EXPERIMENTAL INTRA-UTERINE GROWTH RETARDATION IN THE GUINEA PIG

PROEFSCHRIFT

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To Elly
To my parents

CONTENTS

ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
AMP	adenosine monophosphate
ADP	adenosine diphosphate
ATP	adenosine triphosphate
A/V	arteriovenous relation
CoA	coenzyme A
μCi	microcurie
dpm	desintegrations per minute
DNA	deoxyribonucleic acid
E.C.	enzyme commission number
EDTA	ethylene diamine tetra acetate
E	extinction
M	mole
NAD(P)$^+$	oxidized nicotinamide-adenine dinucleotide (phosphate)
NAD(P) H	reduced nicotinamide-adenine dinucleotide (phospate)
Pi	inorganic phosphate
RNA	ribonucleic acid
S.D.	standard deviation
Tris	tris (hydroxymethyl) aminomethane
v/v	volume per volume
w/v	weight per volume
WHO	world health organization

LIST OF ENZYMES

hexokinase	E.C. 2.7.1.1.
pyruvate kinase	E.C. 2.7.1.40
phosphofructokinase	E.C. 2.7.1.11
aldolase	E.C. 4.1.2.13
α-glycerophosphate dehydrogenase	E.C. 1.1.1.8
enolase	E.C. 4.2.1.11
pyruvate carboxylase	E.C. 6.4.1.1.
phosphoenolpyruvate carboxykinase	E.C. 4.1.1.32
fructose 1,6-diphosphatase	E.C. 3.1.3.11
glucose 6-phosphatase	E.C. 3.1.3.9.
isocitrate dehydrogenase	E.C. 1.1.1.42
citrate synthetase	E.C. 4.1.3.7
lactate dehydrogenase	E.C. 1.1.1.27
ATP citrate lyase	E.C. 4.1.3.8.
glucose 6-phosphate dehydrogenase	E.C. 1.1.1.49
6-phosphogluconate dehydrogenase	E.C. 1.1.1.44
isocitrate dehydrogenase	E.C. 1.1.1.42
malic enzyme	E.C. 1.1.1.40
acetyl-Co A carboxylase	E.C. 6.4.1.2
alanine aminotransferase	E.C. 2.6.1.2.
aspartate aminotransferase	E.C. 2.6.1.1.
tyrosine aminotransferase	E.C. 2.6.1.5.
valine aminotransferase	E.C. 2.6.1.3.
glutamate dehydrogenase	E.C. 1.4.1.3.
carbamylphosphate synthetase I	E.C. 2.6.2.5
ornithine transcarbamylase	E.C. 2.1.3.3
argininosuccinate synthetase	E.C. 6.3.4.5
argininosuccinase	E.C. 4.3.2.1
arginase	E.C. 3.5.3.1
Ca^{++} ATPase	E.C. 3.6.1.3
Na^{+}, K^{+}-ATPase	E.C. 3.6.1.3

PREFACE

In the Western world perinatal mortality rates have been reduced to low levels for various reasons but mainly because of better perinatal care. However morbidity is still relatively high and the concern of every parent for the quality of life of his newborn child demands continuing research into the development of the fetus and the newborn.

One of the major groups of infants at risk are those that are born with a low birthweight. Approximately 5-14% of all live born children in Western Europe and the United States have a birth weight of less than 2500 grams. Of these about two-thirds are born too early and are called "preterm" and one-third is born too small and is called "small for gestational age." Although this division seems logical it took clinicians a long time to realize the differences between these groups that hence require different treatment.

Nearly fifteen years ago the introduction of the so-called "intrauterine growth charts", that indicated the normal fetal growth for a certain population, offered the opportunity of categorising normal or abnormal fetal growth patterns. During recent years it has become possible to assess human fetal growth by means of ultrasound techniques and thus identify and time growth retardation *in utero*. At the same time follow-up studies of infants that were born small for gestational age have demonstrated that growth retardation *in utero* may have permanent effects on later growth and development. For ethical reasons good clinical research studies are difficult. Most research has therefore been concentrated on animals.

During recent years much has become known on normal growth and development of the fetus in several species. There are numerous examples that show that fetal development is for instance under endocrine control. However the precise sequence of causal events leading to tissue differentiation late in fetal development remains unclear. Some parameters that influence fetal development may become more clear by studying abnormal fetal growth. Examples of these are the naturally occuring "runts" or the large fetuses that are frequently seen in diabetic pregnancy.

Another approach to investigate the regulation of fetal growth is to experimentally reduce mean growth rate *in utero*. This is the approach used in this thesis which studies intrauterine growth retardation in the guinea pig and investigates some of the consequences of this condition. Such a comparative approach provides a broad background of information and thus general principles that allow specific questions of the human condition to be asked and interpreted adequately.

The history of the concept of intrauterine growth retardation and of its possible causes is outlined in chapter I. This chapter also summarizes the various experimental approaches that previously have been used. The techniques of the experimental approach in the guinea pig are described in chapter II, together with details of all other methods used. In chapters III-VI the results of measurements on fetal growth and development, nutrient supply and endocrine development and development of fetal liver, heart and skeletal muscle metabolism are given.

CHAPTER I INTRODUCTION

I.1. The study of fetal growth. An historical view

The control of fetal growth is of central importance to perinatal physiology and medicine. To determine the factors regulating normal fetal growth, abnormalities have frequently been studied since they are associated with a high mortality. The first of these studies have concentrated on the relatively easily obtained fetal and placental weights.

In man Adair and Thelander (1925) and in the guinea pig Ibsen (1928) reported a close relationship between fetal and placental weight. Ibsen also noted that the locations and the number of placentas affected the size of the fetal guinea pig. Somewhat earlier Draper (1920) had shown that external factors such as low temperature and a deficient maternal diet had reduced fetal growth in the guinea pig.

During the next two decades most research in this field was performed by Barcroft in England. Studying the sheep he suggested (like Ibsen) that "crowding" in the uterus may limit placental growth and therefore fetal growth, and he concluded that fetal growth was predominantly determined by placental weight (Barcroft, 1939). Then in 1938 Walton and Hammond published their classical studies of the crossing of large Shire horses and small Shetland ponies, showing that the weight of the newborn correlated more closely with maternal than with paternal size. They concluded that intrauterine growth rate is more determined by the maternal control of the fetal environment than by genetic factors (Hammond, 1944).

At this time clinicians thought that newborns were small because they had insufficient time to grow. In 1948 the W.H.O. recommended that all babies with a birth weight < 2500 grams should be called "premature", thus adding to the existing confusion about the relationship between gestational age and birth weight. In 1953 McKeown and Record studying human singleton and twin pregnancies suggested that the variation in fetal weight with litter size is not wholly accounted for by variation in placental weight. In 1955 they demonstrated similar findings in the guinea pig. One of their conclusions was that all these effects could simply be explained as a result of limitation of blood supply to the uterus.

They were among the first to study fetal and placental weight in a large population and at different periods of gestation (Eckstein and McKeown, 1955). In 1954 Kloosterman studying the pathology of two thousand placentas described a correlation between low birth weight and a small, often infarcted, placenta.

Before the second world war external factors influencing fetal growth such as temperature and diet only interested farmers and those involved with animal

reproduction. Clinicians became more interested partly because of the data reported by Smith (1947) on the famine in Holland during 1944 showing a small decrease in the birth weight. From then on there were many reports of the effects of restriction of maternal diet reducing fetal growth and especially brain development (Mendes and Waterlow, 1958; McCance, 1960; Winick, 1969; Dobbing, 1970).

In addition to this concept of maternal nutrition affecting fetal growth Gruenwald (1963), in studying placental pathology, suggested that "fetal malnutrition" or "fetal deprivation" occurs because of poor placental function. He was one of the first to use the concept of the "small for dates" infant (Gruenwald, 1968, 1974, 1975). Until 1961 all babies < 2500 grams were still called "premature" but then the WHO recommended to use the term "low birth weight" for this group of infants. In 1963 Lubchenco *et al.* introduced one of the first so called "intrauterine growth curves" and thus normal and abnormal fetal growth in certain populations could be distinguished. Similar growth curves for different populations soon followed (in Holland for instance that of Kloosterman, 1969).

The analysis of growth was further refined by the introduction of growth curves for head circumference in 1969 by Usher and McLean. The effects of growth retardation on tissue composition were first described by Gruenwald (1963) and Naeye (1965). One of their most important findings was the almost complete maintenance of braingrowth compared with a low liver weight. Numerous other reports on one of the consequences of intrauterine growth retardation have been made.

A high incidence of hypoglycaemia in this group of newborns was reported by Cornblath *et al.*, 1959, 1964 and Neligan *et al.*, 1963. Possibly connected with this was the fact that autopsies of such fetuses and newborns showed depleted glycogen stores in the liver (Shelley and Neligan, 1966) and depleted lipid stores in the brown adipose tissue (Aherne and Hull, 1966).

In 1964 Scott and Usher illustrated poor skeletal and epiphysial growth in newborns that were growth retarded *in utero*. The introduction of semi-quantitative scoring systems such as that of Dubowitz (1970) based on clinical appearance and neurological development of the newborn, helped to estimate the maturity of the newborn. In 1970 a working party of the Second European Congress of Perinatal Medicine recommended division of the low birth weight infant group into those that were "small for gestational age" and those that were "appropriate for gestational age."

Term pregnancy was said to occur between 37 and 42 weeks and births between 28 and 36⅞ weeks were called "premature". As a division of "small and appropriate for gestational age" the 10th percentile of the intrauterine growth curve was suggested. Other groups recommended to use the 5th percentile (Thomson *et al.*, 1968) or all birth weights below 2 standard deviations of the mean (Usher and McLean, 1969). It should be noted that this division is entirely arbitrary. From 1970 onwards the use of ultrasonic techniques has made it possible to continuously follow growth rate *in utero* and not only to identify growth retardation but also its time of onset.

Measurement of biparietal diameter and cross sectional chest area together with the length of the fetus made it possible to distinguish even more parameters of growth (Campbell, 1971, 1974).

Finally after a period of more than 50 years in which clinicians showed interest in low birth weight, newborn infants that are small for their gestational age were considered as a special group and research on its causes and consequences started to develop.

I.2. Intrauterine growth retardation

I.2.1. Clinical aspects

At present about 5-14% of all live born children in Western Europe and the United States weigh 2500 grams or less at birth (Gruenwald, 1964; Geyerstam, 1969; Scott, 1966; Lubchenco, 1972). In other parts of the world this figure can be much higher: in India for instance it is about 27% (Sharma, 1968). Approximately one third of these children are born small for gestational age (Gruenwald, 1964). The identification of "small for dates" as outlined above made it possible to recognize a variety of clinical features associated with this condition. The most important known clinical manifestations of intrauterine growth retardation are listed in table I.1. Most known causes of intrauterine growth retardation are listed in table I.2.

Although in the majority of cases the causes and the mechanism of intrauterine growth retardation remain unexplained, there are often indications of poor uterine or placental circulation, a condition that is frequently associated with toxaemia of pregnancy, hypertension, smoking and multiple pregnancy (Scott and Usher, 1966; Gruenwald, 1970; Butler, 1974; Low and Galbraith, 1974; De Souza *et al.*, 1976; Miller *et al.*, 1976) or with direct evidence of vascular disorders such as abruption and premature separation of the placenta (Gruenwald, 1963, 1968; Fox, 1975).

Apart from measurement of fundus height the best technique for diagnosis and follow-up of intrauterine growth retardation is the ultrasonic measurement of the fetus (Campbell, 1974; Wladimiroff *et al.*, 1978).
No chemical screening techniques available give a satisfactory prediction of intrauterine growth retardation (Robinson, 1978).

I.2.2. Postnatal consequences

Many of the earlier follow-up studies describing the consequences of a low birth weight are completely invalidated by the lack of adequate assessment of gestational age at birth (Dubowitz, 1974). Another complication with the interpretation of the follow-up studies, apart from the diversity of methods, is the heterogeneity of the group of intrauterine growth retarded infants as far as cause and onset of growth restriction are concerned.

Table I. 1. Clinical manifestations of intrauterine growth retardation.

Clinical condition	Incidence	References
Asymmetric organ growth	+++	Gruenwald, 1963, 1968, Naeye, 1965
Hypoglycaemia	++	Cornblath et al., 1959, 1964 Neligan et al., 1963 Blum et al., 1969 Lubchenco et al., 1971
Polycythaemia	++	Haworth et al., 1967 Humbert et al., 1969 Lugo et al., 1971
Haemoglobin rise	++	Haworth et al., 1967 Lugo et al., 1971
Depleted energy stores		Shelley and Neligan, 1966
- glycogen	+	Aherne and Hull, 1966
- fat	++	Brans et al., 1974 Dauncey et al., 1977 Mettau, 1977, 1978
Impaired gluconeogenesis	++	Haymond et al., 1974 Mestyan et al., 1975 Cornblath and Schwartz, 1976a
Delay of skeletal development	++	Scott and Usher, 1964 Wilson et al., 1967
Higher incidence of asphyxia	+	Lugo et al., 1971 Low et al., 1972

In a follow-up study of small for dates infants born at term and more than 2 standard deviations below the mean birth weight, Fitzhardinge and Steven (1972a) showed that at 6 years of age 35% were still below the 3rd percentile of length while the others, due to a greater growth velocity up to six months after birth, were able to "catch up" although only 8% were above the 50th percentile of length. A similar high percentage of small for dates that failed to catch up was found by others (Ounsted and Taylor, 1971; Drillien, 1970; Low *et al.*, 1978). Many small for dates infants show a catch up in head circumference during the first postnatal year (Fancourt *et al.*,

Table I. 2. Causes of intrauterine growth retardation (adapted from Root, 1976).

A. Maternal

- vascular disorders
  - toxaemia, eclampsia of pregnancy
  - maternal smoking
  - hypertensive cardiovascular disease
  - severe diabetes mellitus
  - multiple pregnancy
- hypoxia
  - high altitude
  - sickle cell anaemia
- maternal addiction
  - heroin
  - alcohol
- extreme malnutrition
- socioeconomic factors

B. Placental

- chronic disorders
  - infarctions and fibrosis
  - site of implantation
  - haemangioma
  - parabiotic twinning
- acute disorders
  - abruptio placentae
  - cord ruptures

C. Fetal

- genetic disorders
  - inborn errors of metabolism
- chromosomal disorders
  - trisomic syndrome: 15, 18, 21
  - autosomal deletions: 5, 18, 21
  - sex chromosomes: XO, XXXY
  - chromosomal breakage.
- infections
  - rubella
  - cytomegalic inclusion disease
- twinning
- congenital disorders
  - congenital heart disease
  - single umbilical artery
  - dysmorphic syndromes
- teratogenic agents
  - drugs
  - radiation
- endocrinological disorders
  - hypothyroidism

1976; Babson, 1970, 1974), while a small percentage does not, although this is not necessarily related with a lower intellectual performance (Babson, 1974). Follow-up studies on possible neurological and intellectual sequelae resulting from intrauterine growth retardation in general, show few abnormalities (Fitzhardinge and Steven, 1972b; Low *et al.*, 1978) except in infants that were severely growth retarded. Recently Commey and Fitzhardinge (1979) demonstrated major neurological defects in 49% of a group of preterm small for gestational age children that was followed in a prospective study during the first two postnatal years. A study by Fancourt *et al.* (1976) using ultrasonic techniques, suggests that if growth restriction *in utero* occurs after approximately 26 weeks the brain mass is reduced but there is postnatal compensation to produce a normal sized brain. Growth restriction before 26 weeks causes permanently stunted brain growth and neurological damage.

Hypoglycaemia has been one of the most clearly identified postnatal consequences in infants that suffered from intrauterine growth retardation (Cornblath and Schwartz, 1976a). Because this hypoglycaemia coincides with high levels of gluconeogenic substrates there have been suggestions of a possible delay of development of gluconeogenic enzymes in the liver of the small for dates newborn (Haymond, 1974; Mestyan, 1975). If the impairment of hepatic gluconeogenesis is permanent it may explain the higher incidence of ketotic hypoglycaemia that occurs in such infants later in childhood (Cornblath and Schwartz, 1976b).

