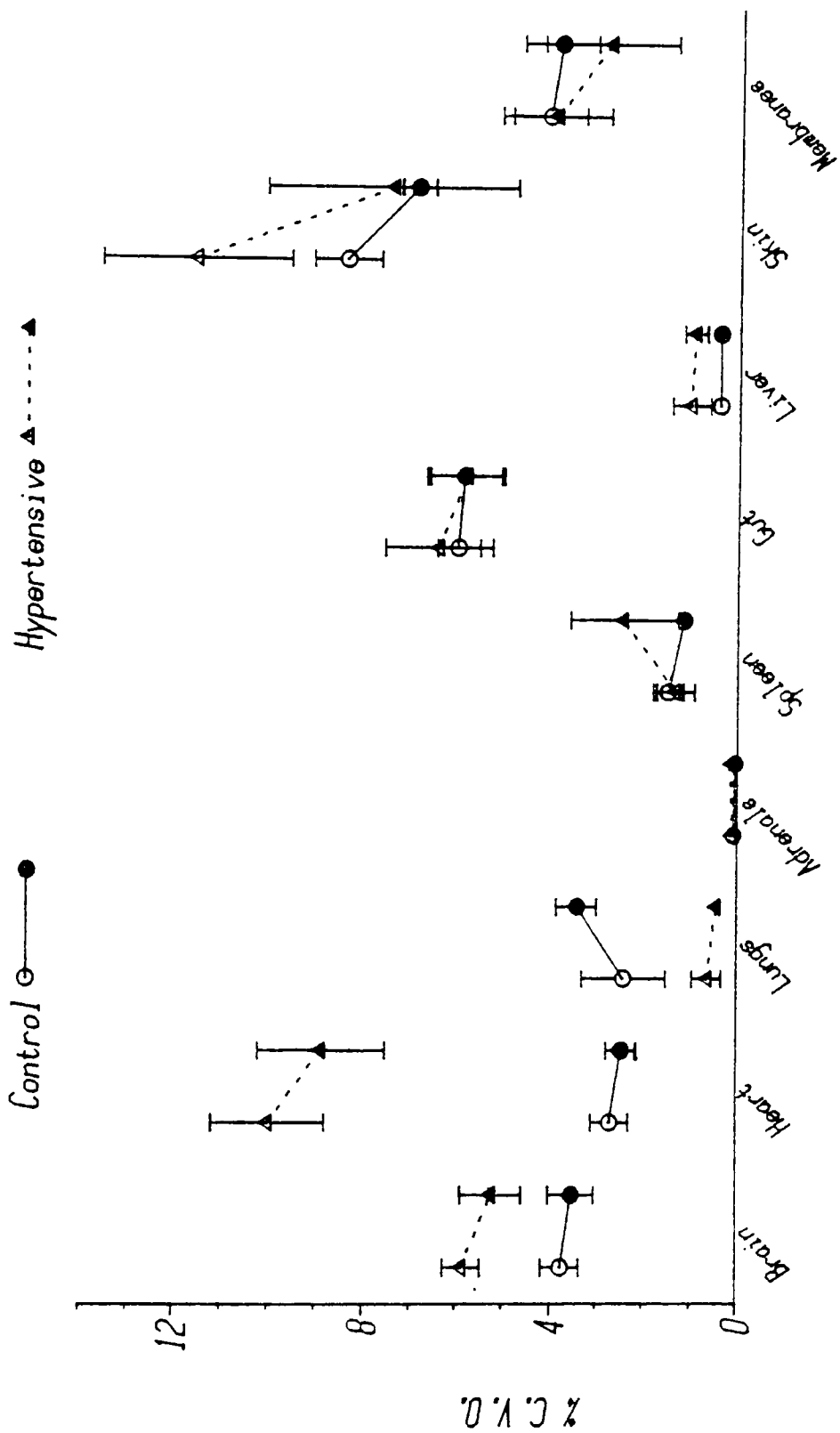


Figure 4.5. Distribution of combined ventricular output (CVO) before (open symbols) and during (closed symbols) infusion of phentolamine to control and hypertensive fetal lambs. All values are means ±SE.

24.4 ± 0.5 %CVO, the mean change being -2.8 ± 1.0 %CVO which just failed to reach statistical significance (P=0.07). There was a fall in the carcass' share in each of the four control fetuses. The change in the hypertensive fetuses was slight, from 29.6 ± 1.5 %CVO to 28.2 ± 1.9 %CVO, (mean change -1.3 ± 3.0 %CVO, not statistically significant).

Of the changes depicted in figure 4.5, only the fall in the share of the CVO flowing to the skin of the control fetuses was statistically significant, a fall from 8.4 ± 0.7% to 6.9 ± 0.4%. The mean fall in the hypertensive fetuses from 11.6 %CVO to 7.5 %CVO (mean change, -4.2 ± 1.6 %CVO) was greater but not statistically significant, though in all three fetuses there was a fall.

The kidneys received 2.1 ± 0.1 %CVO before and 1.83 ± 0.09 % during administration of phentolamine, a mean change of -0.32 ± 0.13 %CVO, but with P=0.09, not significant. All four control fetuses showed falls in the renal share of CVO during phentolamine infusion.

Otherwise, the distribution of cardiac output was little affected by phentolamine in either control or hypertensive fetuses. There was one other slight change in the hypertensive fetuses, the share flowing to the membranes fell from 4.0% to 2.8% (mean change -1.2 ± 0.3 %CVO, P=0.05), all three fetuses showing a fall. There were no signs of a similar change in the control animals.

4.3.2.5 Blood flows

The organ blood flows of control and hypertensive fetuses, before and during phentolamine administration are plotted in figure 4.6, and the mean percentage changes in table 4.5.

There were significant falls in the flows to both the skin and carcass of the control fetuses. The changes were slight, a mean fall in the carcass of 3.1 ± 0.7 $ml.min^{-1}.100g^{-1}$ (P=0.02) from 28.1 to 25.0 $ml.min^{-1}.100g^{-1}$ (mean % change, -10.8 ± 2.1%, P<0.05). The skin blood flow fell from 29.3 to 24.0 $ml.min^{-1}.100g^{-1}$, a mean fall of 5.3 ±0.4 $ml.min^{-1}.100g^{-1}$ or 18.1 ± 0.4% (P<0.001). The individual changes in carcass and skin flow are shown in figures 4.7(a) and (b) respectively. There was no change in the flow to the carcasses of hypertensive fetuses. All three hypertensive fetuses showed falls in cutaneous blood flow but the overall fall of 11.8 ± 5.7 $ml.min^{-1}.100g^{-1}$, from 35.8 to 24.0 $ml.min^{-1}.100g^{-1}$, was not statistically significant. The mean percentage fall in the skin blood flow of the hypertensive fetuses was 32.5 ± 13.6%.

Renal blood flow fell during the administration of phentolamine in all four control fetuses, from 175 ± 16 to 149 ± 17 $ml.min^{-1}.100g^{-1}$ but the mean fall of 25 ± 8.7 (14.9 ± 5.6%) was just outside statistical significance (P=0.06).

Membrane blood flow showed some signs of being decreased by phentolamine in the hypertensive fetuses; it

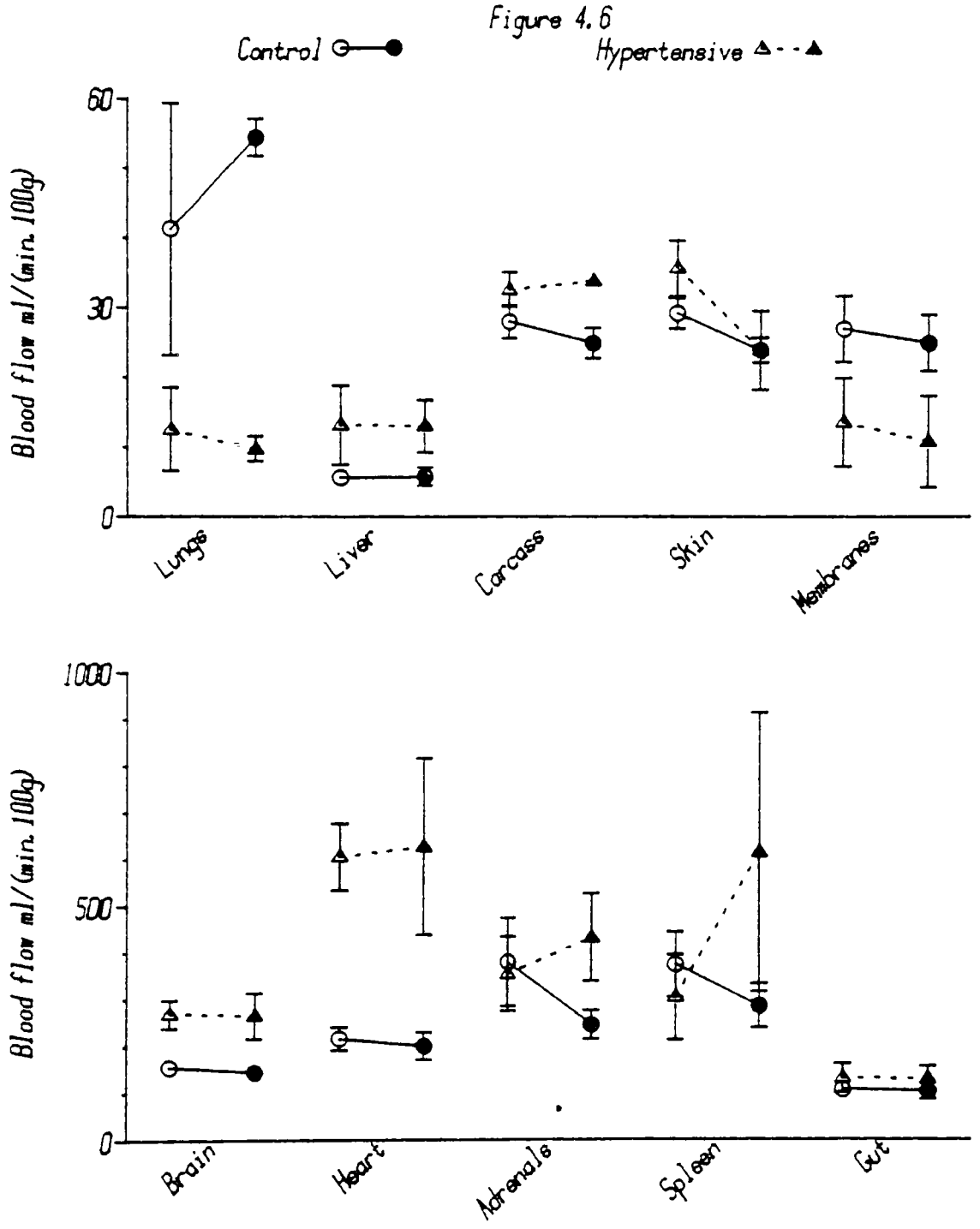


Figure 4.6. Mean (±SE) organ blood flows per unit weight before (open symbols) and during (closed symbols) infusion of phentolamine to control (n=4) and hypertensive (n=3) fetuses.

	Controls (n=4) Mean % change (± SE)	Hypertensives (n=3) Mean % change (± SE)
CVO	-0.6 ± 4.0	9.7 ± 3.6
Brain	-6.5 ± 8.2	-2.5 ± 8.1
Heart	-7.8 ± 7.7	-0.4 ± 18.0
Adrenals	-26 ± 16	24.9 ± 17.9
Spleen	-16 ± 14	156 ± 159
Liver	5.5 ± 25	11.7 ± 24.0
Gut	-2.1 ± 13.4	1.1 ± 6.6
Membranes	-6.6 ± 3.2	-32.1 ± 12.5
Cotyledons	12.0 ± 6.9	39.8 ± 11.6
Carcass	-10.8 ± 2.1*	4.9 ± 7.3
Skin	-18.1 ± 0.4***	-32.5 ± 13.6
Lungs	587 ± 563	8.1 ± 36.2

Table 4.5

Mean (± SE) percentage changes in combined ventricular output (CVO) and organ blood flows during phentolamine infusion

* $p < 0.05$; *** $p < 0.001$

t-test for difference from 0

Figure 4.7

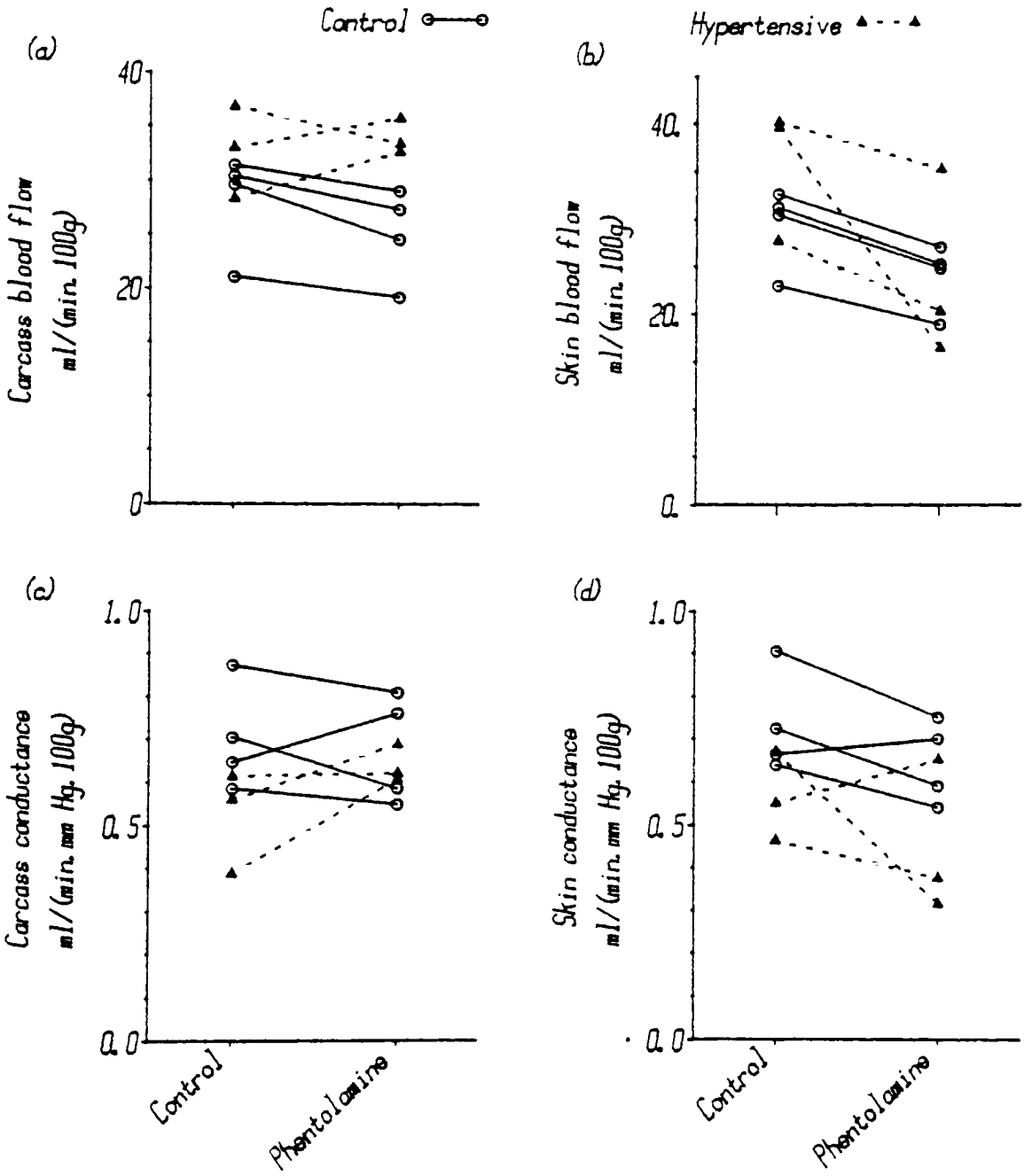


Figure 4.7. Individual changes in (a) carcass and (b) skin blood flows and, (c) and (d), vascular conductances, during phentolamine infusion to control and hypertensive fetuses.

fell in all three but the overall fall of 32.1% was not statisically significant.

There was little change in the blood flows to those organs not mentioned above.

4.3.2.6 Vascular conductances

The changes in mean organ conductances per unit weight are presented graphically in absolute terms in figure 4.8 and as mean percentage changes in table 4.6. The individual changes in carcass and skin conductances are shown in figures 4.7 (c) and (d).

Carcass and skin vascular conductances were unchanged by phentolamine in both control and hypertensive fetuses. It is possible, however, that a slight increase in the carcass conductance of hypertensive fetuses was not uncovered by the present experiments as all three showed an increase, though it was very small in one.

Renal vascular conductance was unchanged, the before phentolamine measurement being 4.33 $\pm$ 0.26 $ml.min^{-1}.mm\ Hg^{-1}.100g^{-1}$ and during the infusion 4.00 $\pm$ 0.45.

Of the remaining organs only the gastointestinal tract of hypertensive fetuses showed any change in conductance.This increased by 20.1 $\pm$ 4.5% ($P<0.05$) from 5.21 to 6.29 $ml.min^{-1}.mm\ Hg^{-1}.100g^{-1}$ (mean 1.07 $\pm$ 0.27 $ml.min^{-1}.mm\ Hg^{-1}.100g^{-1}$, $P=0.03$). The GI vascular conductance of the control fetuses was unchanged.

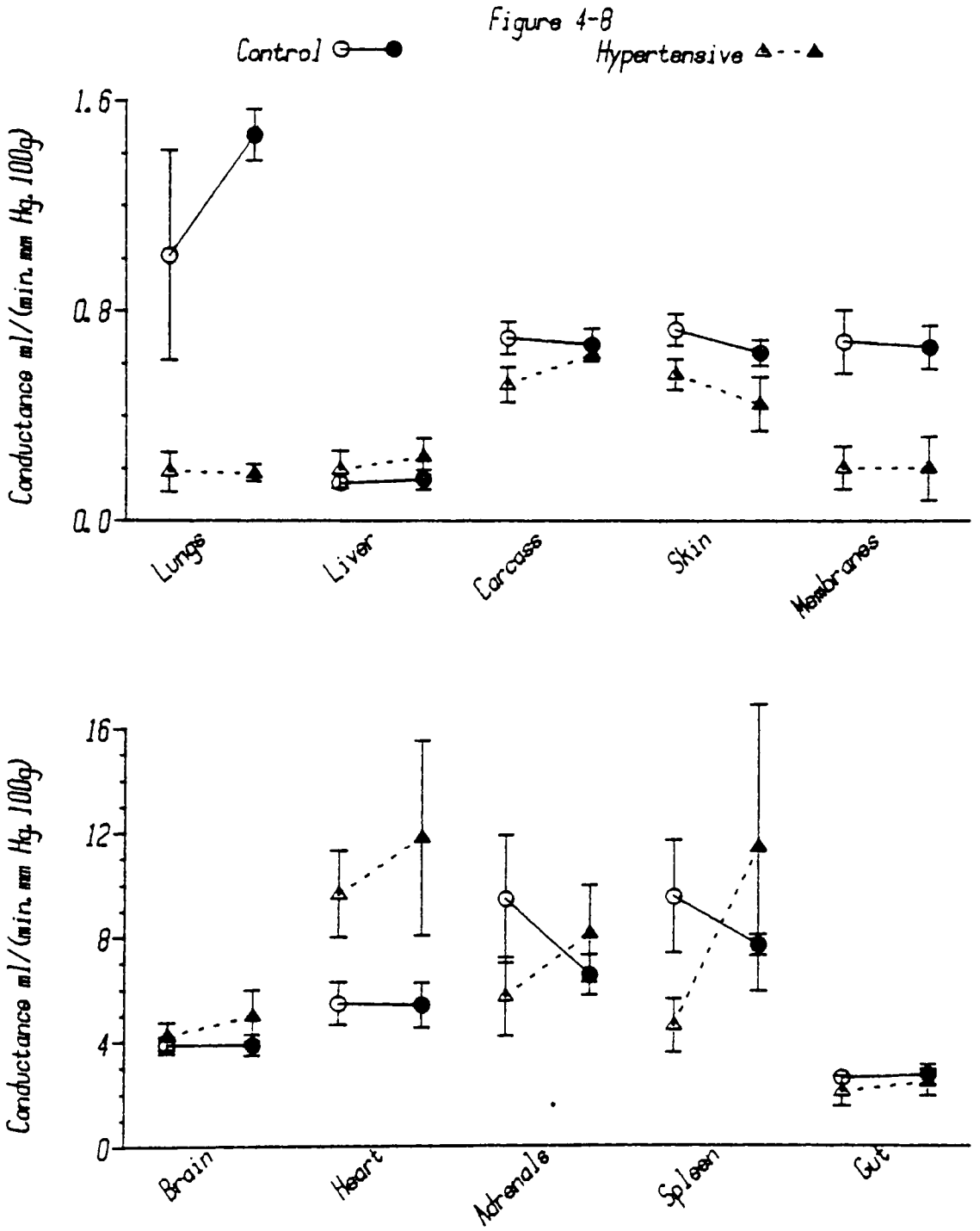


Figure 4.8. Mean (±SE) organ conductances per unit weight before (open symbols) and during (closed symbols) infusion of phentolamine to control (n=4) and hypertensive (n=3) fetuses.