In conclusion, it is evident that many causes and consequences of intrauterine growth retardation have not yet been clearly identified and therefore the basis underlying its treatment is tenuous. Clearly a detailed description of the events occurring in intrauterine growth retardation is unlikely to come from human studies in the near future for various but mainly ethical reasons. Thus animal studies are required to direct specific questions of the human condition.

1.3. Experimental studies of intrauterine growth retardation

1.3.1. Historical review

In order to study the occurrence of "runts" in animal reproduction Ibsen in 1928 investigated the fetal guinea pig and explained the limited fetal growth because of "crowding" in the uterus. One of the first experimental approaches was that of Walton and Hammond in 1938. They investigated genetic versus maternal environmental influences on fetal growth in their experiments in which they crossed large Shire horses and small Shetland ponies.

Barcroft (1939) studying the haemodynamic distribution of blood between the fetus and the placenta in sheep, concluded that fetal weight was mainly determined by placental weight. One of the first studies to experimentally influence fetal growth was that of Eckstein and McKeown in 1955. They studied the effect of transsection of one horn of the guinea pig uterus and concluded that there were at least 2 factors regulating fetal growth: general and local effects in the uterus. The local effect was found in large litters in which there was "crowding" in the uterus and therefore restriction of fetal growth. The general effect was determined by the total number of fetuses present in both uterine horns and not related to their location. In this manner they came to their important conclusion that fetal growth was more likely to be influenced by the blood supply to the uterus. This was confirmed by the experiments of Franklin and Brent (1960, 1964) in which they clamped the uterine artery and vein in rats at various periods of gestation. One of their findings was that this procedure sometimes caused severe growth retardation. This study was followed up in more detail by Wigglesworth in 1964. He specifically ligated the uterine artery at the cervical end of one uterine horn on the 17th day of gestation. This procedure caused intrauterine growth retardation to fetuses adjacent to this ligation. In the same year

Alexander (1964) reported a totally different experimental approach in causing growth retardation in fetal sheep. He reduced the number of placental cotyledons by surgical removal of part of the uterine caruncles before conception. This had the effect of reducing both total placental weight and fetal weight at term. He concluded that the suggestion by Barcroft (Barcroft, 1946) and others that birth weight was restricted if the placenta was very small was not justified. This finding was consistent with that of Eckstein and McKeown who postulated that fetal growth restriction could be explained by a reduction of blood flow to the uterus and therefore to the placenta. In 1968 Emmanouilides *et al.* described a method to create fetal growth retardation in sheep by single umbilical artery ligation of the lamb fetus. Although growth retardation was substantial, mortality was high and this method therefore seemed to be too great an insult to the fetus. Also in sheep Creasy *et al.* (1972) caused intrauterine growth retardation by means of microspheres injection into the maternal arterial blood supply to the uterus and thereby creating infarctions at the maternal side of the placental circulation. In 1971 Hill and Myers published a study on growth retardation in the primate. Here growth retardation was caused by ligating the interplacental vessels of the two placentas of the rhesus monkey.

In general in all experiments described above the experimental procedure has led to a significant fall in fetal growth rate. The associated polycythaemia, hypoxaemia and hypoglycaemia (Oh *et al.*, 1970; Nitzan and Groffman, 1971; Chanez *et al.*, 1972; Robinson *et al.*, 1979) in such conditions supports the view that placental transport is impaired. However it is not, at present, possible to say if the contribution of the placenta to the restricted fetal growth is simply one of impaired transport. The fetal responses to growth restriction are far from simple as indicated by the asymmetric effects on fetal organ growth of conditions thought to result in reduced placental transport. For instance the severe reduction in size of some splanchnic organs such as liver and spleen in relation to the only moderate reduction in brain size in these growth restricted fetal animals is comparable to the situation found in the growth retarded human fetus in cases where there are indications of poor placental function (Hill, 1974). On the other hand results on metabolic properties of the tissues of the growth retarded fetuses are not always consistent in the various experiments. Thus at present a detailed description of the consequences of intrauterine growth retardation is not available while factors such as the effects of differing degrees of growth retardation have not been investigated.

1.3.2. Study of intrauterine growth retardation in the guinea pig

To get a better understanding of the physiological responses of the fetus and the placenta to conditions of reduced nutrient supply the present study on experimental intrauterine growth retardation was undertaken in the guinea pig. The method of Wigglesworth was chosen partly because of its relative simplicity of implementation and partly because of some comparability with the condition in the human where intrauterine growth retardation occurs with evidence of a reduction in

maternal placental blood flow (Wigglesworth, 1964). One of the main disadvantages of studies of the fetal rat, the species that was used by Wigglesworth, is the relatively short period of gestation (22 days) which therefore restricts the period in which fetal growth can be followed, since the time interval between surgery (at 17 days) and moment of birth is short. Of the other small animals used in the laboratory, the guinea pig is one of the few animals with relatively large fetuses that can be followed during a substantial period of gestation (term is approximately 67 - 68 days). Recently many aspects of fetal and neonatal metabolism of the guinea pig have become known (Jones, 1973, 1976; Faulkner and Jones, 1975, 1976, 1978). The bicornuate anatomy of the guinea pig uterus makes it possible to affect surgically one uterine horn while the other can be used as a control.

For practical reasons we performed the uterine artery ligation at mid gestation while the outcome on fetal growth was investigated at two characteristic periods during the last third of fetal development. A few maternal guinea pigs were allowed to deliver in order to study the neonate. In this way we were able to study the effects of various degrees of intrauterine growth retardation at various periods of perinatal development.

CHAPTER II METHODS

II.1. Animal treatment and surgery

II.1.1. Animal treatment

Young virgin female guinea pigs of the Dunkin-Hartley strain weighing 450-500 grams were mated. Gestational age was determined by a system described by Elvidge (Elvidge, 1972). The female guinea pig has an oestrus cycle of 16 days. Usually the vagina is closed for 11-12 days, ¼-½ open for 3-4 days and completely open for 1-2 days. Since mating can only take place when the vagina is totally open, it is possible to determine the day of mating accurately within 1-2 days by checking the vaginal opening every day. The animal is presumed to be pregnant if the vagina remains closed in the subsequent cycle. The date of mating is taken to be the last day the vagina was open. Approximately 70% of the females become pregnant in the first cycle and 25% in the next two cycles. Their diet consisted of Dixon's Diet 18 (Ware, Herts) supplemented with ascorbic acid. Water and hay were supplied *ad libitum*.

II.1.2. Surgery

At day 30 of pregnancy the animals were anaesthetized with sodium pentobarbitone (30 mg/kg) administered via an earvein. The abdomen was shaved and treated with 0.5% chlorohexidine gluconate in ethanol. A midline incision of 2 cm was made in the abdominal wall near the umbilicus. After opening of the peritoneum the mesometrium of one uterine horn was exposed. An uterine artery was ligated near the cervical end of the arterial arcade with a silk ligature (Mersilk no. 1, Ethicon, Edinburgh) (fig. II.1). The other uterine artery was left undisturbed and the fetuses in this horn served as controls. After the ligation the abdominal wall was closed with 15-20 individual stitches, then the skin was closed with continuous overstitching. The surgery lasted 15-20 minutes and the animals recovered in 1-2 hours. For sham operated controls the procedures and treatment were identical except that the uterine artery was not ligated.

II.2. Method of bloodsampling

At days 49-51 and 60-63 of pregnancy the fetuses were delivered by caesarean section after anaesthetizing the mother by the technique as described above. A small group of operated guinea pigs were allowed to deliver at the normal time (66-68 days)

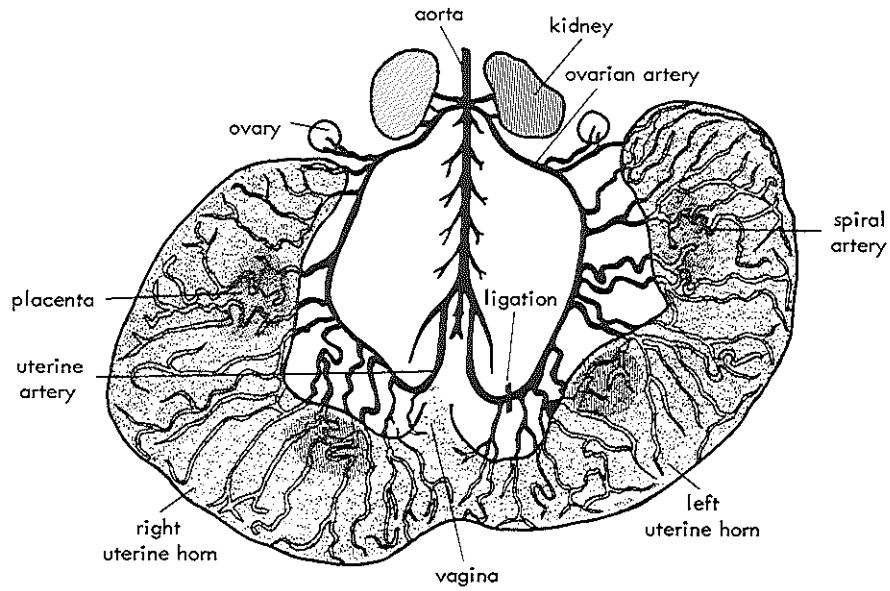

Fig. II. 1. Ventral view of the guinea pig uterus with arterial blood supply (modified from Egund and Carter, 1974). Note the position of the ligation.

in order to study the newborns. In the fetal studies, tissue samples were taken within 2 minutes of anaesthesia and for plasma samples within 30 seconds of fetal delivery. Blood samples of approximately 1 ml were taken from the umbilical vein of a fetus in the uterine horn with the ligated uterine artery and immediately thereafter of a fetus from the control horn by means of a syringe containing ± 10 units of heparin sulphate. Then blood samples were taken from the maternal abdominal aorta. All heparinized blood samples were immediately placed on ice. Apart from some immediate measurements such as blood gases and packed cell volume all samples were centrifuged for 10 minutes and the plasma samples were stored at -20° C.

II.3. Tissue sampling and treatment

Immediately after collecting the blood samples all fetuses were weighed and the length from nose to tail was measured. After this procedure the fetuses were dissected and individual organs were weighed and immediately kept on ice.
In some experiments the liver of the fetus from the horn with the ligated uterine artery was freeze clamped (see below), immediately after anaesthetizing of the mother. In these cases no plasma and further tissue samples were taken.

II.4. Determination of plasma metabolites

Plasma samples were deproteinized by adding half the amount of their volume of

1 M $HClO_4$ on ice. After spinning at 1500 g for 5 minutes at 2° C the supernatant was neutralized by adding ¼ of the amount of their volume of 2M $KHCO_3$ on ice. Plasma metabolites were assayed in the supernatant after spinning at 1500 g for 5 minutes at 2° C.

II.4.1. Determination of glucose

After deproteinization plasma glucose was assayed using the glucoseoxidase reaction. (Huggett and Nixon, 1957).

II.4.2. Determination of lactate

After deproteinization plasma lactate was assayed using the lactate dehydrogenase reaction by spectrophotometric measurement of the conversion of NAD^+ to NADH at 340 nm (Hohorst, 1963).

II.4.3. Determination of acetate

After deproteinization plasma acetate concentrations were measured using acetyl-CoA synthetase. A plasma extract of 0.2 - 0.5 ml was added to 0.3 M Tris HCL containing 30 mM $MgSO_4$, 0.7 mg/ml malate, 20 mM Coenzyme A, 12 mM ATP, 16 mg/ml NAD^+, 5 μl citrate synthetase (final volume 1 ml.), 5 μl of malate dehydrogenase was added and optical density change was measured at E_{340}. After establishing a stable baseline 5 μl of Acetyl-CoA synthetase (5 mg/ml) was added during incubation at 37° C. After the reaction had gone to completion the optical density change was read at E_{340}. In the acetate concentration range measured (0.05 - 1 μmol) recovery was 96-99%.

II.4.4. Determination of free fatty acids

Free fatty acids were extracted from plasma by the method of Dole (1956). Free fatty acids were determined by the colorometric method of Duncombe (1963) by using palmitate as a standard. Palmitate recovery in the Dole (1956) extraction was 92.3 ± 4.7% (n = 6) (± SD); no significant phospholipid extraction occurred.

II.4.5. - II.4.12. Determination of alanine, serine, threonine, glutamine, glutamate, citrate, ammonia and urea

Concentrations in plasma of alanine, serine, threonine, glutamine, glutamate, citrate, ammonia and urea were measured by standard enzymatic methods as described in Bergmeyer 1974.

II.5. Bloodgas analysis and packed cell volume determination

Blood oxygen tension (pO_2), blood carbondioxide tension (pCO_2) and pH were

measured using a Modell 27 Acid-Base analyzer (Radiometer Copenhagen). Packed cell volume (Haematocrit) was determined by means of standard microcapillary determination.

II.6. Determination of plasma hormones

II.6.1. Determination of plasma adrenocorticotrophic hormone

Plasma ACTH was assayed essentially as outlined by Jones *et al.*, 1977, using a similar antibody. The standard used was human $ACTH_{1-39}$ (74/55S) obtained from the National Institute for Biological Standards and Control, London. The intra- and interassay coefficient of variation for 100 pg. of ACTH were $10.6 \pm 3.4\%$ ($n = 6$) and $13.3 \pm 4.2\%$ ($n = 6$).

II.6.2. Determination of plasma growth hormone

Plasma growth hormone was assayed by radioimmunoassay essentially as described by Hart *et al.*, 1975. Porcine growth hormone was used as the standard. Intra- and inter-assay coefficients of variation were $9.6 \pm 3.1\%$ ($n = 6$) and $12.4 \pm 3.7\%$ ($n = 6$) respectively.

II.6.3. Determination of plasma cortisol

Cortisol was determined by radioimmunoassay after extraction from plasma with ethanol. (Abraham, Buster and Teller, 1972). The antibody showed only small crossreactivity with other C_{21} steroids. The assay coefficient of variation was approximately 10% at a concentration of about 5 ng/ml.

II.6.4. Determination of plasma androstenedione

Androstenedione was assayed by radioimmunoassay as described by Abraham *et al.*, 1973 and Challis *et al.*, 1973. Plasma (0.5 ml) was first extracted with 4 ml of diethylether and after resolution of the extract in 1 ml of trimethylpentane it was applied to a column (0.5 cm x 10 cm) of celite to which the androstenedione did not bind. The coefficient of interassay variation was $9.5 \pm 3.5\%$ ($n = 6$) and that for intra-assay variation was $9.2 \pm 3.2\%$ ($n = 6$).

II.6.5. Determination of plasma insulin

The insulin concentrations in fetal plasma were determined by radioimmunoassay using guinea pig insulin prepared by Dr. Steve Wood (Birkbeck college, London) and an anti guinea pig insulin antibody prepared by Dr. M. Lazarus (Wellcome Research Laboratories, Dartford, Kent). Guinea pig insulin was iodininated with

$Na^{125}I$ essentially as described by Greenwood and Hunter (1963) and separated from $Na^{125}I$ by Sephadex G-25 chromatography on a 0.5 cm x 10 cm column. Plasma samples were diluted in 0.1M sodium potassium phosphate (pH 7.4) and to 0.1 ml samples of this was added 0.1 ml of antiserum (1/8000) diluted in the same buffer. This was left for 6 hours at 4° C then 0.1 ml of ^{125}I guinea pig insulin, diluted in 0.1 M sodium potassium phosphate (pH 7.4) was added and the incubation was continued overnight at 4° C. Free and bound hormone were separated by adding 0.1 ml of sheep serum followed by 0.5 ml of 1.5% Dextran T70 and 0.5% (w/v) Norit A charcoal. This was mixed well, left for 30 minutes at 4° C and after separating out the charcoal pellet was counted in a LKB gamma counter. Assays and values were run in triplicate and detected between 0.5 - 50 ng/ml. The interassay coefficient of variation at 5 pg/ml was 12.2 ± 3.4% (n = 12) and the value for intra-assay variation was 10.1 ± 2.6% (n = 12).

II.6.6. Determination of plasma glucagon

Plasma glucagon was measured by radioimmunoassay, using a pancreatic glucagon specific antibody (Unger 30 K), by a modification of the method of Faloona and Unger (1974). Talc was used to separate antibody bound and free glucagon (Rosselin *et al.*, 1966). The interassay coefficient of variation at 0.3 - 3.9 ng/ml was 22.5 ± 5%. The intra- assay coefficient of variation was 7.4%.