	Controls (n=4) Mean % change (± SE)	Hypertensives (n=3) Mean % change (± SE)
TPC	7.2 ± 4.8	31.0 ± 4.0*
Brain	0.6 ± 7.9	16.5 ± 9.5
Heart	1.2 ± 14.9	18.2 ± 18.6
Adrenals	-21.6 ± 14.3	50.0 ± 22.7
Spleen	-7.2 ± 20.5	192 ± 173
Liver	14.3 ± 26.4	31.3 ± 23.6
Gut	4.5 ± 11.4	20.7 ± 68
Membranes	1.6 ± 9.6	-16.6 ± 21.1
Cotyledons	20.1 ± 4.5*	66.0 ± 5.2**
Carcass	-3.3 ± 7.3	26.7 ± 16.0
Skin	-11.4 ± 5.7	-17.7 ± 20.6
Lungs	593 ± 561	25.9 ± 38.5

Table 4.6

Mean (± SE) percentage changes in total peripheral conductance (TPC) and organ conductances during phentolamine infusion

* $p < 0.05$; ** $p < 0.01$

t-test for difference from 0

4.4 Discussion

4.4.1 Effects of microspheres

In addition to the effects of alpha-adrenergic blockade, the before and after phentolamine comparison will include differences due to the effects of the first microsphere injection. These effects, considered in chapter 2, were generally small and did not affect the whole circulation. Briefly summarizing, blood gas tensions, pH, haematocrit, arterial and venous pressures and heart rate were unaffected. Cardiac output was decreased by a mean of 7% though the effect was greater at higher levels. Coronary blood flow fell by 15%, hepatic arterial by 19%, membrane flow by 14% and cutaneous flow by 11%. Total peripheral conductance may have been slightly decreased. The conductances of the liver (arterial) and heart fell by 17% and 14% respectively.

4.4.2 Phentolamine

Phentolamine is a competitive alpha-adrenergic blocker which also has sympathomimetic, histamine-like and cholinomimetic properties. At low doses it exerts a dilator effect by a direct action on vascular smooth muscle (Gilman, Goodman and Gilman, 1980). There are apparently no central effects. Its effect is relatively short lasting so it was given here as an infusion. It was aimed to use the same dose as that used by Jones and Ritchie (1978) (1mg followed by infusion at 100 $\mu g.min^{-1}$) which produced a 12% fall in arterial pressure and blocked the

pressor effects of adrenaline and noradrenaline infusions. The doses used here worked out in terms of body weight as a priming dose of 270-380 $\mu g.kg^{-1}$ and an infusion of about 30-80 $\mu g.kg^{-1}.min^{-1}$.

4.4.3 Effects in control fetuses

4.4.3.1 Pressure and heart rate

Measurements every 10 minutes following the start of phentolamine treatment showed arterial pressure fell only slightly and insignificantly, the change between the pressures at the time of the two microsphere experiments was also insignificant. This is in contrast with the 12% fall found by Jones and Ritchie (1978), and about 10% (after 100 days) by Vapaavouri et al (1973) following phentolamine or phenoxybenzamine (mainly phentolamine, at 100 $\mu g.kg^{-1}$). Alpha blockade by phenoxybenzamine has been found to produce 5% (Joelsson et al, 1972) or 30% falls (Nuwayhid et al, 1975b) or no change (Rankin and Phernetton, 1978) in arterial pressure. There was also no increase in heart rate found here, in contrast with those found by Jones and Ritchie (1978) and Vapaavouri et al (1973) with phentolamine, and by Joelsson et al (1972) with phenoxybenzamine. Nuwayhid et al (1975b) did not find tachycardia in response to phenoxybenzamine.

The doses used here are comparable with those which have shown effects in other studies, so it is puzzling why no effects were seen here. It is possible that the block was incomplete, though this seems unlikely as Jones and

Ritchie (1978) found that this dose blocked the pressor effects of adrenaline and noradrenaline. The alternative explanation is a low level of adrenergic tone.

4.4.3.2 CVO and TPC

The changes in blood flows and conductances, both of the whole circulation and individual organs, following alpha blockade have not previously been investigated in the fetus.

Cardiac output and total peripheral conductance were unchanged by phentolamine in control animals. It is possible that the effects of the first microsphere injection concealed a small increase in $\dot{C}VO_2$ as this was decreased by 7% at the second injection when two control measurements were compared. This indicates that overall there is little peripheral alpha-adrenergic vessel tone, from either adrenergic nerves or the adrenal medulla. There are circumstances however, under which the alpha-adrenergic influence on vascular resistance becomes substantial. Reuss et al (1982) found that many of the increases in vascular resistance during hypoxia could be reversed by phenoxybenzamine.

4.4.3.3 Cotyledonary circulation

Despite total peripheral conductance being unchanged, the conductance of the placental cotyledons was increased by 20%. This is comparable to a 16% lower umbilical conductance in a group of fetuses treated with phenoxybenzamine than in controls by Rankin and Phernetton

(1978). Their difference was not statistically significant, perhaps because their comparison was made between separate groups rather than as paired serial measurements in individual fetuses.

This implies a reactivity of the umbilical circulation to catecholamines though the evidence for this from other sources is equivocal. Dawes et al (1956) found umbilical blood flow passively followed the pressure changes caused by adrenaline and noradrenaline. Barrett et al (1972) found umbilical vascular conductance was decreased by the alpha-agonist methoxamine. Lorijn and Longo (1980) found umbilical blood flow was increased by 27% during noradrenaline infusion, the associated pressure change is not clear. Novy et al (1974) did not find any change in umbilical vascular resistance following intravenous noradrenaline.

At first such a mechanism would not appear to favour the fetus because of the increases in circulating noradrenaline during hypoxia. However, despite placental vasoconstriction during hypoxia, flow is maintained (Reuss et al, 1982).

As with the change described in chapter 3, it is likely that the change in cotyledonary conductance is due to a vasodilatation in the cotyledons themselves rather than the ductus venosus or liver, as a similar change is not seen in the conductance of the fetal membranes. It is possible that the cotyledonary vasodilatation is a direct effect of phentolamine, though if so, it is surprising that

it did not have a similar effect on the systemic circulation as found in adult man by Taylor, Sutherland, MacKenzie, Staunton and Donald (1965).

4.4.3.4 Other organs

The distribution of cardiac output was not greatly affected, though the shares flowing to the carcass and skin decreased slightly and there were signs of a small decrease in the renal share of CVO.

As expected from these changes, carcass and skin blood flows fell slightly (by 11% and 18% respectively). The decreases in conductance which are implied by the absence of a similar fall in pressure did not occur, or rather, were not statistically significant.

The absence of a decrease in carcass conductance caused by phentolamine is unsurprising considering the comparitively low density of alpha receptors in the vessels of skeletal muscle, which forms the bulk of the carcass. Reuss et al (1982) found that phenoxybenzamine did not lower the increased vascular resistance of the carcass during hypoxia. The absence of an increase in the conductance of the skin is more surprising as cutaneous vasoconstriction is one of the prominent alpha-adrenergic effects in the adult. It could be that the decrease in flow is due to the effects of microsphere blocking of vessels, as skin blood flow was 11% lower for the second of two microsphere injections (see chapter 2).

There are also signs that renal blood flow is

decreased during phentolamine infusion. Again the change is slight and there was no change in conductance.

4.4.4 Effects in hypertensive fetuses

The hypertensive fetuses were more sensitive to phentolamine than the controls. Generally, the dose they received was lower and the effects elicited greater.

4.4.4.1 Pressure, heart rate, CVO and TPC

Arterial pressure was decreased by a mean of 14% in the hypertensive fetuses, a fall comparable to that found in control fetuses by other investigators, but greater than that in the control fetuses in this study. Heart rate was increased by phentolamine, a change also observed by others in control fetuses. Cardiac output was increased slightly in all three fetuses, an effect also seen following the administation of phenoxybenxamine to hypoxic fetal lambs (Reuss et al, 1982). Das and Parratt (1971) concluded from experiments in adult cats that phentolamine increased heart rate and myocardial contractility by direct beta-adrenergic effects. Again, why such effects should have been absent in the control fetuses is uncertain.

Total peripheral conductance was increased by 31%, and so the increase in CVO may be due to this rather than myocardial effects.

4.4.4.2 Cotyledonary circulation

The effects of phentolamine on the cotyledonary circulation were similar to those seen in control fetuses

but greater in magnitude. The share of CVO flowing to the cotyledons was increased, and all three hypertensive fetuses showed increases in cotyledonary flow, though the change was not significant. The overall increase in cotyledonary conductance was 66%, though this was not enough to bring it up to control levels. This would seem to indicate substantial adrenergic vasoconstrictor tone in the cotyledons of hypertensive fetuses, but the reservations concerning the reactivity of the cotyledonary vessels to catecholamines, expressed in discussing the effects in control fetuses also apply here. A change induced in the hypertensive fetuses, not seen in the controls was the fall in the share of CVO received by the fetal membranes. Membrane blood flow also showed signs of being decreased by about 30%, though conductance was unchanged. Membrane blood flow was one of the variables affected by the injection of microspheres and this may partly explain the change described here.

4.4.4.3 Other organs

Decreases in the share of cardiac output flowing to the skin were seen in all three hypertensive fetuses, as in the controls, but again the mean change was not significant nor was that for the skin blood flow. Again, blocking of blood vessels by microspheres may be responsible for this decrease.

In contrast to the control fetuses, the hypertensives showed no fall in carcass blood flow during alpha-blockade, and their conductance may have increased slightly, perhaps

indicating some alpha tone in the carcass.

The gastrointestinal tract of the hypertensive fetuses showed an increase in vascular conductance though there was no change in blood flow. In the adult the gastointestinal circulation is one of those particularly affected by alpha-adrenergic vasoconstriction; and Reuss et al (1982) found decreased resistance in the gut when an alpha block was made in hypoxic fetuses.

4.4.5 Contribution of hypoxaemia

The hypertensive fetuses used in this part of the study exhibited a similar degree of hypoxaemia to the whole group described in chapter 3. The effects of phentolamine on the hypertensive fetuses bears some similarities to the effects of alpha-blockade with phenoxybenzamine in hypoxic fetuses described by Reuss et al (1982). It could be that the increased sympathetic influence in the hypertensive fetuses is part of the response to hypoxia rather than a more direct result of nephrectomy.

The similarities of the effects on arterial pressure, cardiac output and peripheral conductance have already been picked out. The decrease in resistance of the placenta found by Reuss et al (1982) was 24%, considerably smaller than the 66% fall found here. There are some changes seen by Reuss et al, not seen here, namely decreased resistances in the spleen, hepatic arterial and pulmonary circulations. Coronary resistance, already decreased by hypoxia, was further decreased by phenoxybenzamine (Reuss et al, 1982),

no change in coronary vascular conductance was seen in the hypertensive fetuses during phentolamine infusion.

4.4.6 Alpha-adrenergic contribution to the vasoconstriction

It would appear that alpha-receptor mediated vasoconstriction plays a small part in the hypertension and in the decreased conductance associated with it. However, this is probably secondary to the hypoxaemia present in the hypertensive fetuses.

Though releasing some of the vasoconstriction in the cotyledons, that in the carcass was hardly affected, indicating the action of some other vasoconstricting influence. Referring back to chapter 3, vasopressin is a possible candidate as is vessel wall thickening.

CHAPTER 5

Changes in vascular volumes in fetal renoprival hypertension

5.1 Introduction

The limited ability of the fetal heart to increase cardiac output in response to increased blood volume was discussed in chapter 3. Nevertheless, increased blood volume does cause increased arterial blood pressure by a combination of slightly increased cardiac output and of right atrial pressure. Faber, Green and Thornburg (1974) varied blood volume by infusion and bleeding and found it was closely related to arterial pressure, even during increases in blood volume. Gilbert (1980) observed an 11% increase and an 8% decrease in blood pressure with increases and decreases in blood volume of about 10%.