II.6.7. Determination of thyroid hormones

Plasma thyroxine (T_4), triiodothyronine (T_3) and 3, 3′, 5′-triiodothyronine (or reverse T_3) (rT_3) were determined by radioimmunoassay, essentially as described by Chopra *et al.*, 1972, 1974. ^{125}I-labelled thyroid hormones were obtained from the Radiochemical Centre, Amersham, U.K. The intra-assay coefficients of variation for T_4, T_3 and rT_3 were 9.6 ± 2.1% (n = 8), 8.7 ± 2.6% (n = 8) and 11.3 ± 2.5% (n = 8) respectively.

II.6.8. Determination of plasma sulphation promoting activity

Fetal plasma samples were assayed on sulphation promoting activity by Dr. A. Price, University of Manchester, Royal Manchester Children's Hospital, Pendlebury, Manchester M27 1HH, England, using a porcine cartilage bioassay. The technique used was a modification of that described by Van den Brande and Du Caju, 1974. Adult male guinea pig plasma served as the standard. The total volumes for each incubation were 100 μl. To each tube 0.1 μCurie of $S^{35}O_4$ was added in 5 μl of the medium. The whole assay was carried out in microtiter plates. Results were analyzed according to the parallel line assay statistical method described by Finney, 1967. Measurements were made in plasma of normal and growth retarded fetal guinea pigs at 60-63 days of gestation. Each fetal stimulation was expressed as a percentage of the standard. Data of stimulation at 20% of the serum were used for statistical analysis using the paired *t*-test.

II.7. Tissue-metabolite determinations

II.7.1. Cellular composition of liver and brain

Tissue samples of the left liver lobe and of the parietal cortex of the brain of 49-51 and 60-63 day fetal guinea pigs from the control and operated uterine horns were homogenized in 10% trichloroacetic acid. The homogenate was kept on ice for 30 minutes. After centrifugation at 1500 g for 15 min. at 2° C the sediment was used for determination of protein, DNA and RNA.

II.7.1.1. Protein measurement

Protein was determined with the Folin-Ciocalteu phenol reagent according to Lowry *et al.*, (1951).

II.7.1.2. Determination of DNA

Tissue deoxyribonucleic acid content was determined by the method of Burton (1956).

II.7.1.3. Determination of RNA

Tissue ribonucleic acid content was determined by the method of Schneider (1957).

II.7.1.4. Microscopy and cellcounting

Fetal liver samples were rapidly fixed with Susa's fixative and after embedding in wax, 5μ sections were stained with haematoxylin and eosin. Parenchymal and haematopoietic cell numbers were counted in a minimum of twelve areas of each liver section. Surface area and volume of the hepatocytes and haematopoietic cells were determined by planimetry.

II.7.2. Determinations in liver, heart and skeletal muscle

II.7.2.1. Tissue glycogen measurement

After homogenization in 0.3M $HClO_4$ tissue glycogen in liver, heart and skeletal muscle was determined as glucose after enzymic digestion with amyloglucosidase (Faulkner and Jones, 1976).

II.7.2.2. Tissue triacylglycerol determination

Tissue samples of liver and heart were homogenized with 9 volumes of 100 mM potassium phosphate (pH 6.1). The homogenate was extracted for 10 min. with 6 vol. of redistilled chloroform (Itaya and Ui, 1965). To remove polar lipids 6 ml of the

chloroform extract was shaken with 0.5 g of silicic acid for 10 min. It was then evaporated to dryness under N_2. Lipids were saponified at 60° C for 30 min. after addition of 0.5 ml of 0.5 M KOH in ethanol (95%, w/v), then 1 ml of 0.15 M $MgSO_4$ was added and the $Mg(OH)_2$ formed removed by centrifugation at 1500 g for 10 min. Glycerol in the supernatant was determined enzymically by using glycerol kinase. Tripalmitin was used as a standard; its mean recovery was 94.2 ± 5.3% (n = 6) (± SD). No significant phospholipid was detected after silicic acid extraction.

II.7.3. Measurement of liver metabolites

Approximately 3-5 min. after anaesthetizing the mother small 49-51 and 60-63 day fetuses of the operated uterine horn were exposed through caesarean section. The left lobes of the fetal liver were freeze-clamped (Wollenberger, 1960) while the umbilical circulation was intact. The time between maternal injection and freeze clamping the fetal liver was 5-7 min. The hepatic tissue was powdered under liquid nitrogen with a ceramic pestle and mortar. Then 1 ml of 30% (w/v) $HClO_4$ was added and after thawing the tissue was homogenized with 5 ml of H_2O. After removing the protein by centrifugating at 75.000 g for 30 minutes the extract was adjusted to pH 3-4 with 20% KOH. Tissue metabolites were assayed on the supernatant by standard methods (Bergmeyer 1974). Hexose phosphates, pyruvate, phosphoenolpyruvate, dehydroxyacetone phosphate and isocitrate were determined in an Aminco Bowman Spectrofluorimeter (Silver Spring, Maryland, USA). The remaining metabolites were assayed spectrophotometrically with the use of a Pye-Unicam spectrophotometer or a Gilford Recording spectrophotometer. Inorganic phosphate was determined colorimetrically (Weil-Malherbe and Green, 1951) and ribulose-5-phosphate was determined by a radiochemical carboxylation assay (Faulkner and Jones, 1978). Results were compared with determination of normal fetal livers at the same gestational ages using the same techniques (Faulkner and Jones, 1976b). These previously published values were identical to those in the livers of fetuses from sham-operated animals.

II.8. Measurement of enzyme activities in liver, heart and skeletal muscle

II.8.1. General tissue preparation

Tissue samples kept on ice were obtained as described in chapter II.3. Samples of fetal liver and samples of fetal heart (after removal of blood) and skeletal muscle after being cut into small pieces were homogenized using a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle. Various homogenizing buffers were used according to the enzyme assayed. Enzyme assays were carried out using a Pye-Unicam SP 1800 or a Gilford 2400 spectrophotometer.

II.8.2. Determination of enzymes in the liver

II.8.2.1. Enzymes of carbohydrate metabolism

For determination of hexokinase activity (E.C. 2.7.1.1.) liver tissue was homogenized in 20 volumes of 25 mM Tris-HCl, pH 7.5, containing 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM EDTA and 0.1 mM dithiothreitol and activity was assayed at 37° C in cytosolic and particulate fractions as described by Faulkner and Jones, 1976a. Pyruvate kinase activity (E.C. 2.7.1.40) was determined in homogenates of liver in 10 mM Tris-HCl, pH 7.5, containing 0.1 mM FDP, 1 mM $MgCl_2$ and 0.1 mM dithiothreitol (50% w/v). Homogenates were centrifuged at 180.000 g for 45 min. at 2° C and the supernatant used for all experiments. Pyruvate kinase was assayed at 37° C as described by Faulkner and Jones, 1975a. For determination of the activities of phosphofructokinase (E.C. 2.7.1.11), aldolase (E.C. 4.1.2.13), α-glycerophosphate dehydrogenase (E.C. 1.1.1.8) and enolase (E.C. 4.2.1.11) liver samples were homogenized in an equal volume of 50 mM Tris HCl, pH 7.5, containing 5 mM $MgCl_2$, 1 mM EDTA and 0.1 dithiotreitol. Homogenates were centrifuged for 1 hr at 150.000 g and the supernatants used for enzyme determinations. All four enzymes were assayed by continuous spectrophotometric recording of pyridine nucleotide oxidation or reduction at 37° C as described by Faulkner and Jones (1975b). Pyruvate carboxylase (E.C. 6.4.1.1.) and phosphoenolpyruvate carboxykinase (E.C. 4.1.1.32) were assayed at 30° C by radiochemical carboxylation assays as described by Jones and Ashton (1976a), after extraction from the liver as described by Ballard and Hanson (1967). For the determination of fructose 1,6-diphosphatase (E.C. 3.1.3.11) and glucose 6-phosphatase (E.C. 3.1.3.9.) liver tissue was prepared as described by Jones and Ashton (1976a). Fructose 1,6-diphosphatase was assayed at 37° C as described by Jones and Ashton (1976a) and glucose 6-phosphatase was assayed at 37° C as described by Harper (1963).

II.8.2.2. Enzymes of lipid biosynthesis

For determination of some enzymes involved in lipid biosynthesis and NADPH-production liver tissue was prepared as described by Jones and Ashton, (1976b). ATP citrate lyase (E.C. 4.1.3.8.), glucose 6-phosphate dehydrogenase (E.C. 1.1.1.49), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44), isocitrate dehydrogenase (E.C. 1.1.1.42) and "malic" enzyme (E.C. 1.1.1.40) were extracted and assayed spectrophotometrically at 25° C as described by Jones and Ashton, (1976b). Acetyl-Co A carboxylase (E.C. 6.4.1.2.) was extracted and measured by radiochemical carboxylation at 25° C as described by Jones and Ashton, (1976b). Fatty acid synthetase was determined at 37° C in liver extracts by a modified method of Wakil *et al.* (1958) as described by Jones and Ashton, (1976b).

II.8.2.3. Enzymes of amino acid metabolism

The activities of alanine aminotransferase (E.C. 2.6.1.2.) and aspartate

aminotransferase (E.C. 2.6.1.1.) were determined by the method of Bergmeyer and Bernt, (1963). Tyrosine aminotransferase (E.C. 2.6.1.5.) activity was assayed as described by Diamondstone, (1966).
Valine aminotransferase (E.C.2.6.1.3) activity was determined by the method of Aki and Ichihara, (1970). Glutamate dehydrogenase (E.C. 1.4.1.3.) was assayed as described by Hogeboom and Schneider, (1953).

II.8.2.4. Enzymes of urea synthesis

Liver was homogenized in 9 volumes of ice cold water, for 30 seconds in an Ultra-Turrax homogeniser. The resulting homogenate was used for the determination of enzyme activity. Carbamylphosphate synthetase I (E.C. 2.6.2.5.) was assayed essentially as described by Marshall, Metzenberg and Cohen, (1958). The assay contained 100 mM triethanol amine (pH 7.4), 50 mM NH_4HCO_3, 10 mM L-ornithine, 10 mM ATP, 30 mM $MgSO_4$, 20 mM phosphoenolpyruvate, 10 mM N-acetyl-L-glutamate, 2 units of ornithine transcarbamylase, 10 units of pyruvate kinase and extract to 1.0 ml. This was incubated at 37° C and gassed with CO_2 for 20 min. The assay was stopped and citrulline determined as described for ornithine transcarbamylase. Ornithine transcarbamylase (E.C. 2.1.3.3.) was assayed essentially as described by Snodgrass (1968). The assay contained 200 mM triethanolamine-HCl (pH 7.6), 5 mM carbamylphosphate, 2 mM L-ornithine and extract to 1 ml. After starting the reaction with substrate the suspension was incubated for 10 minutes at 37° C. The reaction was stopped with 1 ml of 10% (w/v) trichloroacetic acid. After removing protein, 0.5 ml of the supernatant was added to 3 ml of an acid solution prepared from 2 parts of a solution containing in 1 liter, 250 ml of concentrated H_3PO_4, 250 ml of concentrated H_2SO_4, 2.5 g of ferric ammonium sulphate and 3.7 g of antipyrine and 1 part of a solution containing 0.4% (w/v) diacetylmonoxine in 7.5% (w/v) NaCl. This was placed in boiling water for 15 minutes then cooled in water to room temperature. Absorbance at 464 nm was proportioned to citrulline concentration. Argininosuccinate synthetase (E.C. 6.3.4.5.) was assayed essentially as described by Schimke (1962). The assay medium contained 100 mM potassium phosphate (pH 7.4), 8 mM citrulline, 8 mM aspartate, 5 mM ATP, 10 mM $MgSO_4$, 20 mM phosphoenolpyruvate, 10 units of pyruvate kinase, 8 units of argininosuccinase (E.C. 4.3.2.1.) and 20 units of arginine and extract to 0.5 ml. The reaction was started with substrate and terminated as described for arginase except that urea standards were run in the presence of citrulline. Arginase (E.C. 3.5.3.1.) was assayed essentially as described by Schimke (1970). The assay contained 100 mM glycine (pH 9.6), 5 mM $MnCl_2$, 200 mM L-arginine and extract to 1 ml. Before the assay the extract was preincubated in 150 mM glycine (pH 9.6) and 15 mM $MnCl_2$ for 20 minutes at 55° C. The arginase reaction was started by adding substrate and after incubation at 37° C for 10 minutes the reaction was stopped with 0.25 ml of 20% (w/v) trichloroacetic acid.

After removal of protein 0.3 ml of the supernatant was added to 2 ml of an acid

solution, containing in 1 liter, 250 ml of concentrated H_3PO_4, 100 ml of concentrated H_2SO_4 and 1 ml of 1 M $FeCl_3$, than 0.1 ml of 3% (w/v) α-isonitroso propiophenone in 95% (v/v) ethanol was added. This was incubated in a boiling water bath for 60 minutes, cooled in iced water and then urea was determined by reading absorbance at 540 nm.

II.8.3. Determination of enzymes in the heart and skeletal muscle

For the determination of enzyme activities fetal hearts were removed, cut open and blotted to remove blood. For enzyme activity measurements in skeletal muscle the upper hind limb muscles were removed. Tissue treatment, homogenization and separation into particulate and cytosolic fraction was as described by Rolph, Jones and Parry, (1981). The activities of isocitrate dehydrogenase (E.C. 1.1.1.42) citrate synthetase (E.C. 4.1.3.7.), alanine aminotransferase (E.C. 2.6.1.2.), lactate dehydrogenase (E.C. 1.1.1.27), phosphofructokinase (E.C. 2.7.1.11), glucose 6-phosphate dehydrogenase (E.C. 1.1.1.49) and 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44) were assayed at 25° C in a Pye-Unicam SP 1800 spectrophotometer as described by Rolph, Jones and Parry, (1981). Ca^{++}-ATPase (E.C. 3.6.1.3.) was assayed at 30° C and Na^+, K^+-ATPase (E.C. 3.6.1.3.) at 45° C, both also as described by Rolph, Jones and Parry, (1981).

II.9. Liver incorporation experiments

II.9.1. Carbohydrate metabolism

For incorporation experiments liver slices (appr. 0.3 mm thick) were incubated in 3 ml of Krebs bicarbonate buffer containing 10 mM glucose and the radioactive label for 1 hour at 37° C, with shaking at 1.5 Hz and under an atmosphere of 95% O_2 and 5% CO_2. The incubation condition and measurement of incorporation was generally as described by Jones and Ashton (1976 a, b). For the measurement of glucose synthesis the incubate was deproteinized with 0.4 M $HClO_4$ and after neutralization with 2 M KOH an aliquot was applied to a column (1 x 4 cm) of Dowex 50 (H^+ form, 100-200 mesh) on top of a column (1 x 8 cm) of Dowex 1 x 8 (acetate form, 100-250 mesh) and ^{14}C glucose was recovered in 20 ml of H_2O which was then freezedried. When glycerol was used as precursor neutralized extract was first incubated for 60 min. at 30° C with 4 mM ATP, 5 mM $MgCl_2$, 1 mM phosphoenolpyruvate, pyruvate kinase (4 units/ml) and glycerol kinase (1 unit/ml) before application to the columns. Pretreatment of extracts from incubations with the other precursors did not influence the yield of ^{14}C glucose although it depressed the blank values. Blank values for each incubation were obtained by converting the glucose to glucose 6-phospate prior to column separation and these values were subtracted from the values for total incorporation into glucose. Between 88-95% of the total counts could be converted to glucose 6-phosphate. The rate of ^3H_2O production was determined as described by Katz *et al.*, (1975).

For the measurement of O_2-consumption and CO_2 production, liver slices were incubated in 3 ml of Krebs phosphate buffer containing 10 mM glucose for 1 h. at 37° C in Warburg flasks with an atmosphere of 100% O_2. The liberated CO_2 was collected in 0.2 ml. of 2 m. NaOH, contained within the centre well. This CO_2 was precipitated as $BaCO_3$ by adding the contents of the centre well and its washings to 5 ml. of 1 M Barium acetate. The precipitate was washed twice with 5 ml. of H_2O, then counted in 10 ml. of a dioxane based scintillation fluid.

II.9.2. Lipid metabolism

The synthesis of lipids by liver slices of fetal guinea pigs was determined as previously described by Jones and Ashton, (1976a) and Jones and Fellows, (1981).

II.9.3. Amino acid metabolism and urea synthesis

Liver slices of about 100 mg with a thickness of 0.3 mm were incubated in Krebs phosphate buffer containing 10 mM (1 μCi) of the appropriate amino acid and 10 mM glucose in Warburg flasks at 37° C with shaking at 1.5 Hz. Oxygen consumption was linear for at least 2 hrs. The production of $^{14}CO_2$ was determined as described in II.9.1. and this was linear for at least 60 minutes.

For determination of urea synthesis liver slices of about 100 mg with a thickness of 0.3 mm were incubated in Krebs-bicarbonate buffer containing 60 mM NH_4Cl, 20 mM lactate, 4 mM L-ornithine, 10 mM glucose and 27 mM $H^{14}CO_3$ (1 μCi) in 3 ml under 95% O_2 and 5% CO_2. The slices were incubated for 60 minutes at 37° C and with shaking at 1.5 Hz. At the end of the incubation the suspension was homogenized after adding 0.2 ml of 30% (w/v) $HClO_4$ and protein was removed. The supernatant was neutralized with KOH. The supernatant was then placed into a scaled Warburg flask (which contained 0.2 ml of 20% KOH in the centre well) together with 0.5 units of urease for 2 hrs. The contents of the centre well containing liberated $^{14}CO_2$ were added to 5 ml of 2M Barium acetate. The precipitated $Ba^{14}CO_3$ was washed twice with 2 ml of 2M Barium acetate and once with 2 ml of water.

Then radioactivity was determined, after suspension in 10 ml of aquasol, by counting in a Philips PW 4510 liquid-scintillation counter. Counting efficiency was estimated by the channels ratio method using an external standard and a series of carbon tetrachloride quenched [^{14}C] hexadecane standards.

II.10. Histology

II.10.1. Electron microscopic studies

For electron microscopy liver, heart and skeletal muscle of the fetal guinea pigs were fixed with 3% glutaraldehyde in 0.1 M sodiumphosphate (pH 7.4), postfixed in 1% OsO_4, then after dehydration and embedding in epoxy resin, post stained in

methanolic uranyl acetate and lead citrate (Reynolds, 1963). A Philips 301 electron microscope was used.

II.10.2. Light microscopic studies

Samples of the upper limbs of fetal guinea pigs were treated as described for the liver in section II.7.1.4.

II.11. X-ray photography

For X-ray photography of the skeleton guinea pigs were frozen at -20° C in standard positions of the four limbs. Anteroposterior X-ray pictures were taken using a Philips standard X-ray apparatus. Photographic prints were made of the left fore limb for detail studies. Epiphysial development of the fetuses was studied and rated by a scoring system giving 1 to 5 points for central ossification and appearance of the epiphysial disk of the left upper and lower limbs and the ossification of carpal and tarsal bones.

II.12. Statistical analysis

Where appropriate results are expressed as means ± standard deviation with the number of observations in parentheses; significance was determined by using a two tailed Student's *t* test. Linear regression was determined by standard statistical procedure.

CHAPTER III THE INFLUENCE OF INTRA-UTERINE GROWTH RETARDATION ON ORGAN GROWTH AND COMPOSITION

III.1. Introduction

During the approximately 67 days of intrauterine development of the guinea pig the fetus grows at a linear rate from approximately day 40 onwards. There is a short decrease just before birth and again a linear growth rate during neonatal development at approximately one week after birth. This perinatal growth pattern is comparable to that of many other species including man. In this chapter the effects of the maternal surgery on this growth pattern are described. The growth of individual fetal organs and of the placenta together with the effect of uterine artery ligation on their growth was also investigated.

Earlier reports on the results of experimental intrauterine growth retardation in other species (Wigglesworth, 1964, Roux *et al.*, 1970, Myers *et al.*, 1971) mention various differences between cellular composition of organs from normal and growth retarded fetuses. For comparison similar measurements of protein, DNA, RNA and microscopic observations of various fetal tissues were made and are described in this chapter. The cellstructure was in addition more closely studied by means of electron microscopy.

Tissue glycogen and fat in fetal liver, heart and skeletal muscle, both become important energy stores in the last fase of fetal life and the beginning of neonatal life. Therefore both these metabolites were measured in normal and small fetuses. The results of these measurements are also mentioned and discussed in this chapter.

III.2. Fetal size and weight

Of 372 pregnant female guinea pigs that were operated (table III.1) 7 animals died, 9 aborted during the weeks following the surgery and 22 animals were misdatings. 334 animals had their fetuses delivered. In 108 cases the fetuses from the operated horn had a birth weight that was not significantly different from that of the fetuses from the control uterine horn.

In 116 cases all fetuses from the operated horn were small but had recently died or were already partially reabsorbed. Both these groups of fetuses were not studied. In 110 cases the operated horn of the uterus contained at least one live fetus weighing approximately 60% or less than that from the other horn (fig. III.3.). Of these fetuses

Table III. 1. Results of uterine artery ligation.

Results of operations	Number	Percentage
Fetuses within the normal weight range	108	29
Fetuses <60% of the normal mean weight	110	30
Fetuses small but dead or reabsorbed	116	31
Total no. of deliveries	334	90
Maternal death	7	2
Abortion	9	2
Misdating	22	6
Total no. of operations	372	100

	Fetal weight (g.)	
	49 - 51 days	60 - 63 days
Control horn	39.6 ± 4.3 (34)	85.0 ± 11.0 (60)
Ligated horn	19.4 ± 3.4 (34)°	38.6 ± 7.7 (60)°

°P<0.001

Guinea pigs were operated at day 30 of pregnancy and fetuses were delivered at days 49 - 51 or 60 - 63 of pregnancy as described in chapter II. Results are expressed as means ± S.D. with the number of observations in parentheses.

Table III. 2. Sex distribution of normal and small fetuses.

	49 - 51 days		60 - 63 days	
	Normal	Small	Normal	Small
Bodyweight (g.)				
Total group	39.6 ± 4.3 (34)	19.4 ± 3.4 (34)	85.0 ± 11.0 (60)	38.6 ± 7.7 (60)
Male	40.0 ± 4.5 (21)	19.3 ± 3.3 (18)	84.1 ± 10.3 (28)	38.3 ± 7.6 (27)
Female	38.8 ± 4.0 (13)	19.5 ± 3.5 (16)	85.7 ± 11.6 (32)	38.7 ± 7.9 (33)
Length nose-tail (cm.)				
Total group	10.8 ± 0.6 (32)	8.5 ± 0.7 (32)	14.6 ± 0.8 (60)	11.1 ± 1.0 (60)
Male	10.8 ± 0.6 (19)	8.5 ± 0.7 (17)	14.7 ± 0.6 (28)	11.1 ± 1.1 (27)
Female	10.7 ± 0.5 (13)	8.4 ± 0.8 (15)	14.5 ± 0.9 (32)	11.1 ± 0.9 (33)

The results are means ± S.D. with the number of observations in parentheses. All differences between normal and small fetuses are significant ($P < 0.001$). All differences between male and female fetuses are not significant.

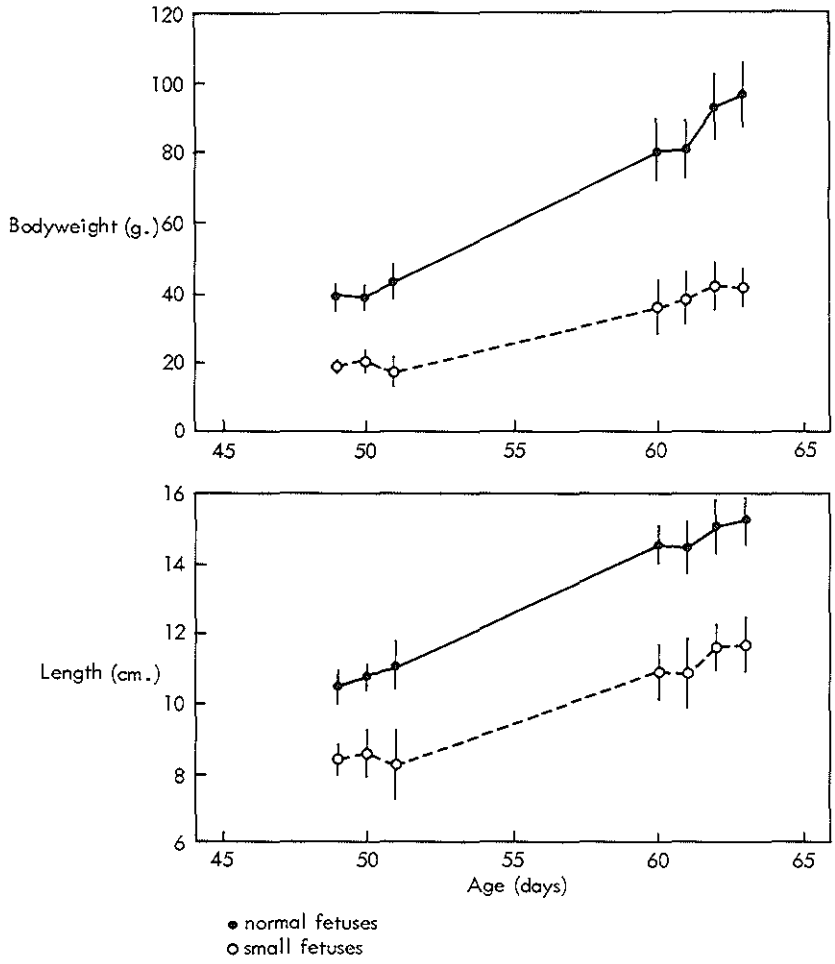

Fig. III. 1. The growth of fetal guinea pigs between 49 and 63 days of gestation. Normal fetuses were taken from the unoperated uterine horn and small fetuses from the uterine horn in which the uterine artery was ligated at day 30 of pregnancy.
The vertical bars represent 2 S.D.

94 were used for fetal studies. 34 were delivered at 49-51 days and 60 at 60-63 days of gestational age. Their mean weights and lengths are given in table III.1 and fig. III.1. There were no significant differences in weight or length of males or females from either of the groups (table III.2). All fetuses from the control horn were within the normal weight range for this strain of guinea pig (fig. III.2).

In this and the following chapters it will be shown that the numerous effects of intrauterine growth retardation are much more pronounced in the very small fetuses. Therefore the groups of small fetuses have been subdivided arbitrarily in those that

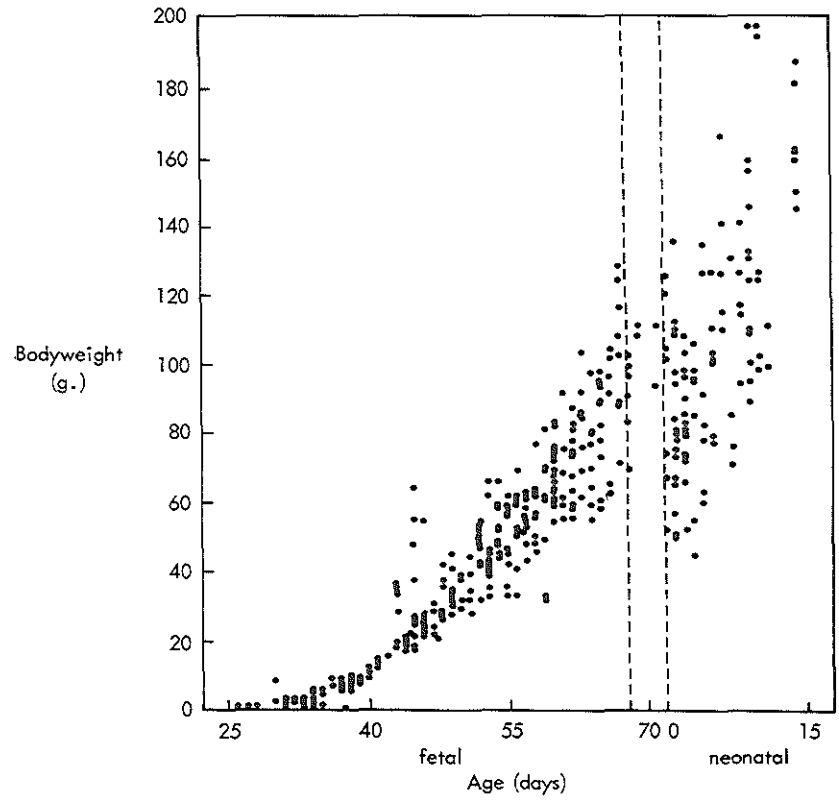

Fig. III. 2. Fetal and neonatal growth curve of the Dunkin-Hartley strain guinea pigs used in this study.

were "moderately" and "severely" growth retarded (table III.3). For practical reasons weight groups of 20-25 g and < 20 g have been used at 49-51 days representing approximately 60 and 40% of normal weight at that age. At 60-63 days weight groups of 35-50 g and < 35 g were used (respectively 50 and 35% of normal weight) (table III.3). If a sufficient number of observations was available this subdivision was used in this and following chapters.
The results of 8 "sham" operated animals are shown in table III.4. The fetuses from both uterine horns were all within the normal weight range (table III.4).

III.3. Neonatal growth

Nineteen operated guinea pigs were allowed to deliver at the normal time (66-68 days). Fourteen of these gave birth to at least one small newborn, which in 6 cases had died recently. From the other 8 deliveries one small newborn (mean birthweight 51.7 ± 7.4 g) together with one normal weight newborn (mean birthweight 100.3 ±

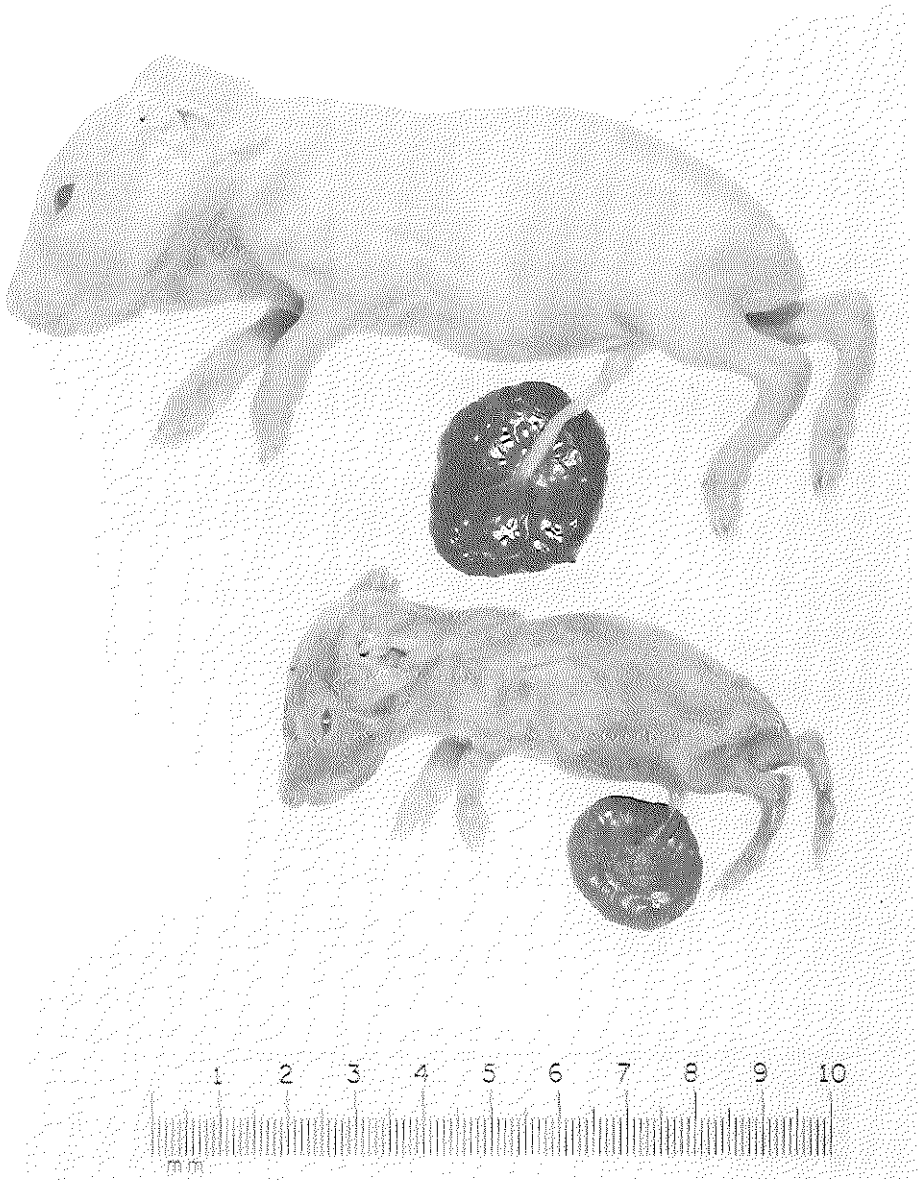

Fig. III. 3. Normal and small 61 days fetal guinea pigs after 30 days of uterine artery ligation. The weight of the small fetus was 18 g. and that of its normal littermate from the unoperated horn was 61 g. Both were born alive (reproduced from Lafeber *et al.*, 1977).