It seems likely from the fall in haematocrit, the apparent oedema and increased central venous pressure in the hypertensive fetuses, that plasma volume and blood volume are increased and this may be an important part of the pathogenesis of the hypertension. It was therefore decided to measure plasma volume in the hypertensive fetuses. Total body water was also measured in three fetuses.

5.1.1 Choice of method

Several techniques have been used for measuring fetal plasma and red cell volumes. If only one of these

variables is measured, the other, and blood volume, can be calculated if the haematocrit is known, though ideally a correction should be made for the amount of plasma trapped between red cells when the latter is measured. Differences between central and peripheral haematocrits also affect the calculation.

Methods for measurement of fetal plasma or red cell volumes suffer from similar weaknesses to those used in adults, and there is the additional problem of the placenta, from which no indicator must be lost. Evans blue dye (Prystowsky, Hellegers, Meschia, Metcalfe, Huckabee and Barron, 1960; Caton, Wilcox, Abrams and Barron, 1975) and radiolabelled albumin (Creasy, Drost, Green and Morris, 1970) have been used to label plasma, and ^{51}Cr labelled red cells to measure red cell volume (Creasy et al, 1970; Broughton Pipkin and Kirkpatrick, 1973). As plasma volume was the volume of interest, and because inaccuracies are introduced in calculating it from red cell volume and haematocrit, it was decided to measure it directly. The Evans blue method is technically more simple and appears to give accurate results (Caton et al, 1975) so this method was used.

5.2 Methods

5.2.1 Measurement of vascular volumes

Plasma volume was measured in four control fetuses and two nephrectomized fetuses, both of which developed hypertension. The measurement of plasma volume was made

before the first microsphere injection, except in one control fetus, in which it was made after the second microsphere procedure was complete.

A 4 ml blood sample was taken from a carotid or femoral artery for a plasma blank and standards, haematocrit was measured on this sample for the calculation of red cell and blood volumes. A 2 ml syringe was filled with 0.5-1.0 ml of Evans blue (Harvey laboratories, Philadelphia) and weighed before and after emptying into a 250 ml volumetric flask. The syringe was refilled with 1-1.3 ml of Evans blue which was injected into the fetus via the inferior vena caval catheter, and flushed in with 3-4 ml of heparinised saline. The weight of Evans blue injected into the fetus was obtained as for that in the standard. Blood samples (1 ml) were taken from the same catheter as the blank sample at 10, 20 and 40 minutes after injection of the indicator.

All blood samples were centrifuged at 2,000 rpm for 20 minutes and the plasma pipetted off. To 0.5 ml of each plasma sample (including the blank) was added 3.5 ml distilled water. Standards were prepared using 0.5 ml aliquots of blank plasma and known volumes of the standard solution, the total sample volume being made up to 4 ml with distilled water. The absorbance of all samples and standards was read at a wavelength of 620 um in a Pye Unicam SP600 spectrophotometer. The extrapolation of the indicator concentration to zero time is considered in the results section. Two to four standards were obtained for

each fetus, the volumes of Evans blue solution being chosen empirically so the zero-time absorbance was included within the range of the standard curve, or lay only just outside it. The relationship between absorbance and the concentration of Evans blue was linear, the line of best fit was calculated by linear regression. The zero-time plasma concentration (C_o) of Evans blue was calculated from the regression line and the plasma volume (V_p) obtained using the following equation.

$$V_p = \frac{W_i}{C_o} \qquad (5.1)$$

where W_i= weight of Evans blue injected. Blood volume (V_b) and red cell volume (V_r) were calculated from the plasma volume and haematocrit (h) using the following equations.

$$V_b = \frac{V_p}{1-(h/100)} \qquad (5.2)$$

$$V_r = \frac{V_p\ h}{1-h} \qquad (5.3)$$

5.2.2 Total water content

The total water contents of two control fetuses and one hypertensive fetus and their placentas (cotyledons and membranes) were obtained by drying to constant weight at 80°C. Weighed aliquots of the minced larger organs were dried, rather than the whole organs.

5.3 Results

5.3.1 Evans blue clearance curves

Semi-logarithmic clearance curves of absorption against time are shown in figure 5.1; one curve from a hypertensive fetus and one from a control fetus, both being representative of their groups. In the four control fetuses the three points did not lie on a straight line, the 10 minute point lying above a line drawn between the 20 and 40 minute points as in figure 5.1(a). However, for both hypertensive fetuses, all three points lay on a straight line (see fig. 5.1(b)). It would appear that mixing is incomplete by 10 minutes in the control fetuses, therefore a line drawn between the 20 and 40 minute points has been extrapolated to zero-time to obtain the initial value for absorption. The half-times for the clearance of Evans blue were slightly lower in the two hypertensive fetuses (79 and 97 minutes, mean 88 minutes) than in the four controls (114, 103, 108 and 209 minutes, mean ($\pm$SE) 134 $\pm$ 25).

5.3.2 Plasma volume

The absolute and relative plasma, red cell and blood volumes for the six fetuses are given in table 5.1, and the absolute volumes plotted against body weight in figure 5.2. Statistical tests are inappropriate as the hypertensive group consists of only two fetuses.

Plasma volume expressed in terms of body weight was similar in the hypertensive and control groups, the mean

Figure 5.1

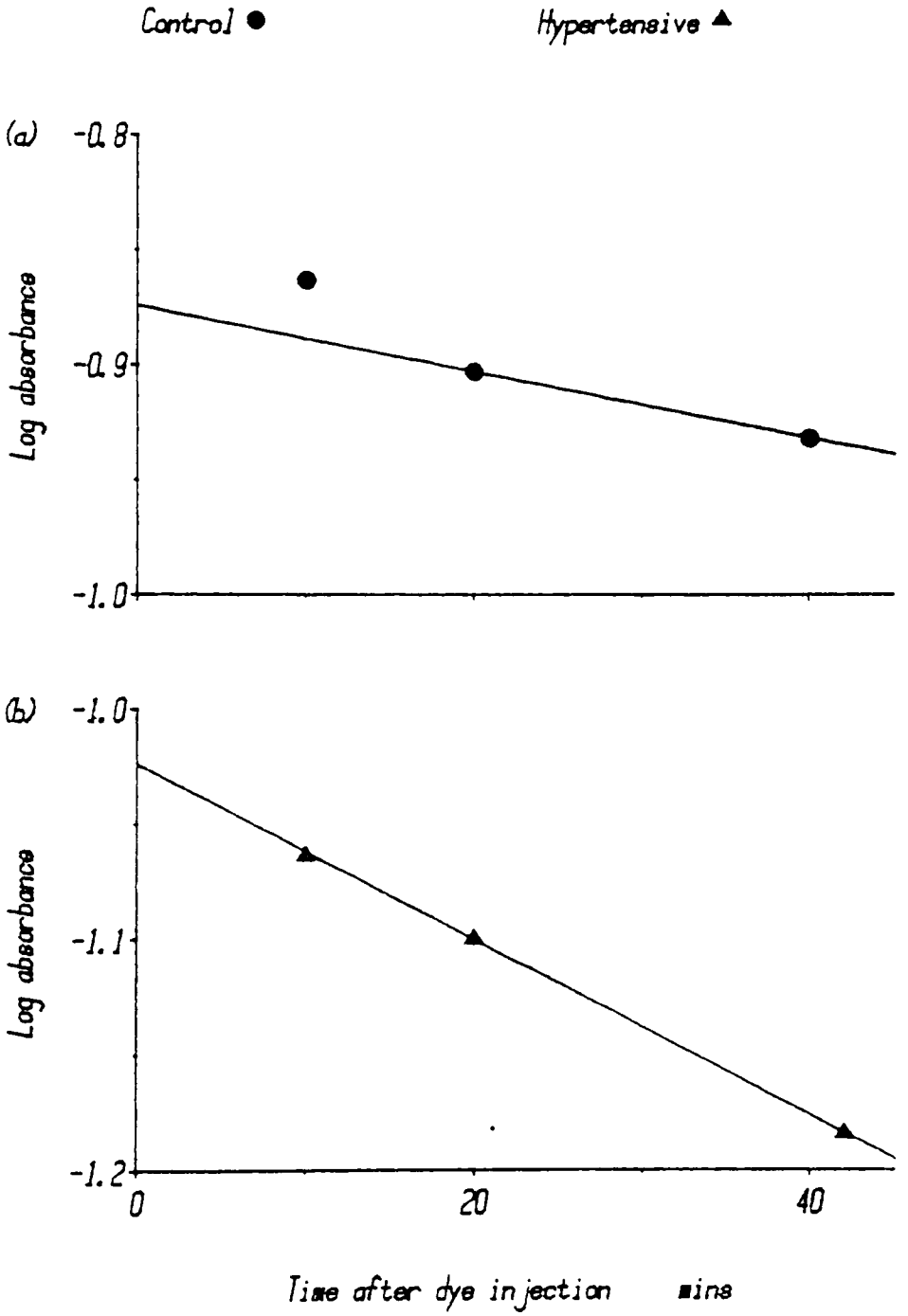


Figure 5.1. Clearance of Evans blue from the plasma of (a) a control fetus and (b) a hypertensive fetus. The regression lines are fitted to the 20 and 40 minute points.

a) Plasma, red cell and blood volumes per kg body weight

Animal Controls	Plasma $_{-1}$ ml.kg	Red cells ml.kg^{-1}	Blood $_{-1}$ ml.kg
164	136	67	203
111	122	60	182
182	136	77	200
42	150	60	210
Hypertensives			
99	123	37	160
301	134	57	191

b) Absolute plasma, red cell and blood volumes

Animal Controls	Body weight kg	Plasma ml	Red cells ml	Blood ml
164	3.43	468	231	699
111	3.15	384	189	573
182	2.61	355	213	555
42	3.46	519	207	726
Hypertensives				
99	3.73	457	137	638
301	3.58	481	206	641