Table III. 3. Subdivision of small fetuses.

49 - 51 days				
	Normal	Small	20 - 25 g.	<20 g.
Mean body weight (g.)	39.6 ± 4.3	19.4 ± 3.4	22.6 ± 1.1	16.8 ± 2.0
Range (g.)	31.6 - 53.5	11.5 - 25.0	20.6 - 25.0	11.5 - 19.5
Mean % of normal	100	49	57	42
Range (%)	80 - 135	29 - 63	52 - 63	29 - 49
Number	34	34	15	19

60 - 63 days				
	Normal	Small	35-50 g.	<35 g.
Mean body weight (g.)	85.0 ± 11.0	38.6 ± 7.7	42.8 ± 4.9	30.2 ± 4.8
Range (g.)	60.9 - 114.7	18.1 - 50.0	35.7 - 50.0	18.1 - 34.9
Mean % of normal	100	45	50	35
Range (%)	72 - 135	21 - 60	42 - 60	21 - 41
Number	60	60	40	20

Mean bodyweight ± S.D.

Table III. 4. Results of "sham" operated pregnant guinea pigs.

	Fetuses from control uterine horn	Fetuses from "sham" operated uterine horn
Bodyweight (g.)	75.8 ± 10.5 (8)	72.2 ± 6.9 (8)
Bodylength (cm.)	13.6 ± 0.3 (8)	13.5 ± 0.3 (8)
Placentaweight (g.)	4.71 ± 0.95 (8)	4.04 ± 0.36 (8)
Sex distribution		
Males	6	4
Females	2	4

Guinea pigs were "sham" operated at day 30 of pregnancy and fetuses were delivered at 60 - 63 days of pregnancy as described in chapter II. The results are means ± S.D. with the number of observations in parentheses. All differences between fetuses from the control horn and the "sham" operated horn are not significant.

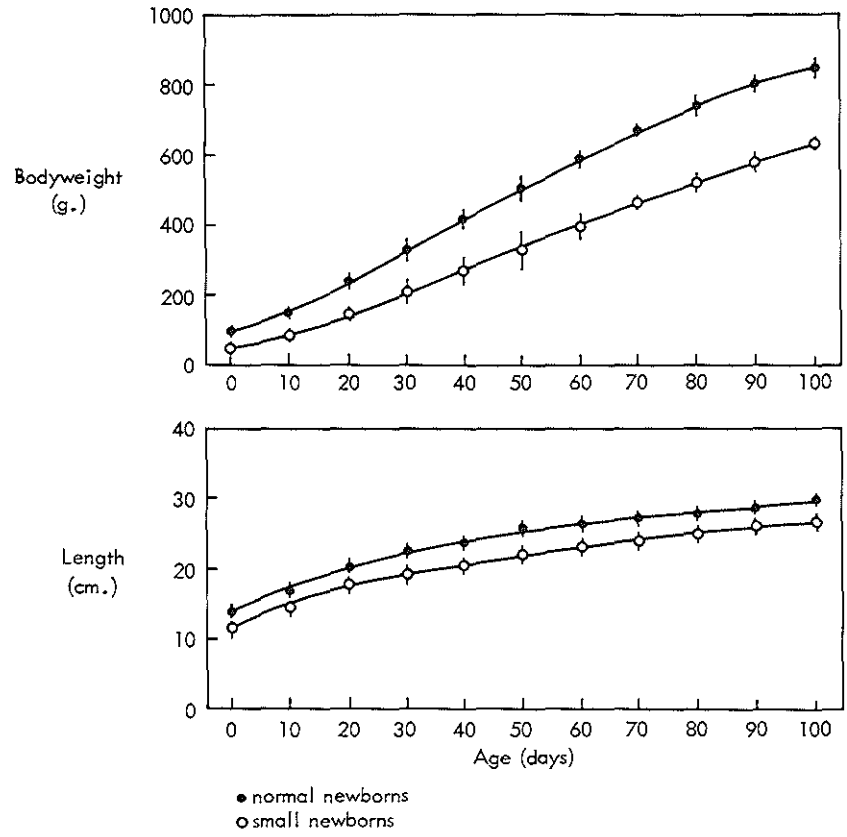

Fig. III. 4. The growth of newborn guinea pigs. The results represent mean weight and length of 2 - 8 neonates. Vertical bars represent 2 S.D.

4.4 g) were in each case selected and kept at 20° C with the mother for 4 weeks. Of the small newborns 2 died within 48 hours and a further 4 died within 17-31 days. The growth rate for both normal and small newborns was almost linear for up to 100 days (fig. III.4).

In the normal newborn the growth rate increased from a mean fetal rate of 4.2 g/day between 50 and 60 days of pregnancy to 10.6 g/day between 10 and 20 days after birth (fig. III.5). By comparison the small fetuses had a growth of 1.7 g/day. This increased over 5 fold to 9.1 g/day during the same postnatal period (fig. III.5). Growth velocity expressed in cm/day (bodylength measured from nose to tail) decreased slightly in normal newborn guinea pigs over the first ten days, increased again and then progressively decreased (fig. III.6).

Small fetuses had a lower growth velocity during 50 and 60 days of pregnancy, but showed no decrease after birth and grew even faster during the first 10 postnatal days (fig. III.6).

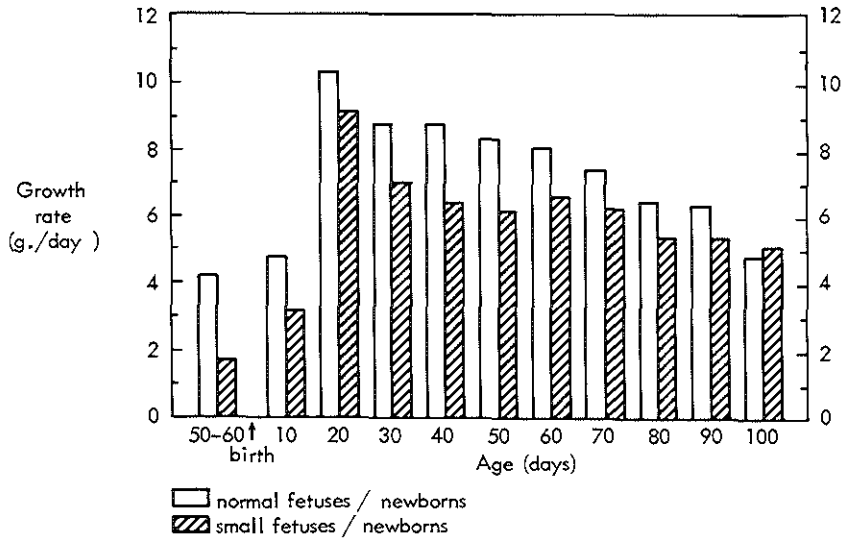

Fig. III. 5. Growth rate of normal and small fetal and newborn guinea pigs.

Fig. III. 6. Growth velocity of normal and small fetal and newborn guinea pigs.

Table III. 5. The weights of individual fetal organs and the placenta of normal and small fetuses at 49 - 51 days of gestation.

	49 - 51 days								
	Normal			Small					
				20 - 25 g.			< 20 g.		
Fetus									
Bodyweight	39.6	± 4.3	(34)	22.6	± 1.1 $^{\circ}$	(15)	16.8	± 2.0 $^{\circ}$	(19) $^{\bullet}$
Liver	2.79	± 0.54	(30)	1.23	± 0.12 $^{\circ}$	(13)	0.91	± 0.17 $^{\circ}$	(17) $^{\bullet}$
Heart	0.22	± 0.03	(27)	0.13	± 0.02 $^{\circ}$	(12)	0.11	± 0.03 $^{\circ}$	(15) $^{\blacktriangle}$
Lungs	0.97	± 0.15	(25)	0.50	± 0.14 $^{\circ}$	(12)	0.38	± 0.09 $^{\circ}$	(13) $^{\blacktriangle}$
Kidney	0.21	± 0.03	(25)	0.11	± 0.03 $^{\circ}$	(11)	0.10	± 0.02 $^{\circ}$	(14)
Spleen	0.090	± 0.025	(26)	0.037	± 0.008 $^{\circ}$	(12)	0.022	± 0.006 $^{\circ}$	(14) $^{\bullet}$
Perirenal adipose	0.77	± 0.22	(24)	0.31	± 0.09 $^{\circ}$	(10)	0.23	± 0.08 $^{\circ}$	(14) $^{\blacktriangle}$
Pancreas (mg.)	88	± 19	(24)	49	± 11 $^{\circ}$	(11)	27	± 8 $^{\circ}$	(13) $^{\bullet}$
Adrenal (mg.)	8.1	± 2.5	(10)	5.5	± 1.2 $^{\circ}$	(6)	6.5	± 2.4 $^{\triangle}$	(4)
Brain	1.82	± 0.16	(29)	1.57	± 0.34 $^{\circ}$	(13)	1.44	± 0.11 $^{\circ}$	(16) $^{\bullet}$
Cerebrum	1.55	± 0.10	(19)	1.35	± 0.10 $^{\circ}$	(9)	1.17	± 0.14 $^{\circ}$	(10) $^{\blacksquare}$
Cerebellum	0.13	± 0.02	(19)	0.11	± 0.01 $^{\triangle}$	(9)	0.12	± 0.03	(10)
Medulla	0.15	± 0.04	(19)	0.15	± 0.05	(9)	0.12	± 0.02	(10)
Brain/liver ratio	0.67	± 0.08	(27)	1.39	± 0.22 $^{\circ}$	(18)	1.60	± 0.29 $^{\circ}$	(19) $^{\blacktriangle}$
Placenta	4.15	± 0.54	(32)	2.70	± 0.57 $^{\circ}$	(15)	2.16	± 0.38 $^{\circ}$	(17) $^{\blacksquare}$
Fetal/placental weight ratio	9.5	± 1.0	(32)	8.4	± 1.8 $^{\triangle}$	(15)	7.8	± 1.3 $^{\circ}$	(17) $^{\blacksquare}$

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$ for comparison of normal and small fetuses

$^{\blacktriangle}P<0.05$ $^{\blacksquare}P<0.01$ $^{\bullet}P<0.001$ for comparison of the two groups of small fetuses

All results are expressed in grams (unless stated otherwise) as means ± S.D. with the number of observations in parentheses.

III.4. Effects on organ and placental weight

The effects of the ligation of the uterine artery on placental and fetal organ weight are summarized in tables III.5 and III.6. The small fetuses were subdivided (as

Table III. 6. The weights of individual fetal organs and the placenta of normal and small fetuses at 60 - 63 days of gestation.

	60 - 63 days		
	Normal	Small	
		35 - 50 g.	<35 g.
Fetus			
Bodyweight	85.0 ± 11.0 (60)	42.8 ±4.9° (40)	30.2 ±4.8° (20)●
Liver	4.30 ± 0.95 (57)	1.76 ±0.35° (36)	1.20 ±0.26° (21)●
Heart	0.47 ± 0.08 (59)	0.26 ±0.04° (37)	0.21 ±0.04° (22)●
Lungs	1.66 ± 0.26 (48)	0.87 ±0.18° (29)	0.51 ±0.20° (19)●
Kidney	0.35 ± 0.04 (44)	0.18 ±0.04° (29)	0.13 ±0.04° (15)●
Spleen	0.140 ± 0.026 (49)	0.056 ±0.014° (31)	0.039 ±0.015° (18)●
Perirenal adipose	1.71 ± 0.30 (43)	0.81 ±0.21° (29)	0.56 ±0.23° (14)■
Pancreas (mg.)	134 ±31 (44)	63 ±18° (27)	58 ±20° (17)
Adrenal (mg.)	17.6 ± 5.1 (36)	13.3 ±4.4° (19)	12.6 ±4.1° (17)
Brain	2.65 ± 0.18 (59)	2.37 ±0.17° (38)	2.16 ±0.22° (21)●
Cerebrum	2.11 ± 0.15 (37)	1.82 ±0.25° (22)	1.67 ±0.16° (15)▲
Cerebellum	0.27 ± 0.05 (35)	0.22 ±0.02° (20)	0.21 ±0.03° (15)
Medulla	0.22 ± 0.04 (35)	0.20 ±0.04□ (20)	0.17 ±0.04° (15)▲
Brain/liver ratio	0.68 ± 0.13 (56)	1.19 ±0.18° (31)	1.82 ±0.36° (25)●
Placenta	5.09 ± 0.89 (58)	2.63 ±0.39° (40)	2.29 ±0.36° (18)■
Fetal/placental weight ratio	16.7 ± 2.2 (58)	16.3 ±2.3 (40)	13.2 ±2.1° (18)●

△ P<0.05 □ P<0.01 °P<0.001 for comparison of normal and small fetuses

▲ P<0.05 ■ P<0.01 ●P<0.001 for comparison of the two groups of small fetuses

All results are expressed in grams (unless stated otherwise) as means ± S.D. with the number of observations in parentheses.

described in III.2) into those that were moderately and those that were severely retarded. The weights of all organs of the small fetuses were less than normal. The most striking effect of the uterine artery ligation was a large reduction in the weight of the visceral organs such as the liver and spleen with virtually no effect on the weight of organs like particularly the brain (tables III.5, III.6). The asymmetric nature

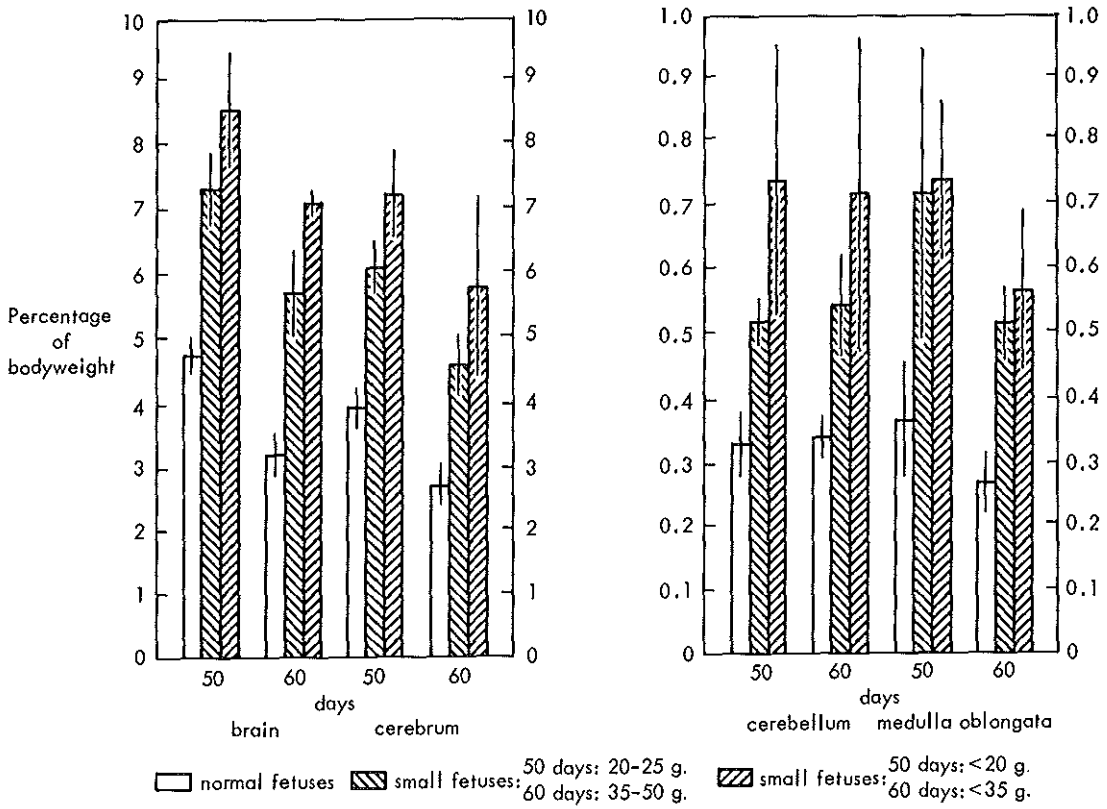

Fig. III. 7.The relative size of the total brain, cerebrum, cerebellum and medulla oblongata in normal and small fetuses at 50 days (49 - 51 days) and 60 days (60 - 63 days) of gestation. The results are means of 19 - 59 determinations. The vertical bars represent 2 S.D.

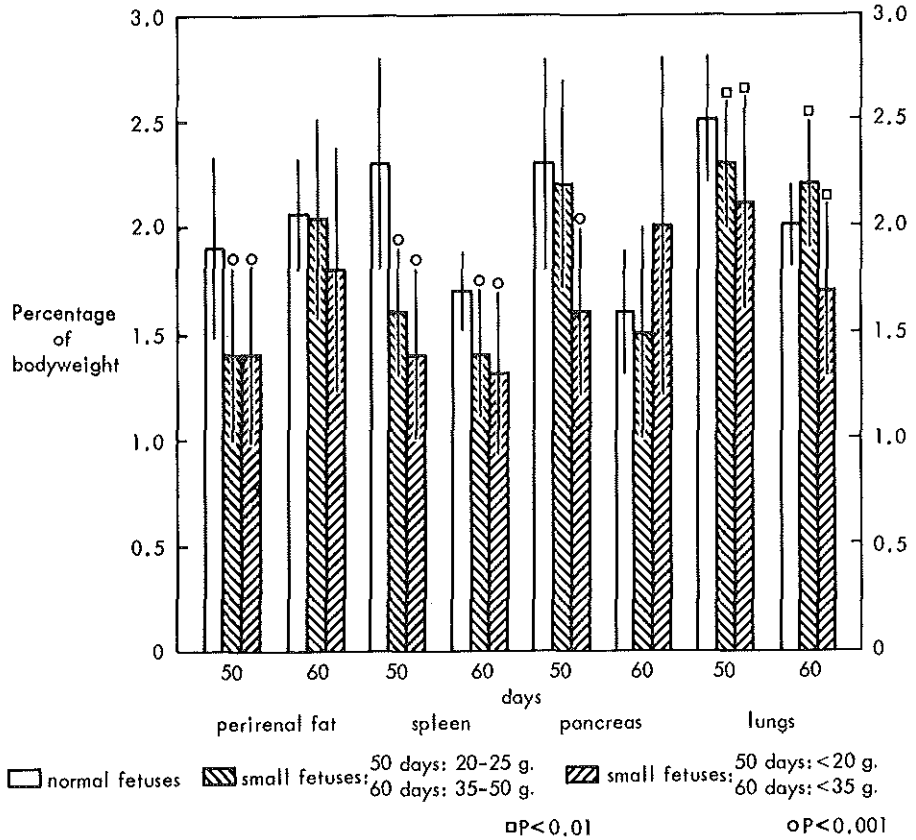

Fig. III. 8. The relative size of perirenal fat, spleen, pancreas and lungs in normal and small fetuses at 50 days (49 - 51 days) and 60 days (60 - 63 days) of gestation. The results are means of 24 - 49 determinations. The vertical bars represent 2 S.D.

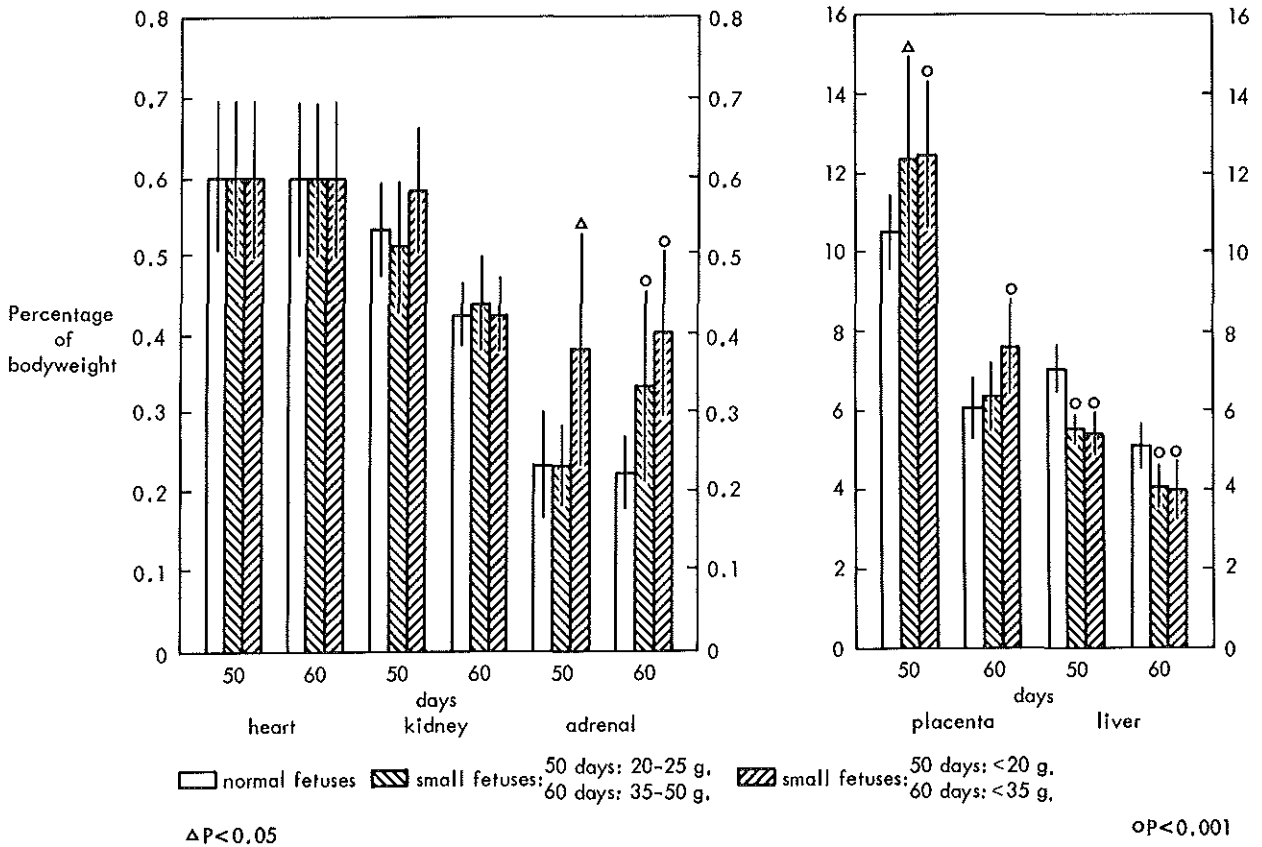

Fig. III. 9. The relative size of heart, kidney, adrenal gland, liver and placenta of normal and small fetuses at 50 days (49 - 51 days) and 60 days (60 - 63 days) of gestation. The results are means of 10 - 59 determinations. The vertical bars represent 2 S.D.

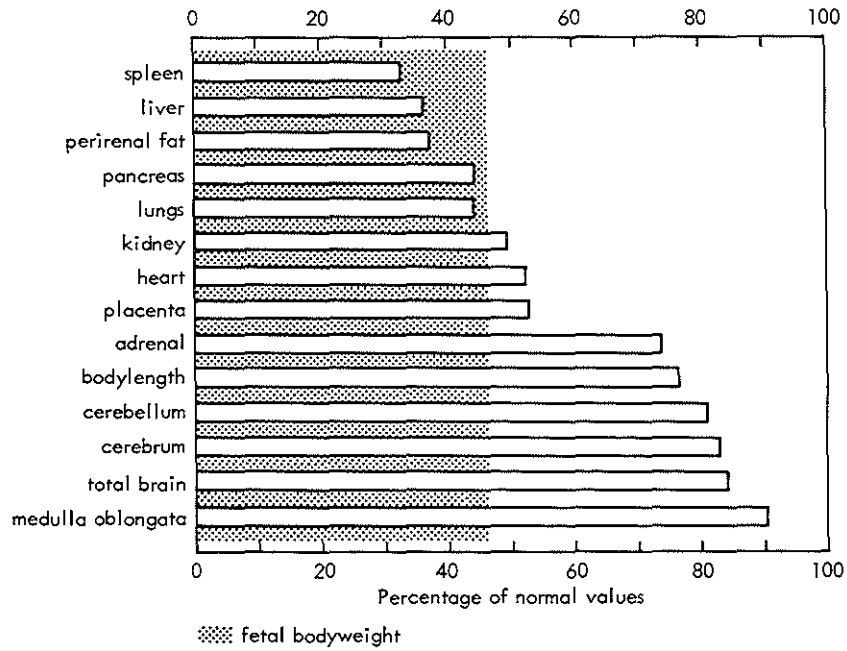

Fig. III.10. The weights of several fetal organs and placentas and body length in relation to fetal bodyweight of intrauterine growth retarded fetal guinea pigs both at 49-51 days and 60-63 days, expressed as a percentage of the values observed in their normal littermate fetuses.

of these changes is better shown when the weights are expressed as a percentage of bodyweight (fig. III.7, III.8, III.9). Brain and adrenal weight increased in proportion to bodyweight. The fall in the weight of the lungs, kidney and heart is more or less in proportion to bodyweight (fig. III.7, III.8, III.9).

The weight of the liver and the spleen fall to a much greater extent than the fall in bodyweight (fig. III.8, III.9). Some of the changes (e.g. brain) were more pronounced the smaller the fetus (fig. III.7).

The placental weight was reduced in small fetuses (tables III.3, III.6). The effect of this is a reduction in the fetal to placental weight ratio at 49-51 days and even more at 60-63 days in the severely retarded group (tables III.5, III.6). Although the placenta of small fetuses was always lower in weight, there was a considerable variability. Often the placenta contained large infarctions (fig. III.11). The correlation between placental and fetal bodyweight is shown in fig. III.12 for normal and small 49-51 and 60-63 days old fetuses. Although this correlation was good in the normal fetuses it was rather poor in the small fetuses (fig. III.12).

When the weights of organs from all normal fetuses are compared with those from all small fetuses (both at 49-51 and 60-63 days) the asymmetric differences become even more clear (fig. III.10).

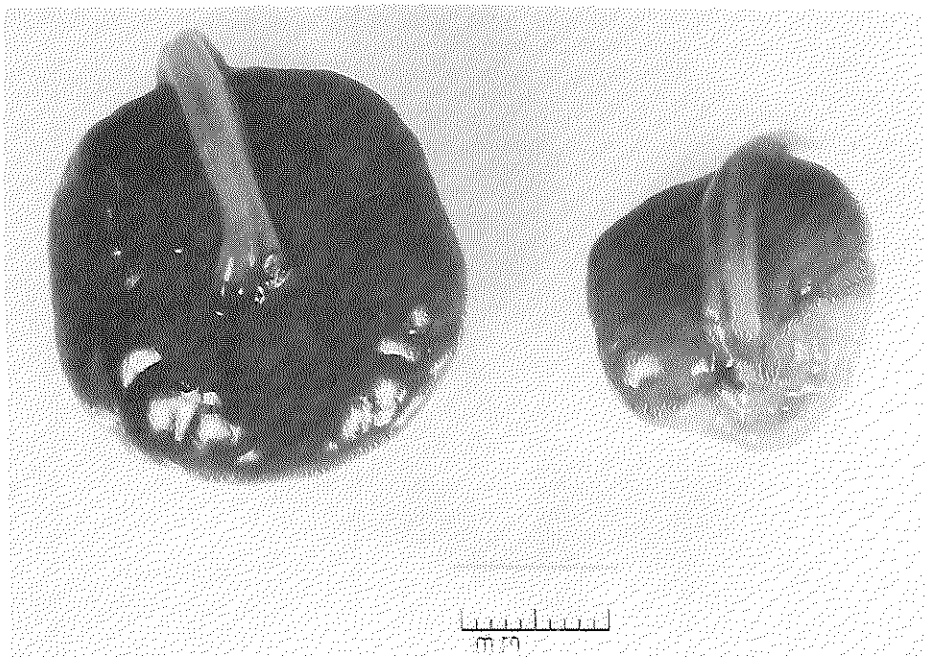

Figure III.11. Placentas of a normal (left) and intrauterine growth retarded (right) fetal guinea pig at 60 days of gestation (percentage of normal fetal bodyweight was 42%). Note the infarction in the placenta of the small fetus. (reproduced from Lafeber *et al.*, 1979).

III.5. Effects on organ composition

III.5.1. Cellular structure of liver and brain

III.5.1.1. Protein and nucleic acids

The protein concentration in the liver of the small fetuses at 49-51 days was significantly less than that in the normal (table III.7). At 60-63 days the variation between fetuses was too large to show a significant difference between the means. The livers of the fetuses < 35 g had a protein concentration that was $9.8 \pm 8.2\%$ (8) ($p < 0.01$) less than their littermate controls. Liver DNA concentrations were not significantly different (table III.7), but the 60-63 day fetuses of < 35 g had $11.9 \pm 8.9\%$ (8) less DNA/g of liver than their littermate controls ($p < 0.01$). The livers of these very small 60-63 day fetuses had a significantly higher RNA concentration (table III.7). Cerebral protein concentrations were not significantly different in the two groups of fetuses, while DNA and RNA concentrations were significantly lower in the small 60-63 day but not in the 49-51 day fetuses (table III.7).

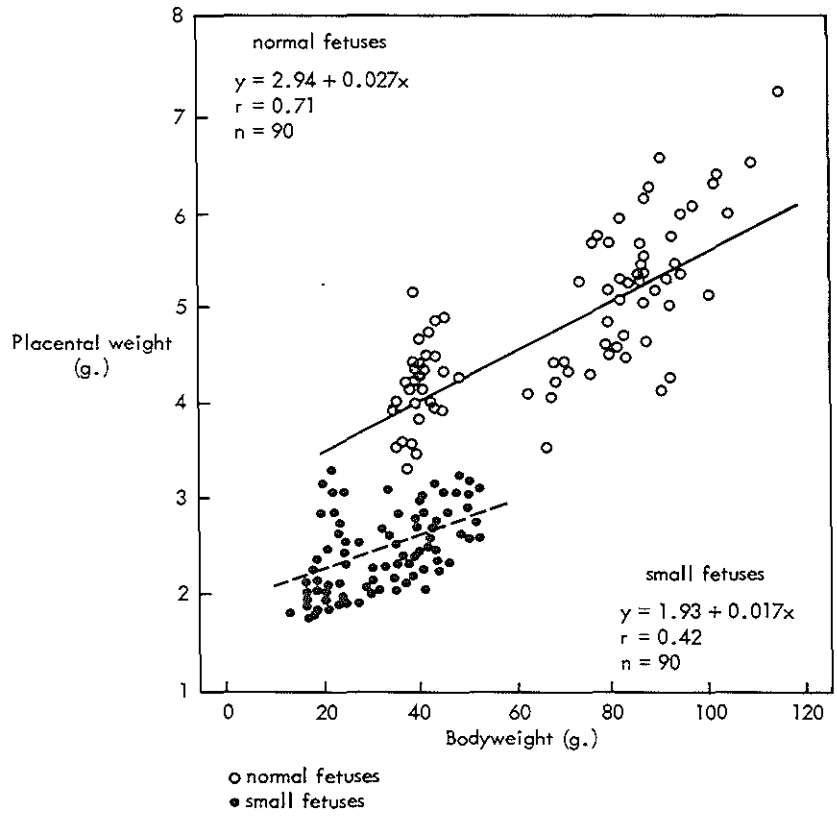

Fig. III.12. Relationship between placental and fetal bodyweights in normal and small fetal guinea pigs at 49-51 and 60-63 days of gestation (reproduced from Lafeber *et al.*, 1979).