Table 5.1

Relative and absolute vascular volumes
in control and hypertensive fetuses

Figure 5.2

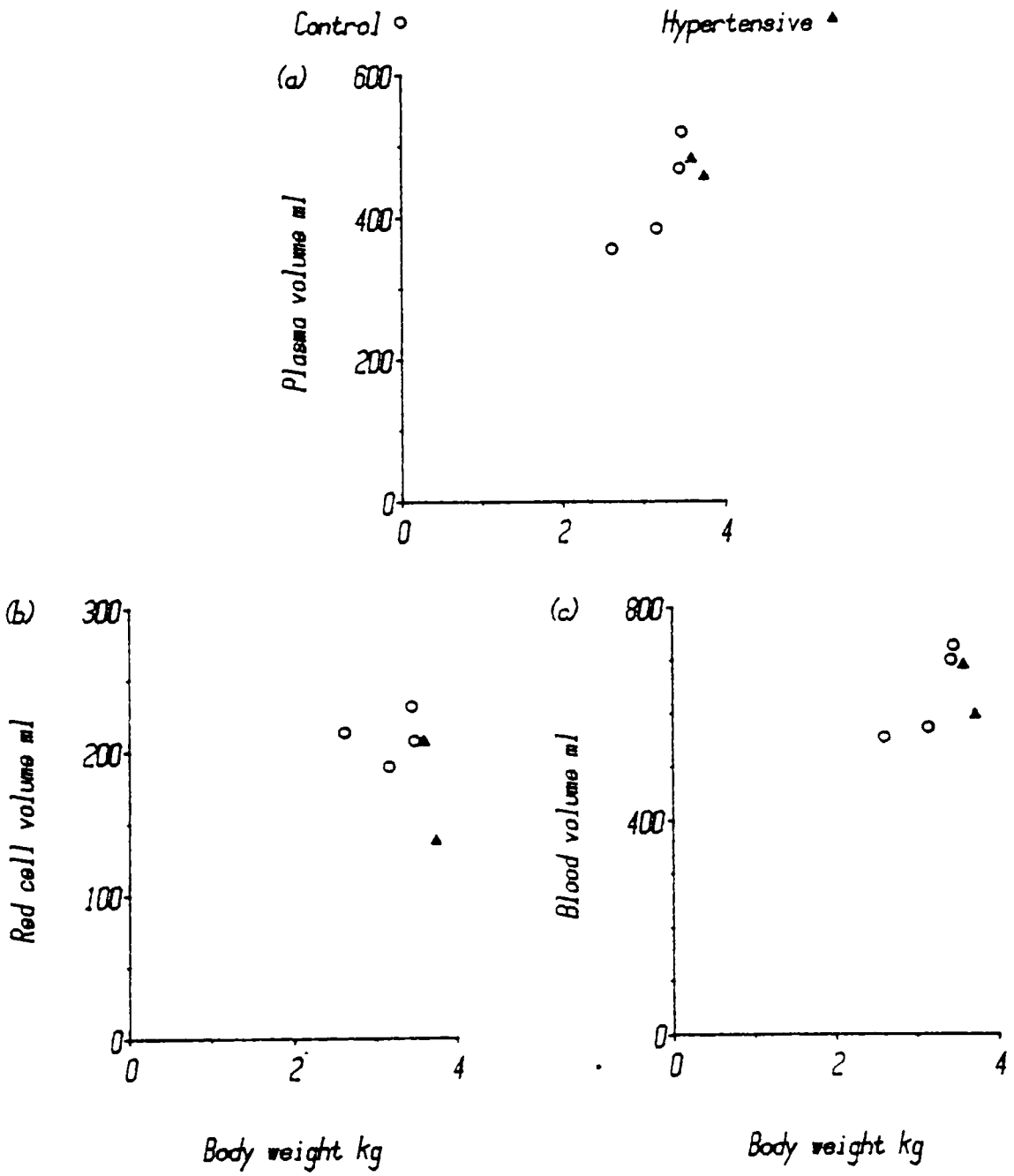


Figure 5.2. Vascular volumes plotted against body weight for individual control and hypertensive fetuses. (a)plasma volume, (b) red cell volume and (c) blood volume.

(±SE) for the control group being 136 ± 6 $ml.kg^{-1}$, and 129 $ml.kg^{-1}$ for the hypertensives. Both hypertensive fetuses were heavier than any of the controls, so absolute plasma volume was slightly higher, the mean being 469 ml compared with 432 ± 38 ml in the controls.

5.3.3 Red cell volume

Red cell volume was lower in relative terms in the hypertensive group, fetus 99 having a particularly low red cell volume, and fetus 301 being at the lower end of the control range, the mean was 47 $ml.kg^{-1}$ compared with 66 ± 4 $ml.kg^{-1}$ in the controls, though that of 99 was low. The mean absolute red cell volume was 210 ± 9 ml for the controls, and 172 ml for the hypertensives.

5.3.4 Blood volume

Absolute blood volume was comparable in control and hypertensive fetuses despite the low red cell volume of the latter. The means were 638 ± 43 ml for the controls and 641 ml for the hypertensives. In terms of body weight, blood volume was lower in the two hypertensive fetuses (mean 176 $ml.kg^{-1}$) than in the controls (199 ± 6 $ml.kg^{-1}$).

The plots in figure 5.2 illustrate these relationships graphically, and figures 5.2 (b) and (c) demonstrate well that while fetus 301 is comparable to the controls, fetus 99 lies apart from this grouping.

Both the hypertensive fetuses were characteristic of their groups; despite its lower blood volume, fetus 99 had

a mean arterial pressure of 74 mm Hg, and fetus 301 of 72 mm Hg. No relationship between blood volume and arterial pressure or cardiac output was apparent in either controls or hypertensives, but the range of blood volume spanned was small.

5.3.5 Total water content

The dry and wet weights and the percentage dry weight for fetuses 182, 42 and 301 are given in table 5.2. Fetuses 182 and 301 were at 127 and 128 days gestation and fetus 42 was at 132 days.

The one hypertensive fetus (42) had a lower dry body weight as a percentage of wet weight (14.4%) compared with either control fetus (18.1% and 15.1%). The difference in percentage dry weight of the placenta was even more marked; hypertensive, 5.2%; controls 8.1% and 11.7%. This reflects the increased wet weight of the membranes in particular, as in the hypertensive fetus their dry weight was 3.4% of their wet weight, and the cotyledonary percentage dry weight was 8.6%.

5.4 Discussion

5.4.1 Measurement of vascular volumes

Because indicator is lost from the circulation before mixing is complete, it is necessary to extrapolate its clearance curve to the time of injection (zero-time) to calculate its volume of distribution. The conventional

Fetus	Body weight			Placental weight		
	Wet g	Dry g	% Dry	Wet g	Dry g	% Dry
Controls						
182	2610	395	15.1	571	46.4	8.1
42	3460	627	18.1	714	83.6	11.7
Hypertensive						
301	3580	517	14.4	1908	100	5.2

Table 5.2

Wet and dry, body and placental (membranes and cotyledons) weights and percentage dry weights in control and hypertensive fetuses

way of doing this is to extrapolate the curve which sets in once the initial phase of rapidly falling indicator concentration, which supposedly corresponds to mixing, has ended.

As found by Prystowsky et al (1960), mixing did not appear to be complete 10 minutes after the injection of dye into the control fetuses, and so a line between the 20 and 40 minute points was extrapolated to obtain the zero-time concentration. This corresponded well with the concentration at 10 minutes, a coincidental relationship but useful for obtaining the zero-time concentration from a single sample, as performed by Prystowsky et al (1960).

In the two hypertensive fetuses, however, the 10, 20 and 40 minute points all lay on the same straight line, which suggests mixing occurred more quickly in these fetuses. Dye was also cleared more quickly from the plasma in these fetuses, though this should not greatly affect the extrapolation to zero-time. This faster clearance could be due to the higher arterial pressure and increased capillary filtration of plasma proteins. The congruence of the 10 minute concentration and the zero-time concentration obtained by extrapolation breaks down in the hypertensive fetuses. It would be inappropriate in these circumstances to use the 10 minute concentration to calculate plasma volume.

Plasma volume was similarly and closely related to body weight in both control and hypertensive fetuses. The values of around 130 $ml.kg^{-1}$ are high compared with other

measurements made with Evans blue (Caton et al, 1975; 97 ± 8 $ml.kg^{-1}$) and radio labelled albumin (89.9 ± 2.0 $ml.kg^{-1}$; Creasy et al, 1970). There is no apparent technical difference which would explain this high plasma volume. Caton et al (1975) used the 10 minute concentration, and Creasy et al (1970) extrapolated the logarithm of 15 and 30 minute concentrations to obtain the zero-time concentration. Evans blue binds to albumin in the plasma, and should therefore label the same compartment as radio-labelled albumin. Albumin is filtered by the capillaries and so will eventually be distributed through the extracellular space. However, this does not occur instantaneously, and this route of clearance from the vascular space should be accounted for in the extrapolation to the time of injection.

5.4.1.2 Red cell and blood volume

Errors in the measurement of plasma volume will be reflected in the calculated red cell and blood volumes. In addition, overestimation of these two volumes will arise from two factors affecting the haematocrit. Firstly, some plasma will be trapped between red cells during centrifugation, leading to a slightly high value for the haematocrit. Secondly, where both plasma and red cell volumes have been measured it has been found that the haematocrit calculated from these volumes (mean haematocrit) is consistently less than that measured in samples taken from central blood vessels (central haematocrit). The error arising from the trapped plasma

is probably quite small; Owen and Power (1953) found that about 4% of the red cell column was trapped plasma. Over the 20-40% haematocrit range this would lead to a 1-3% overestimation of blood volume and a 5-6% overestimation of red cell volume. The error arising from the uneven distribution of cells and plasma is quantitatively more serious.

The ratio of the mean haematocrit to the central haematocrit (termed F_{cells}) is 0.80 in newborn lambs (Longo, Allen, Niswonger, Pagel, Wieland and Gilbert, 1978); and 0.83 in fetal lambs (calculated from the data of Creasy et al, 1970). At a measured haematocrit of 33% (control level) this would lead to an 8% overestimation of blood volume and a 31% overestimation of red cell volume. At the lower haematocrit in the hypertensive fetuses (about 25%) the overestimation will be slightly less, about 6% and 27% for blood and red cell volumes respectively. This assumes that the relationship between central and mean haematocrits, described by F_{cells}, remains the same in the hypertensive fetuses. F_{cells} was constant over a wide range of central haematocrits (14-56%) in patients with haemorrhage, anaemia or polycythaemia (Verel, 1954); and over the range 18-66% in human neonates (including some with haemolytic disease of the newborn) (Mollison, Veall and Cutbush, 1950). The range of central haematocrit spanned by the data of Creasy et al (1970) in the fetus was only 30-56%, but despite F_{cells} being quite variable, no relationship with haematocrit was apparent.

As would be expected, the calculated red cell volume was high (66 $\pm$ 4 ml.kg^{-1}) compared with those found by Creasy et al (1970) (45 ml.kg^{-1}) and Broughton Pipkin and Kirkpatrick (1973) (49 ml.kg^{-1}) both with ^{51}Cr labelled red cells. The lower red cell volume in terms of body weight of the hypertensive fetuses would be expected if there were an expansion of plasma and extracellular fluid volumes. According to this hypothesis, absolute red cell volume should be similar; though that of one hypertensive fetus was comparable with the controls, that of the other was low. This fetus (no 99) did show signs of slight haemmorhage in the renal fat, and this may explain the low red cell volume. As mentioned in chapter 3, nephrectomy does not appear to reduce erythropoiesis.

Blood volume was also high in the control fetuses (199 $\pm$ 6 ml.kg^{-1}) compared with that found by Creasy et al (1970), again to be expected from the high plasma volumes and error due to F_{cells}. The slightly lower blood volume found by Broughton Pipkin and Kirkpatrick (1973) may be due to the reverse effect of F_{cells} as blood volume was calculated from red cell volume and haematocrit.

The lower blood volume per kg in the hypertensive fetuses is largely due to fetus 99. Even if the red cell volume were lower because of haemorrhage, maintenance of blood volume would be expected.

5.4.2 Changes associated with hypertension

As measurements of plasma volume were only made in two

hypertensive fetuses, the conclusions on the changes during hypertension are tenuous. If the low red cell and blood volumes in fetus 99 can be explained by haemmorhage, then it might be concluded that blood volume is maintained in terms of weight in the hypertensive fetuses, and slightly increased in absolute terms. This could be assigned to an increased absolute plasma volume again in parity with the weight increase. Red cell volume would be maintained in absolute terms, but be lower when normalised for body weight.