III.5.1.2. Cell content of the liver

The fetal liver has many haematopoietic cells in addition to hepatocytes and the number of these falls between 40 days and term (table III.8, fig. III.14). In the small fetuses the number of haematopoietic cells per g. liver was higher than in the normal fetuses and this increase was more pronounced the smaller the fetuses (table III.8, fig. III.15, III.16, III.17, III.18).

Between 40 days and term the number of hepatocytes rises to a peak at about 50 days then falls at a time when the cell volume increases (table III.8, fig. III.13). In the livers of the small fetuses these changes are problably delayed (table III.8, fig. III.16, III.17, III.18).

Table III. 7. The protein and nucleic acid content of liver and brain of normal and small fetal guinea pigs.

49 - 51 days

	Normal	Small	
		20 - 25 g.	<20 g.
Bodyweight (g.)	40.6 ± 5.5 (18)	22.7 ± 1.6°(9)	17.0 ± 2.4°(10)
Liver			
Protein	127.0 ± 4.9 (18)	110.0 ± 6.3°(9)	107.0 ± 4.9°(10)
DNA	24.8 ± 2.5 (18)	25.2 ± 2.6 (9)	27.0 ± 2.9 (10)
RNA	14.2 ± 1.6 (18)	14.4 ± 1.6 (9)	15.7 ± 1.5 (10)
Brain			
Protein	57.6 ± 2.9 (6)		58.4 ± 4.9 (6)
DNA	3.9 ± 1.0 (6)		3.7 ± 1.1 (6)
RNA	6.8 ± 0.3 (6)		6.9 ± 0.3 (6)

60 - 63 days

	Normal	Small	
		35 - 50 g.	<35 g.
Bodyweight (g.)	84.6 ± 11.3 (16)	40.7 ± 2.4°(8)	30.8 ± 5.7°(8)
Liver			
Protein	92.1 ± 7.5 (16)	93.0 ± 9.1 (8)	86.1 ± 12.8 (8)
DNA	34.2 ± 5.8 (16)	32.9 ± 7.6 (8)	31.7 ± 6.0 (8)
RNA	12.5 ± 0.6 (16)	12.7 ± 0.7 (8)	13.3 ± 0.4□(8)
Brain			
Protein	74.0 ± 6.4 (16)	79.6 ± 7.6 (8)	67.2 ± 5.7 (8)
DNA	8.6 ± 1.0 (16)	7.1 ± 1.3△(8)	7.5 ± 0.7□(8)
RNA	9.3 ± 0.4 (16)	8.7 ± 0.6□(8)	8.7 ± 0.6□(8)

△P<0.05 □P<0.01 °P<0.001

All results are expressed in mg./g. wet tissue weight as means ± S.D. with the number of observations in parentheses.

Table III. 8. The cellular composition of the liver of normal and small fetal guinea pigs.

	49 - 51 days	
	Normal	Small
Bodyweight (g.)	41.4 ± 5.8 (6)	22.1 ± 5.1° (6)
Hepatocytes		
Cell number/ mm^3 (x 10^{-6})	0.95 ± 0.03 (6)	0.75 ± 0.13$^{\square}$ (6)
Cell volume (μ^3 x 10^{-3})	0.73 ± 0.02 (6)	0.61 ± 0.05° (6)
Fractional volume *)	0.70 ± 0.04 (6)	0.46 ± 0.10° (6)
Haematopoietic cells		
Cell number/ mm^3 (x 10^{-6})	1.14 ± 0.11 (6)	1.72 ± 0.14° (16)
Cell volume (μ^3)	29.2 ± 0.6 (6)	26.2 ± 2.5 (6)
Fractional volume *)	0.033 ± 0.004 (6)	0.045 ± 0.002° (6)

	60 - 63 days		
	Normal	Small	
		35 - 50 g.	<35 g.
Bodyweight (g.)	84.5 ± 10.1 (14)	43.8 ± 3.8° (8)	29.4 ± 6.3° (6)
Hepatocytes			
Cell number/ mm^3 (x 10^{-6})	0.55 ± 0.06 (10)	0.63 ± 0.09$^{\triangle}$ (8)	0.65 ± 0.04$^{\triangle}$ (6)
Cell volume (μ^3 x 10^{-3})	1.48 ± 0.23 (10)	1.05 ± 0.23° (8)	0.91 ± 0.09° (6)
Fractional volume *)	0.83 ± 0.06 (10)	0.64 ± 0.07° (8)	0.59 ± 0.03$^{\square}$ (6)
Haematopoietic cells			
Cell number/ mm^3 (x 10^{-6})	0.54 ± 0.22 (10)	0.76 ± 0.26$^{\triangle}$ (8)	1.00 ± 0.20$^{\triangle}$ (6)
Cell volume (μ^3)	38.1 ± 5.4 (10)	34.3 ± 4.0 (8)	32.8 ± 4.2 (6)
Fractional volume *)	0.021 ± 0.008 (10)	0.026 ± 0.010 (8)	0.034 ± 0.011 (6)

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are means ± S.D. with the number of observations in parentheses.

*) Proportion of section occupied by cell type.

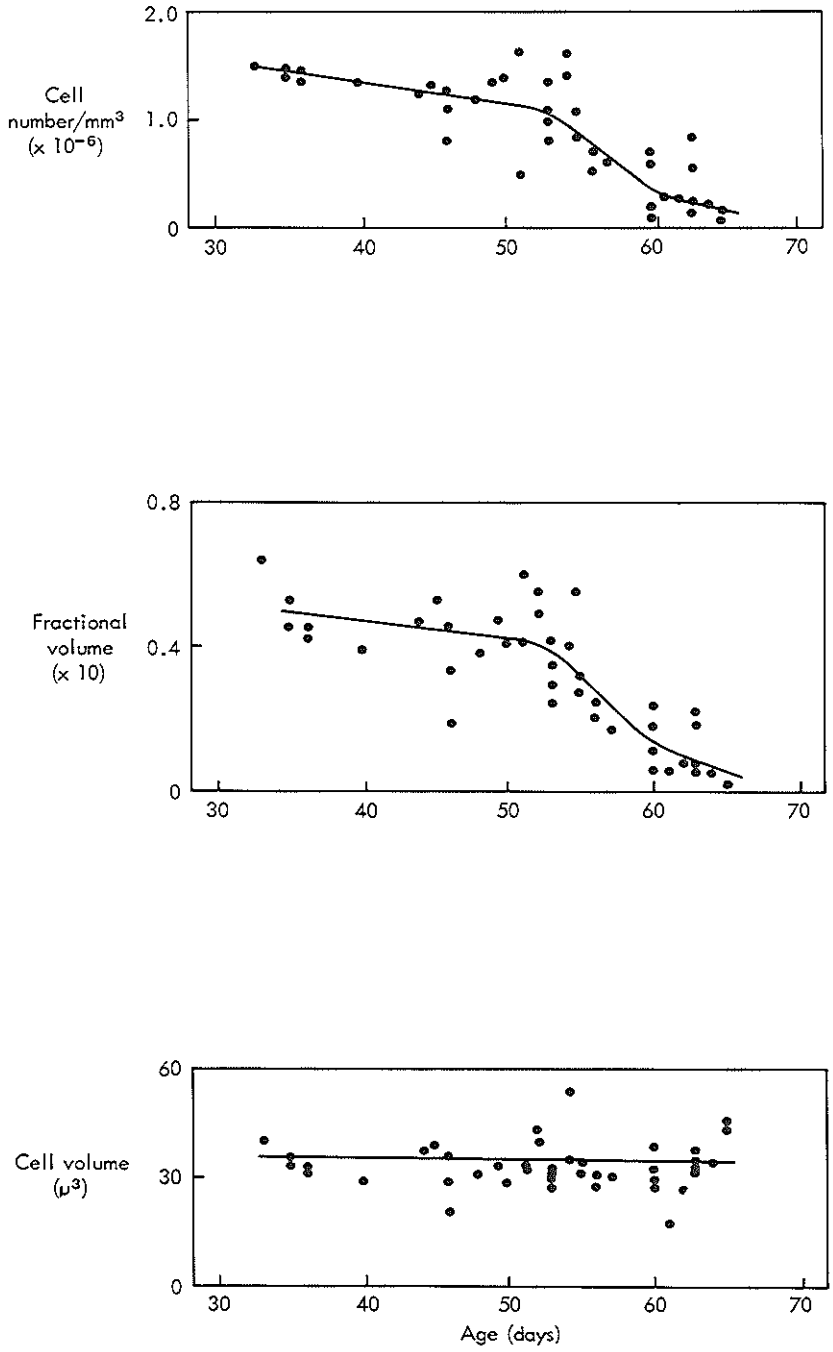

Fig. III.13. Volume, number and fractional volume of hepatocytes in the liver of fetal guinea pigs. Fractional volume refers to the proportion of the liver occupied by this cell type (reproduced from Faulkner and Jones, 1979).

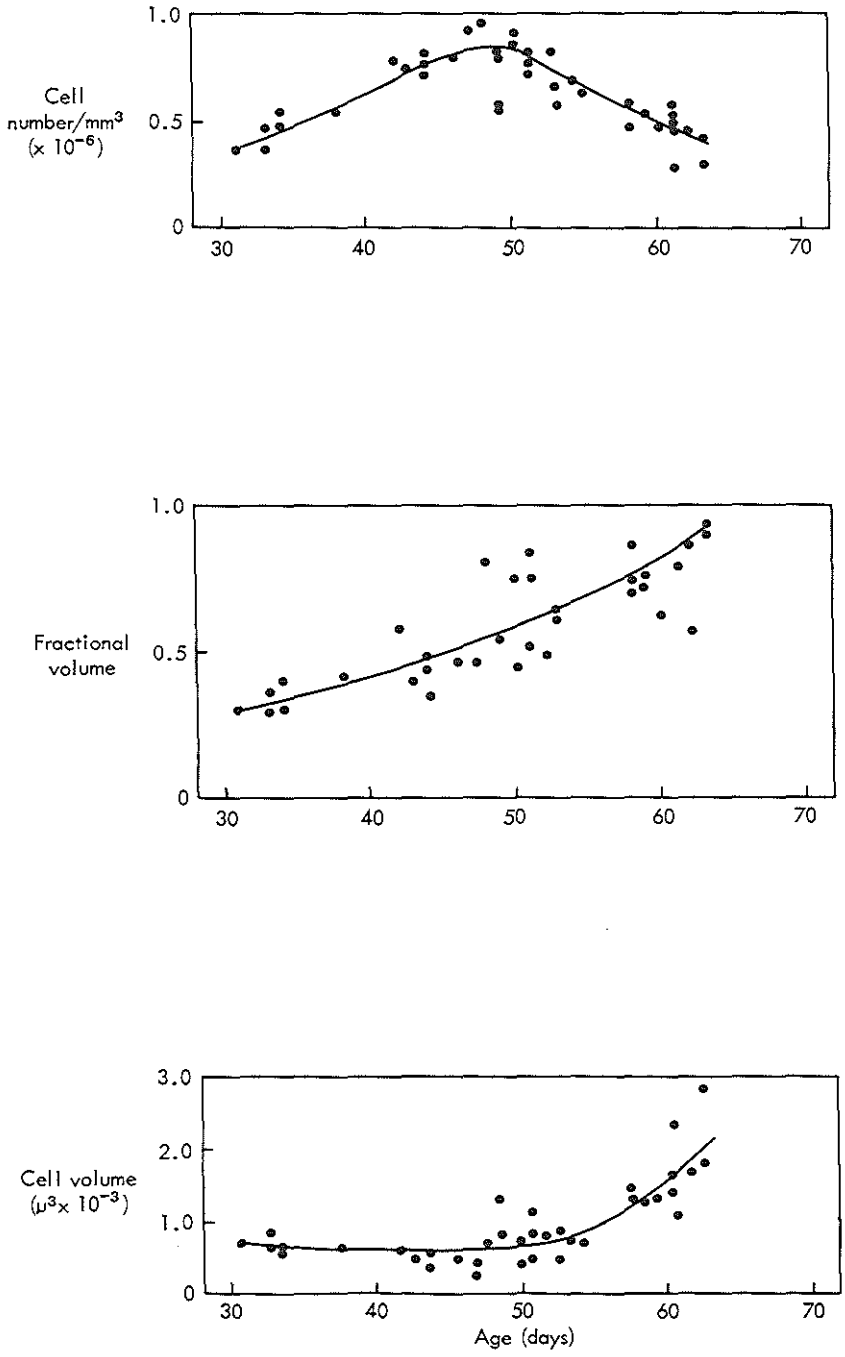

Fig. III.14. Volume, number and fractional volume of haematopoietic cells in the liver of fetal guinea pigs. Fractional volume refers to the proportion of the liver occupied by this cell type (reproduced from Faulkner and Jones, 1979).

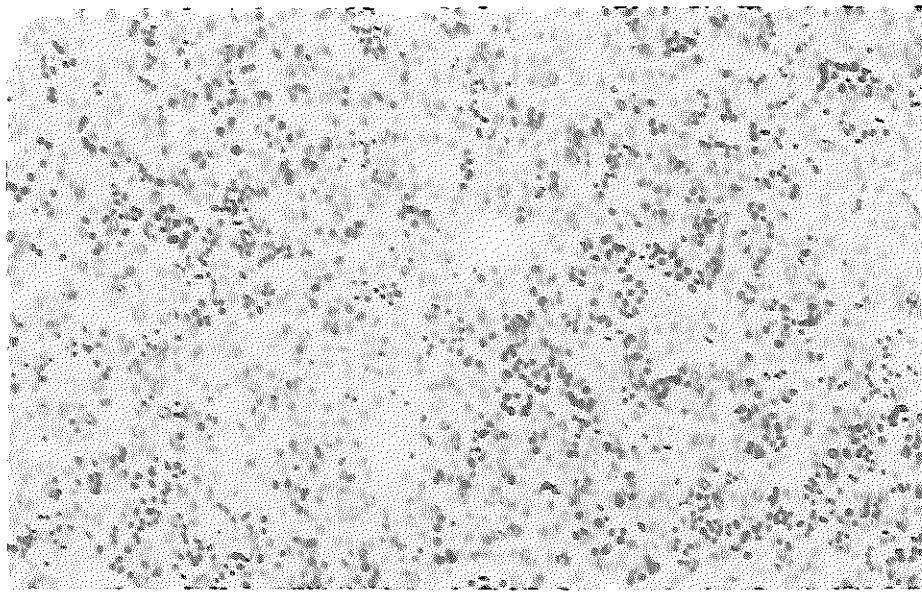

Fig. III.15. Light micrograph of a section of a normal fetal guinea pig liver at 50 days of gestation (magnification is x 320). Liver sections were stained with haematoxylin and eosin.

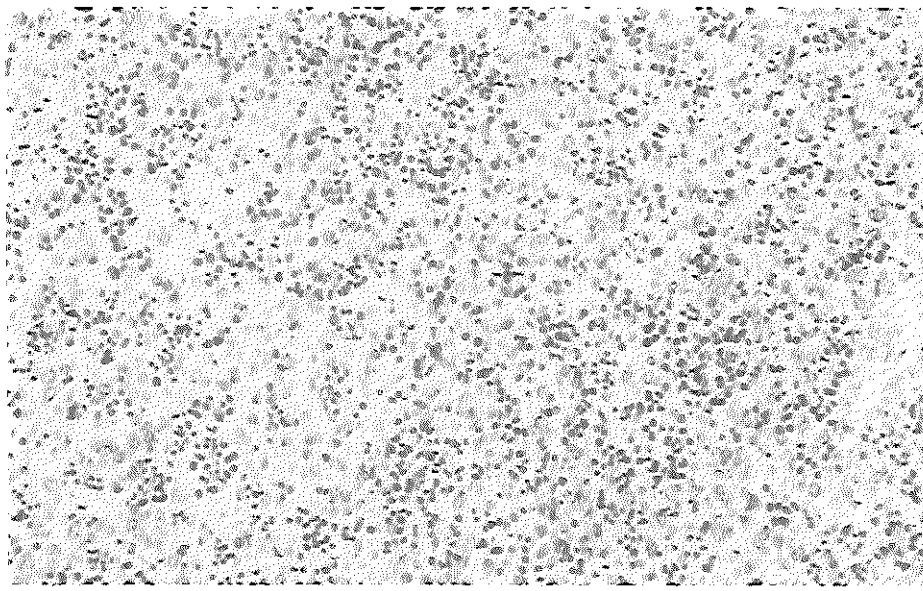

Fig. III.16. Light micrograph of a section of a small fetal guinea pig liver at 50 days of gestation. Further details as for Fig. III.15. Note the larger number of haematopoietic cells in comparison with the normal fetal liver at this age.