There is no reason to suppose increased growth in the hypertensive fetuses (indeed nephrectomy at 84-88 days results in growth retardation, Thorburn, 1974), in which case the increase in body weight would be due to increased interstitial fluid volume. The apparent oedema seen in fetuses with renoprival hypertension (Mott, 1980), including those described here, provides support for this hypothesis. If the volume changes measured here are to be explained by such a mechanism, then there must be a greater proportional increase in interstitial fluid volume than in plasma volume. Guyton (1980, chapter 10) presents a relationship based on anecdotal data which suggests that as extracellular fluid volume is increased, a maximum blood volume is quickly reached, after which further increases are accomodated virtually entirely in the interstitial fluid space.

Experiments in dogs by Guyton (1965) suggest that the compliance of the interstitial space reaches a point at

which it increases dramatically; large volumes of fluid can be taken up by the space with only small increases in interstitial fluid pressure and oedema results.

Capillary filtration is determined by the following relationship

$$F = K_f(P_c + \pi_i - \pi_c - P_i) \qquad (5.4)$$

where F= net fluid movement, K_f= capillary filtration coefficient, P_c and P_i are the capillary and interstitial fluid hydrostatic pressures and π_c and π_i the capillary and interstitial fluid colloid osmotic pressures. Capillary hydrostatic pressure is dependent on arterial and venous pressures and resistances (P_a, P_v, r_a and r_v respectively) as follows

$$P_c = \frac{P_a\, r_v + P_v\, r_a}{r_a + r_v} \qquad (5.5)$$

An increase in arterial pressure, especially if due solely to increased arterial resistance, will cause only a slight increase in capillary pressure and hence in capillary filtration. Increases in venous pressure have substantially greater effects on capillary pressure and filtration as may be appreciated from the above relationship, arterial resistance being substantially higher than venous resistance.

As stated above, only small increases in capillary pressure will result in the exudation of large volumes of

fluid from the capillaries once a certain level of vascular filling has been reached. Fetal vascular filling would appear to be greater than in the adult; even though some of the high fetal blood volume (135 $ml.kg^{-1}$, Creasy et al, 1970) can be attributed to extra blood contained in the placenta, it remains high (104 $ml.kg^{-1}$) following tying of the umbilical cord (Creasy et al, 1970). However, if the degree of vascularity of the fetus were higher than the adult this may partially explain the higher blood volume per unit body weight. The high mean systemic pressure (see chapter 3 introduction) of the fetus (Gilbert, 1977) is a stronger indicator of high vascular filling in the fetus.

5.4.3 Total water content

Measurement of total water content does not distinguish between intracellular and extracellular water, but would be increased if either of these two were increased. The percentage dry body weight was slightly lower in the one hypertensive fetus in which it was measured than in the two controls, but this is obviously not conclusive evidence for increased water content.

CHAPTER 6

General Discussion

6.1 Summary of haemodynamic findings

To summarize the results described in the previous chapters concerning fetal renoprival hypertension; 7-9 days after nephrectomy the hypertension is associated with a decrease in total peripheral conductance involving most of the organs including, and perhaps to a slightly greater degree, the placental cotyledons. Some of the decreased conductance can be attributed to an alpha-adrenergic vasoconstriction which may be part of a response to the mild hypoxaemia in the hypertensive fetuses. Plasma volume is perhaps slightly increased, as is blood volume.

The main cause of the vasoconstriction remains unknown, though increased vasopressin secretion or vessel wall thickening are attractive hypotheses. An increase in vessel wall tension is necessary to trigger the latter, increases in arterial pressure due to hypoxaemia, adrenergic or other hormonal mechanisms are possibilities. Though the capacity to increase fetal cardiac output is limited, a transient increase in response to increased blood volume, causing an increased arterial pressure is a remote possibility for a trigger.

6.2 Other examples of fetal nephrectomy

Not all investigators who have nephrectomized fetal lambs have observed hypertension. Faber, Green and

Thornburg (1974) found a mean (±SE) arterial pressure of 35 ±4 mm Hg about 4 days following nephrectomy in 110-145 day fetal lambs compared with 43 ±2 mm Hg in controls. Heart rate was similar in both groups. Haematocrit did show some signs of being lower at 28 ±3% compared with 37 ±2%.

Thorburn (1974) produced growth retardation by nephrectomy at 84-88 days; this was assessed by decreased body weight and retardation of osseous maturation at 134-138 days. No differences in electrolyte concentrations were found in the nephrectomized fetuses. Nephrectomy at 105-125 days did not result in lower fetal weight at 134-140 days, though total body length was slightly lower at 47 cm compared with 52 cm in controls (Brinsmead, Waters and Thorburn, 1980). This suggests the presence of oedema as well as growth retardation. Cardiac hypertrophy was also observed, heart weight, measured in three fetuses, was 49 ±12 g compared with 24 ±3 g in controls. The description of the replacement of amniotic fluid by "glairy material" suggests a similarity to the changes observed in the fetal membranes here. Arterial blood pressure was not measured, but the cardiac hypertrophy in particular suggests that it may have been increased.

Robillard, Gomez, Meernik, Kuehl and Van Orden (1982a) measured a mean (±SD) arterial presssure of 52 ±30 mm Hg in four 131-141 day old fetal lambs at least 6 days post nephrectomy. A value of 47 ±5 mm Hg was found in control fetuses of comparable age; the difference between the

means is not great, however, the considerably greater variability in the nephrectomized group suggests some of the fetuses may have been hypertensive. The haematocrit was slightly lower in the nephrectomized fetuses (29 ±9%) than the controls (38 ±2%), though in contrast with the results from this laboratory (Dutton and Mott, 1980) plasma aldosterone was slightly lower. Plasma sodium and potassium concentrations and osmolality were not different from controls.

Zanjani and Banisadre (1979) nephrectomized fetal lambs at 100-110 days gestation and about three weeks later found no difference in haematocrit from control fetuses.

Nathanielsz has not seen hypertension in a number of fetal lambs nephrectomized at comparable ages to those described here (personal communication). However, Moutquin and Liggins (personal communication) state that they have measured high fetal blood pressures following nephrectomy.

There are some indications that the differences in the response to nephrectomy may be due to the amount of sodium in the maternal diet. The ewes in this laboratory are fed hay ad libitum and a pelleted dietary supplement (up to 1 kg/day) which contains ~150 mM/kg NaCl. When pellets with no added salt were given to two ewes from 12 and 14 days before nephrectomy of their fetuses, the pressure rise was slight in one and absent in the other (Dutton and Mott, 1982). Fetal nephrectomy following a reduced maternal sodium diet for 26 days resulted in a progressive fall in

the arterial pressure of one fetus. Sodium restriction for 6-7 days before nephrectomy in two fetuses did not prevent the development of hypertension (Dutton and Mott, 1982).

6.3 Nephrectomy and fluid and solute balance

It seems a reasonable possibility that the increased extracellular fluid volume and the hypertension, whether or not the latter is dependent on the former, are the results of the inability of the placenta to excrete what is normally excreted by the kidneys. Overall it would not appear that the kidneys play as an important an excretory role in the fetus as in the adult as they receive only 2% of cardiac output rather than 20%.

6.3.1 Renal excretion in the fetus

The excretory function of the fetal lamb kidney was investigated in chronic preparations by Gresham, Rankin, Makowski, Meschia and Battaglia (1972a) from 117-130 days for varying periods until term. After initial post-operative variations, large volumes of hypotonic urine were produced; mean urine flow was 0.14 $ml.min^{-1}.kg^{-1}.min^{-1}$ and urine osmolality 65-160 $mOsm.kg^{-1}$ H_2O. Sodium ions accounted for about 30% of the total osmolality, K^+ about 6%; Cl^-, 17%; fructose, 12%; urea, 18%; creatinine, Ca^{2+} and PO_4^{2-}, 5% and other solutes about 9%.

When urine is continuously drained from the bladder, as in these experiments, one cannot be certain that urine

production will be the same as when it is allowed to pass by its normal routes into the amniotic and allantoic sacs. However, continuous drainage of urine from one fetus for 18 days did not appear to affect growth, despite the excretion of 9.25 l of urine (Gresham et al, 1972a). In addition, it seems unlikely that build-up of amniotic and allantoic fluids would affect renal excretion.

Robillard, Matson, Sessions and Smith (1979) measured fetal urine output over a more extensive age range, comparing groups from 101-119, 120-130, and 131-142 days gestation. Absolute urine flow increased from 0.46 ml/min (660 ml/day) before 120 days to 0.79 ml/min at 120-130 days (1137 ml/day) and 0.62 ml/min (892 ml/day) after 130 days. Urine osmolality increased after 130 days, in some cases being hypertonic to plasma.

6.3.2 Other non-placental fluid fluxes

In considering what might be the effects of removing the renal route of excretion in the fetus, it is necessary to appreciate the position of this in respect to other exchanges of water and solutes between fetus and mother.

6.3.2.1 Lung liquid production

Appreciable volumes of lung liquid are secreted by the fetus. Dawes, Fox, Leduc, Liggins and Richards (1972) observed outward movements of about 2 ml of tracheal fluid once or twice an hour. Fetal breathing movements were not associated with appreciable shifts of tracheal fluid. Adamson, Brodecky, Lambert, Maloney, Ritchie and Walker

(1973) found secretion of 6.6-15.4 ml/hr of lung liquid when it was drained to the outside from fetuses of 110 days to near term. When a tracheal loop was used, and lung liquid returned to its normal route, lower flows of 0.3-11.1 ml/hr (mean 5 ml/hr) were seen. Normand, Olver, Reynolds and Strang (1971) observed a mean flow of ~2 $ml.kg^{-1}.min^{-1}$ in acute exteriorized preparations of 123 days to term.

Compared with plasma, lung liquid is low in protein and bicarbonate, approximately equal in sodium and potassium concentrations and high in chloride (Olver and Strang, 1974).

It is not clear what proportion of the secreted lung liquid passes into the amnion and how much is swallowed.

6.3.2.2 Cutaneous exchanges

Exchanges with amniotic fluid via the fetal skin may be important early in gestation, but are negligible following keratinization of the skin (Seeds, 1980).

6.3.2.3 Fetal swallowing

An extremely important, though often neglected exchange is the swallowing of amniotic fluid by the fetus. Bradley and Mistretta (1973) examined swallowing in chronic fetal lamb preparations by an electromagnetic flowmeter placed in the oesophagus. Swallowing occurred in 2-7 bouts per day, during each of which 20-200 ml were swallowed. More frequent swallowing of smaller volumes (1-10 ml) was

also observed. Over the age range 109-143 days, 79-491 ml were swallowed per day. These estimates may be low because fluid may have passed around the outside of the flowmeter head which was of the cannulating type and simply lay in the oesophagus. In addition, implantation of the flowmeter may have impaired peristalsis. As body weights were only available at termination of the experiments, it was not possible to relate these flows to weight.

6.3.2.4 Summary of fluid fluxes

In a three kilogram fetus of about 120-130 days gestation, the following fluid exchanges are reasonable estimates. About 200-380 $ml.kg^{-1}.day^{-1}$ of urine are produced (Gresham et al, 1972a; Robillard et al, 1979); about 40-50 $ml.kg^{-1}.day^{-1}$ of lung liquid (Adamson et al, 1973; Normand et al, 1971). The volume of amniotic fluid swallowed is about 25-160 $ml.kg^{-1}.day^{-1}$ (Bradley and Mistretta, 1973).

The difference between the volume of fluid drunk and the volumes produced must be made up by the placenta. In addition, about 60 ml of water per day is required for growth by the fetal lamb at this stage of gestation (Barcroft, 1947).

Nephrectomy removes the major route of excretion from the fetal body, and though the resultant fall in amniotic fluid volume means drinking is probably decreased, a reduction in the maternal-fetal flux of water across the placenta is required if balance is to be preserved.

6.3.3 Placental transfer

6.3.3.1 Water

The placenta acts as a passive barrier to water transfer through which the flux of water will be determined by the resultant of the osmotic and hydrostatic pressure gradients and the placental filtration coefficient (L_p) (Conrad and Faber, 1977; Power, Roos and Longo, 1978) according to the following relationship

$$J_v S = L_p S \ (\Delta P + \sigma RT\Delta C) \qquad (6.1)$$

where J_v= volume flow per unit area membrane, ΔP is the hydrostatic pressure difference and ΔC the solute concentration difference across the membrane, S is the membrane surface area and σ is the reflection coefficient, a dimensionless constant from 0-1 which describes what proportion of the osmotic pressure predicted from solute concentration ($RT\Delta C$) is actually exerted across the membrane. A reflection coefficient of 0 describes a solute which is not impeded by the membrane and therefore exerts no osmotic pressure, and $\sigma = 1$ a solute which is completely excluded by the membrane and exerts the osmotic pressure expected from its concentration. R and T are the gas constant and absolute temperature respectively. The osmotic component of the equation is in fact the sum of separate $\sigma RT\Delta C$ components for each solute.

Plasma osmolality is lower in the fetus than in the ewe by a mean of 2 mosmol measured by freezing point

depression, and 6 mosmol when calculated from solute concentrations. Faber and Thornburg (1981) explain the transfer of water against the apparent osmotic gradient by low reflection coefficients for the major electrolytes (Na^+, Cl^-, K^+) such that they do not exert their full osmotic potential. These electrolytes are present in higher concentrations in the mother.

Hydrostatic gradients would appear to be quantitatively less important than osmotic forces as 1 mosmole corresponds to about 20 mm Hg at body temperature. Measurements of the transplacental hydrostatic pressure gradient vary, but generally it seems that the gradient is from the fetus to mother (Power et al, 1978).

Reduction of the materno-fetal water transfer following nephrectomy should not, therefore, be a problem; the fact that extracellular fluid volume is increased suggests that the osmotic balance is interfered with, probably due to increased fetal concentrations of some solute which cannot pass across the placenta. The renal excretion of possible solutes and their placental transfer are considered below.

6.3.3.2 Solute transfer

6.3.3.2.1 Electrolytes

The amounts of sodium and chloride excreted by the fetal lamb kidney at ~120-130 days are about 4-35 $mEq.kg^{-1}.day^{-1}$ and 1-20 $mEq.kg^{-1}.day^{-1}$ respectively (Robillard, Sessions, Kennedey, Robillard and Smith, 1977).

A large reduction in the amount of the gastrointestinal intake of these solutes will occur following nephrectomy because of the reduced amniotic fluid volume. From the estimate of fetal swallowing given above and the electrolyte concentrations in amniotic fluid measured by Alexander, Nixon, Widdas and Wohlzogen, (1958) the normal intake by this route is about 3-18 $mEq.kg^{-1}.dqy^{-1}$ of both Na^{+} and Cl^{-1}.

The factors which determine the transfer of charged particles across the placenta, particularly whether they include active transport or electrical factors are controversial. Faber and Thornburg (1981) maintain that no electrical gradient can exist across the exchange membranes on the basis of the transplacental distribution of endogenous (Armentrout, Katz, Thornburg, and Faber, 1977) and exogenous (Thornburg, Binder and Faber, 1979a) elecrolytes. Their conclusion rests on the unlikelihood of active pumps for all the ions investigated. Boyd, Canning, Stacey and Ward (1981) cite a number of points which suggest the simple passive model of electrolyte transfer proposed by Faber and coworkers is unlikely.

Under the passive model (Faber and Thornburg, 1981) solute flux is made up of a diffusional component ($PS\Delta C$) and a filtration component ($J_v SC(1-\sigma)$) as follows

$$J_s S = PS\Delta C + J_v SC(1-\sigma) \qquad (6.2)$$

where J_s is the solute flux per unit area; P, the diffusion permeability; C_s, the mean of the solute

concentrations on both sides of the membrane, and other variables as defined for equation 6.1.

Thornburg, Binder and Faber (1979b) found diffusion-permeability area surface area products of 5.2 $x10^{-3}$ $ml.s^{-1}.kg^{-1}$ and 9.8 $x10^{-3}$ $ml.s^{-1}.kg^{-1}$ for sodium and chloride respectively. On theoretical grounds they felt it was necessary to incorporate an electrical term into the calculation of reflection coefficients, and found the data was best fitted by a system in which there was a 1 mV difference across the barrier. Reflection coefficients of 0.83 forNa^{+} and 0.79 for Cl^{-} were calculated. From these values it is found that diffusion and filtration fluxes of sodium are roughly equal, whereas the diffusional flux of chloride is approximately double the filtration flux. In looking for the cause of water flux changes clearly the diffusion component is of more interest, unless changes in reflection coefficients occur. The reduction in transplacental sodium flux required following nephrectomy is at most 30 $mM.kg^{-1}.day^{-1}$ which at the diffusion permeability for sodium given above would require a reduction in the transplacental concentration difference of only 0.07 mM. The required reduction in chloride flux is lower and placental chloride permeability is higher (see above). Reductions in sodium and chloride transplacental flux can therefore occur with only small changes in concentration differences.

The feto-maternal clearance of other electrolytes

(including K^+) are higher than those for sodium and chloride (Boyd et al, 1981).

Under a system where active transport and appreciable transplacental potential differences are present the situation is more complex and less well understood (Boyd et al, 1981). As these authors conclude, insufficient data has been accumulated to build a model of transplacental electrolyte transfer.

6.3.3.2.2 Urea

The urea which is normally excreted by the kidneys will be transferred across the placenta in nephrectomized fetuses. Placental clearance and excretion of urea are high (19 $ml.min^{-1}.kg^{-1}$ and 0.54 $mg.min^{-1}.kg^{-1}$ respectively) and increasing plasma urea concentration by the infusion of ammonium lactate can result in a three-fold increase in placental excretion of urea, (Gresham, James, Raye, Battaglia, Makowski and Meschia, 1972).

6.3.3.2.3 Fructose

Fructose is a major constituent of foetal urine (Gresham et al, 1972a), being excreted at a rate of ~700 $mg.kg^{-1}.day^{-1}$ (calculated from clearances and plasma concentrations given in Gresham et al, 1972a). Fructose is produced by the ruminant placenta (Huggett, Warren and Warren, 1951) but as it appears to be in state of dynamic equilibrium (Alexander, Huggett and Widdas, 1951; Alexander, Andrews, Huggett, Nixon and Widdas, 1952) it is unlikely that its concentration would increase

substantially if its excretion were prevented.

None of the above seems a likely candidate for altering the materno-fetal osmotic balance. There remains the possibility of some other constituent of fetal urine having this effect. In addition, it is not clear to what extent placental transfer is under the control of hormones responsible for fluid and elecrolyte balance, either those which serve the same function in the adult (e.g. vasopressin and aldosterone) or perhaps some unique to the fetus and placenta. Thornburg et al (1979), for example, have postulated control of Na^+ and Cl^- placental reflection coefficients by vasopressin.

6.4 Further investigation

On the haemodynamic front, serial studies of cardiac output and perhaps organ blood flows would establish whether or not there was a transient increase in th development of the hypertension.

The cause of the vasoconstriction requires further investigation. specific vasopressin antagonists do not appear to be available (Gilman, Goodman and Gilman, 1980) but the role of this substance might be indicated by more extensive measurements of vasopressin concentration than have so far been carried out. Vessel wall thickening could be assessed histologically; careful control of fixing would be necessary.

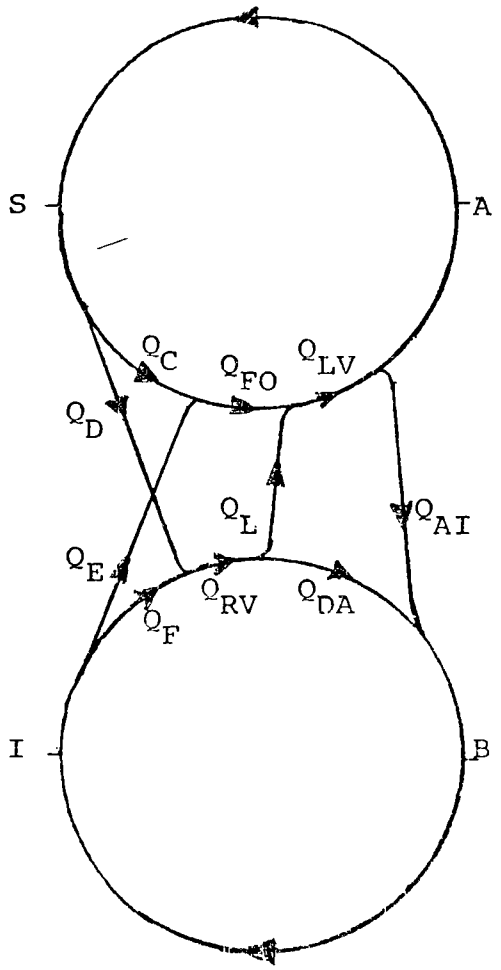
More extensive measurements of vascular volumes, including direct measurement of red cell volume with ^{51}Cr

would be informative. Measurement of extracellular fluid volume has not been done in the fetus. Inulin seems a good candidate as an indicator for this space as it does not cross the placenta (Rankin, Gresham, Battaglia, Makowski and Meschia, 1972).

Appendix 1

Verification of the Four-way thermodilution equation proposed by Gilbert et al (1980).

The following model represents the true course of the blood through the fetal heart, lungs and great vessels.



Where Q represents flow, and the subscripts; LV, left ventricle; RV, right ventricle; DA, ductus arteriosus; FO, foramen ovale; AI, aortic isthmus; L, lungs; and Q_C to Q_F represent the flows from the inferior and superior venae cavae to right and left atria as indicated.

Using H for the amount of "cold" injected (the same amount

for inferior and superior injections) and A and B for the integrals of the dilution curves recorded in the brachiocephalic artery and descending aorta (subscripts I and S indicate whether for inferior or superior vena caval injection), the areas under each curve are related to true flows as follows (assuming no cold indicator passes through the lungs.

$$A_S = \frac{H.Q_C}{Q_{LV}.Q_{SVC}} \qquad (A1.1)$$

$$B_S = \frac{H.\left(\frac{Q_{DA}.Q_D}{Q_{RV}} + \frac{Q_{AI}Q_C}{Q_{LV}}\right)}{Q_{SVC}\ Q_{IVC}} \qquad (A1.2)$$

$$A_I = \frac{H.Q_E}{Q_{IVC}.Q_{LV}} \qquad (A1.3)$$

$$B_I = \frac{H.\left(\frac{Q_{DA}.Q_F}{Q_{RV}} + \frac{Q_{AI}.Q_E}{Q_{LV}}\right)}{Q_{IVC}^2} \qquad (A1.4)$$

Equations A1.1-4 are substituted into Gilbert et al's equation

$$Q_{CO} = \frac{H.(A_S + B_I - A_I - B_S)}{A_SB_I - A_IB_S} \qquad (A1.5)$$

which then simplifies to

$$Q_{CO} = Q_C + Q_D + Q_E + Q_F + Q_L \qquad (A1.6)$$

which is a true measure of cardiac output

Appendix 2

Temperature correction of blood gas tensions and pH

The following equations correct for anaerobic temperature changes ($\Delta T\ ^{o}C$)

pH

$$pH = -0.0147\ \Delta T \qquad \text{(A2.1)}$$

(Bradley, Stupfel and Severinghaus, 1956)

Po_2

This correlation was obtained by taking the direct temperature effect on the oxygen dissociation curve as

$$\log Po_2 = 0.024\ \Delta T \qquad \text{(A2.2)}$$

(Severinghaus, 1966)

and calculating the indirect effect caused by the pH shift, using the pH correction above, and the pH sensitivity of the fetal oxygen dissociation curve obtained by Cassin et al (1964). This gives an overall correction of

$$\log Po_2 = 0.03\ \Delta T. \qquad \text{(A2.3)}$$

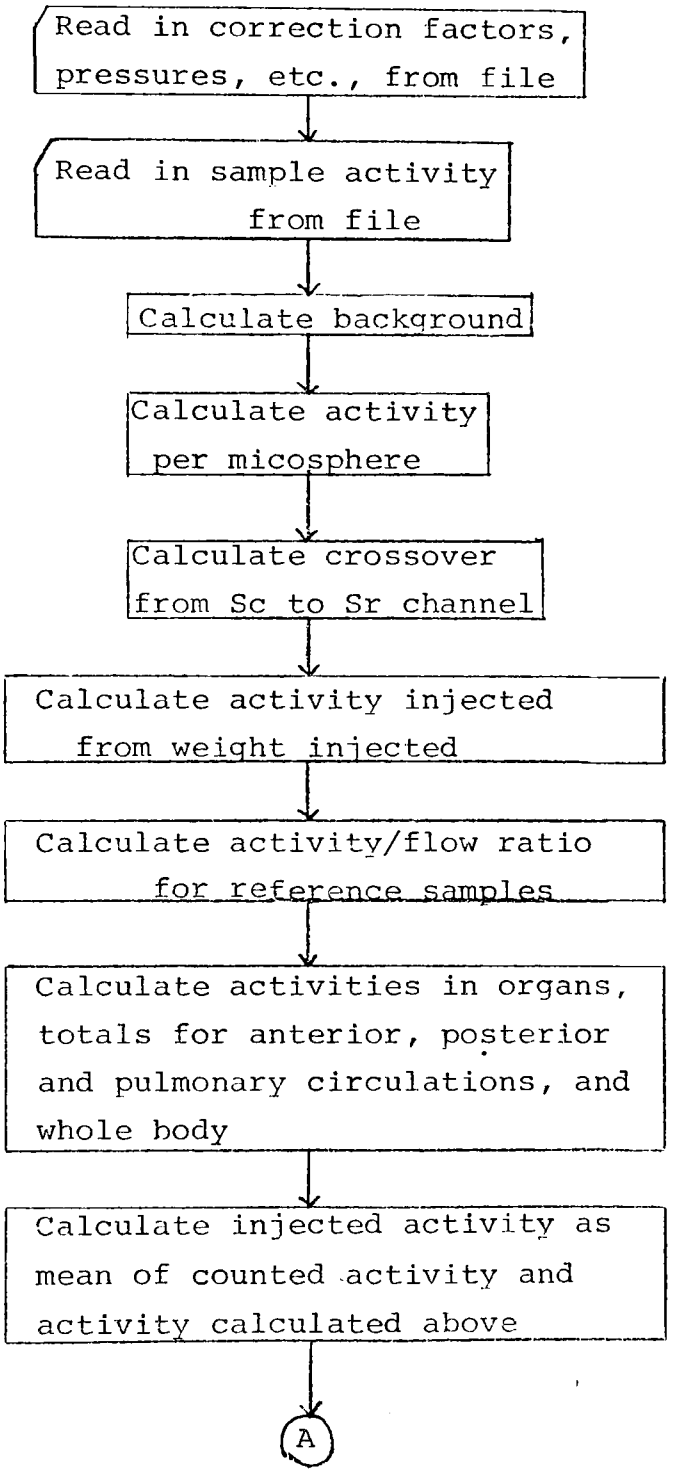
Pco_2

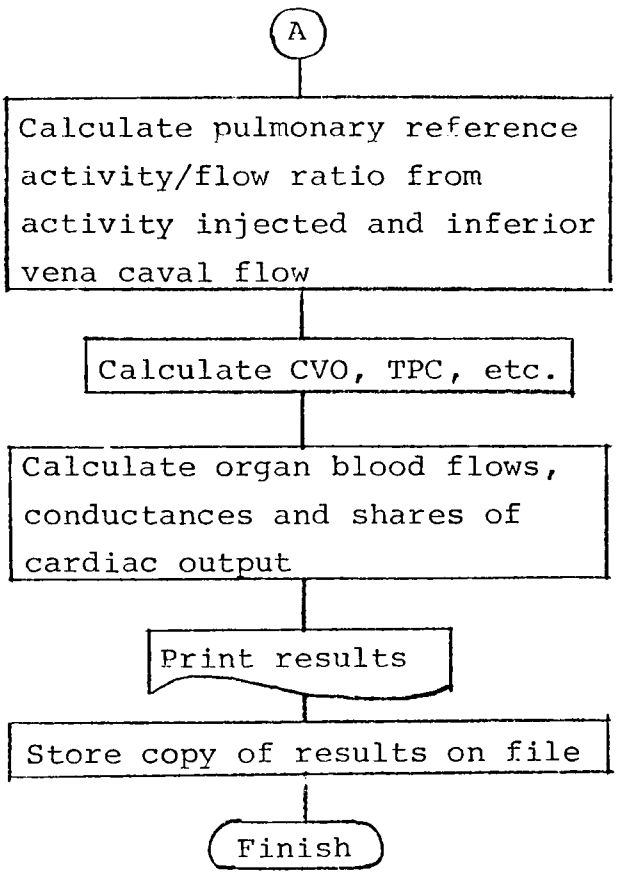
$$\log Pco_2 = 0.0185\ \Delta T \qquad \text{(A2.4)}$$

(Bradley et al, 1956)

Appendix 3 (a)

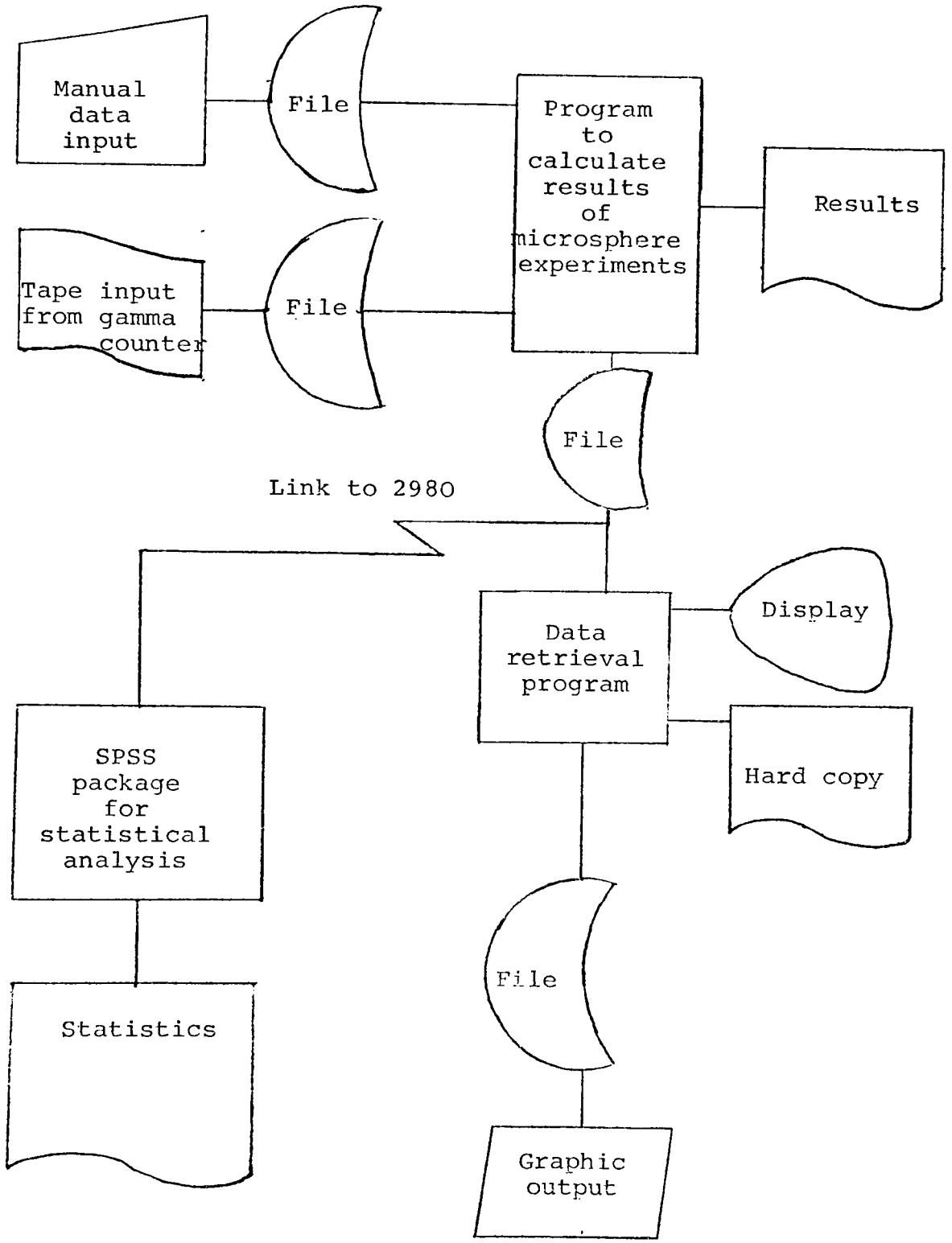
Flow diagram of program to calculate results from microsphere experiments




A
Calculate pulmonary reference activity/flow ratio from activity injected and inferior vena caval flow
Calculate CVO, TPC, etc.
Calculate organ blood flows, conductances and shares of cardiac output
Print results
Store copy of results on file
Finish

Appendix 3 (b)

Flow diagram showing routes of data analysis on CTL 8050 and ICL 2980 computers



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