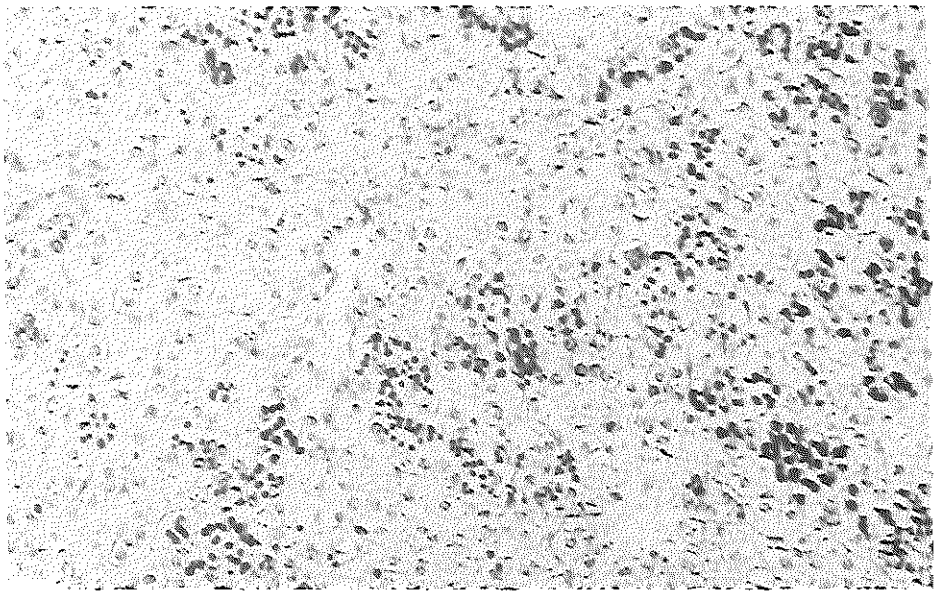

Fig. III.17. Light micrograph of a section of a normal fetal guinea pig liver at 60 days of gestation. Further details as for Fig. III.15.

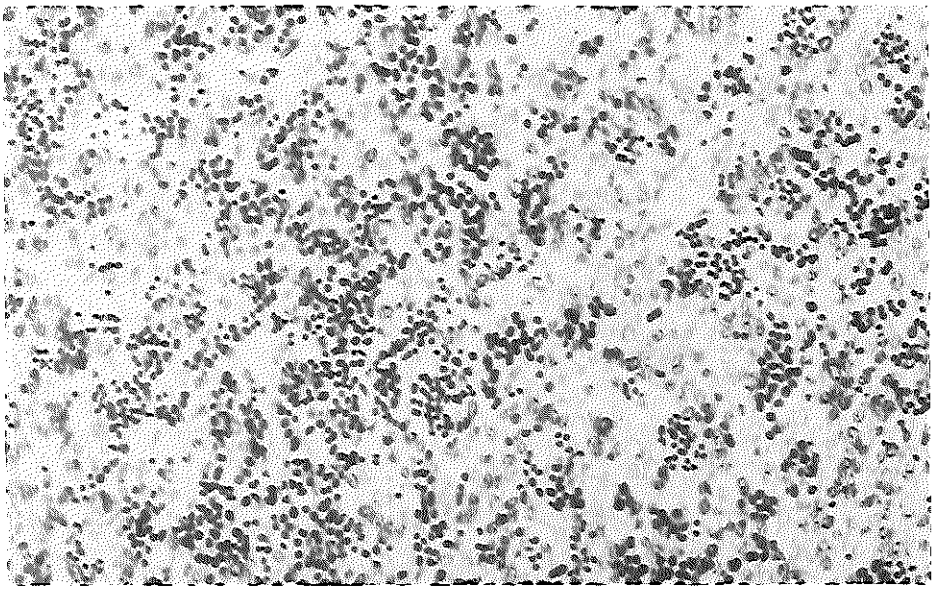

Fig. III.18. Light micrograph of a section of a small fetal guinea pig liver at 60 days of gestation. Further details as for Fig. III.15. Even at this gestational age still a fairly large number of haematopoietic cells are present in comparison with the normal liver at this age (Fig. III.17.).

Table III. 9. The gas tensions, pH and packed cell volume of venous cord blood from normal and small fetal guinea pigs.

49 - 51 days		
	Normal	Small
Bodyweight (g.)	41.5 ±4.6 (7)	22.4 ±2.5° (7)
Packed cell volume	0.37 ± 0.07 (7)	0.49 ± 0.04□ (7)

60 - 63 days			
	Normal	Small	
		35 - 50 g.	< 35 g.
Bodyweight (g.)	89.8 ± 10.9 (27)	43.6 ±4.5° (18)	31.6 ±3.6° (9)
Packed cell volume	0.45 ± 0.04 (27)	0.53 ± 0.04° (18)	0.55 ± 0.05° (9)

60 - 63 days		
	Normal	Small
Bodyweight (g.)	80.6 ± 2.5 (6)	39.7 ±6.8° (6)
pO_2	26 ± 7	25 ±4
pCO_2	47 ±10	45 ±5
pH	7.31 ± 0.05	7.31 ± 0.06

□ $P<0.01$ ° $P<0.001$

Gas tensions are expressed in mm. Hg. The results are means ± S.D. with the number of observations in parentheses.

III.5.2. Blood gas values and packed cell volume

The blood gas and pH values for small and normal weight fetuses were not significantly different (table III.9) at both 49-51 and 60-63 days. The packed-cell volume was significantly higher in blood from small fetuses than in that from normal fetuses (table III.9).

III.5.3. Energy stores in liver, heart and skeletal muscle

III.5.3.1. Glycogen

In the fetal guinea pig near term glycogen is deposited rapidly in the liver (fig.

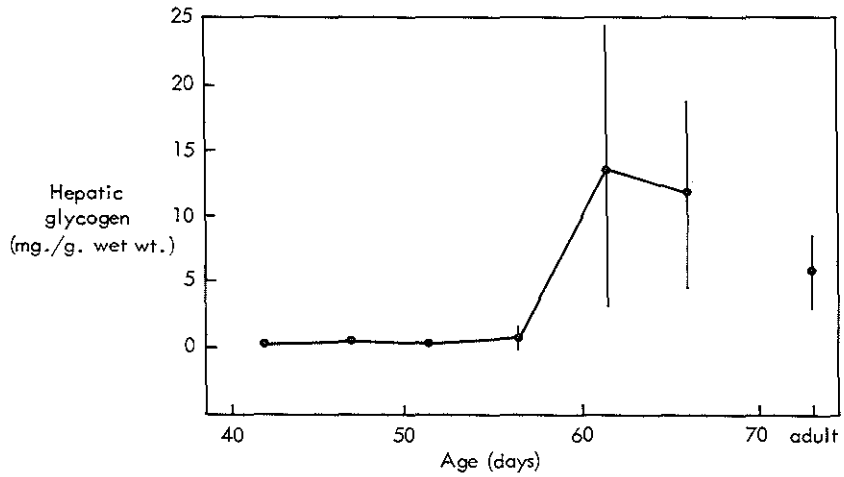

Fig. III.19. Glycogen content of fetal and adult guinea pig liver. The values are the means ± S.D. of 6-11 determinations. (reproduced from Faulkner and Jones, 1976b).

III.19). The concentration of glycogen in the liver of small fetuses was higher than in that of the normal fetuses. Because of the smaller liver the total quantity of hepatic glycogen was not significantly higher (table III.10). At 49-51 days the quantity of hepatic glycogen per g bodyweight was significantly higher in the small fetuses while at 60-63 days there were no significant differences in these quantities between normal and small fetuses.

The glycogen contents of the hind limb skeletal muscle and heart were similar in the two groups of fetuses (table III.10). Only for the heart at 60-63 days was the quantity of glycogen per g bodyweight significantly higher in the small compared with the normal fetus (table III.10).

III.5.3.2. Triacylglycerol

In the guinea pig there is normally a large increase in hepatic triacylglycerol content in fetuses between 57 days and term (fig. III.20). At 49-51 days the concentration in the liver of the small fetuses was almost twice that in the normal, although the total quantity and quantity per g bodyweight was not significantly different (table III.11). At 60-63 days the fetuses smaller than 35 g had much less hepatic triacylglycerol and the total liver store was thus substantially reduced. There was an increase in both the concentration and quantity of triacylglycerol in the hearts of the fetal guinea pigs between 50 and 60 days (table III.11). The small fetuses had a concentration that was 2-3 times higher than the normal values although surprisingly in the different weight groups the quantity of triacylglycerol per heart was constant (table III.11). The quantity of cardiac triacylglycerol per g bodyweight was thus 2-3 fold higher in the small fetuses.

Table III. 10. The glycogen concentration in the tissues of normal and small fetal guinea pigs.

49 - 51 days

	Normal	Small
Bodyweight (g.)	39.6 ± 4.1 (8)	19.8 ± 3.1° (8)
Liver		
mg./g.	0.7 ± 0.3 (8)	2.2 ± 0.8□ (8)
mg./liver	1.9 ± 0.7 (8)	2.3 ± 0.7 (8)
mg./g. of fetus	0.05 ± 0.02 (8)	0.11 ± 0.04□ (8)
Heart		
mg./g.	18.3 ± 2.3 (7)	19.0 ± 6.1 (7)
mg./heart	3.9 ± 1.3 (7)	1.9 ± 0.7 (7)
mg./g. of fetus	0.10 ± 0.03 (7)	0.10 ± 0.02 (7)
Skeletal muscle		
mg./g.	7.6 ± 1.1 (6)	6.9 ± 0.7 (6)

60 - 63 days

	Normal	Small
Bodyweight (g.)	81.8 ± 12.9 (15)	38.7 ± 10.9° (15)
Liver		
mg./g.	14.8 ± 7.2 (13)	24.2 ± 6.2□ (13)
mg./liver	66.3 ± 34.3 (13)	44.6 ± 18.0 (13)
mg./g. of fetus	0.8 ± 0.5 (13)	1.0 ± 0.5 (13)
Heart		
mg./g.	5.3 ± 2.9 (15)	8.7 ± 5.9 (15)
mg./heart	2.3 ± 1.4 (15)	2.1 ± 1.5 (15)
mg./g. of fetus	0.03 ± 0.02 (15)	0.06 ± 0.04△ (15)
Skeletal muscle		
mg./g.	7.8 ± 2.3 (11)	9.9 ± 2.6 (11)

△P<0.05 □P<0.01 °P<0.001

The results are means ± S.D. with the number of observations in parentheses.

Table III. 11. The triacylglycerol content of liver and heart of normal and small fetal guinea pigs.

49 - 51 days

	Normal	Small
Bodyweight (g.)	39.2 ± 3.3 (10)	20.5 ± 3.7° (10)
Liver		
μmol./g.	11.1 ± 6.2 (10)	19.6 ± 8.2$^{\triangle}$ (10)
μmol./liver	28.7 ± 16.7 (10)	22.2 ± 12.4 (10)
μmol./g. of fetus	0.74 ± 0.36 (10)	1.03 ± 0.49 (10)
Bodyweight (g.)	38.5 ± 2.5 (6)	20.0 ± 3.5° (6)
Heart		
nmol./g.	754 ± 267 (6)	1687 ± 855$^{\triangle}$ (6)
nmol./heart	175 ± 54 (6)	201 ± 102 (6)
nmol./g. of fetus	4.6 ± 1.3 (6)	9.3 ± 4.5$^{\triangle}$ (6)

60 - 63 days

	Normal	Small	
		35 - 50 g.	<35 g.
Bodyweight (g.)	80.4 ± 11.3 (24)	44.4 ± 7.1° (15)	29.1 ± 6.7° (9)
Liver			
μmol./g.	78.3 ± 27.4 (24)	84.1 ± 25.2 (15)	43.6 ± 32.9$^{\triangle}$ (9)
μmol./liver	305.0 ± 125.0 (24)	150.0 ± 55.0$^{\square}$ (15)	56.4 ± 52.9$^{\square}$ (9)
μmol./g. of fetus	3.8 ± 1.4 (24)	3.3 ± 1.0 (15)	2.0 ± 1.6$^{\triangle}$ (9)
Bodyweight (g.)	86.4 ± 12.0 (16)	42.1 ± 4.0° (8)	29.5 ± 5.8° (8)
Heart			
nmol./g.	1354 ± 803 (16)	2378 ± 1113$^{\triangle}$ (8)	3051 ± 1904$^{\triangle}$ (8)
nmol./heart	686 ± 481 (16)	640 ± 306 (8)	672 ± 303 (8)
nmol./g. of fetus	8.0 ± 4.9 (16)	15.4 ± 7.7$^{\triangle}$ (8)	24.2 ± 10.4$^{\square}$ (8)

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are means ± S.D. with the number of observations in parentheses.

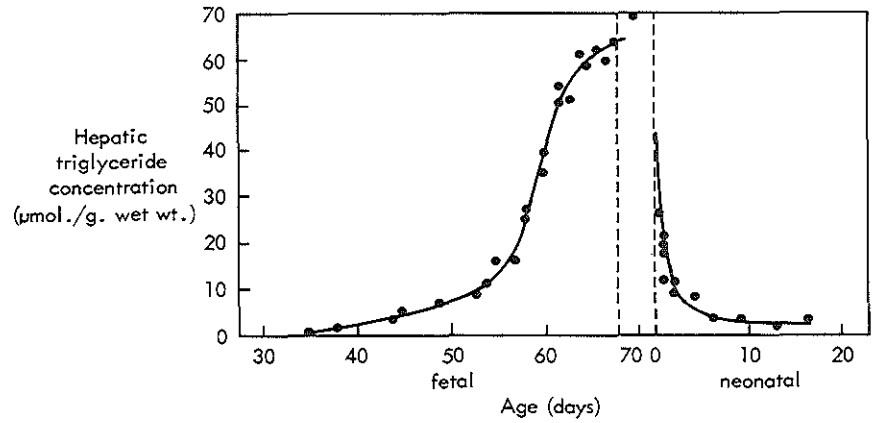

Fig. III.20. Triacylglycerol content of fetal and neonatal guinea pig liver (reproduced from Jones, 1976a).

III.5.4. Electron microscopic studies of liver, heart and skeletal muscle.

III.5.4.1. Liver

The development of the fetal hepatocyte of the guinea pig is clearly shown in electron microscopic pictures taken at 40, 50 and 60 days of gestation (Fig. III.21-III.31). At 40 days the hepatocyte still has a relatively small amount of cytoplasma with few less well developed mitochondria and few endoplasmic reticulum (fig. III.22, III.23).

At 50 days the number of mitochondria increases together with the cytoplasmic volume. The smooth endoplasmic reticulum is well developed at this stage (fig. III.24, III.25). The mitochondria become more dense and contain more cristae (fig. III.25). At 60 days the cell number has relatively decreased while the cytoplasmic volume has dramatically increased (fig. III.28). At this time larger deposits of glycogen and fat can be seen in the cytoplasma (fig. III.28). At 60 days the mitochondria are better developed together with the endoplasmic reticulum, although relatively less smooth endoplasmic reticulum can be noticed in comparison with the situation at 50 days (fig. III.25, III.29). The adult guinea pig liver shows much less glycogen and fat in its relatively larger cytoplasma (fig. III.21). There are many mitochondria that together with all other cell constituents are well developed (fig. III.21).

In the growth retarded liver at 50 days still less cytoplasma and fewer mitochondria are visible than in the normal hepatocyte at this stage (fig. III.26, III.27). The mitochondria are less dense and there is less smooth endoplasmic reticulum (fig. III.27). At 60 days on the other hand there seems to be more smooth endoplasmic reticulum than normally can be seen at this age (fig. III.30, III.31). The 60 day growth retarded hepatocyte has less cytoplasma that contains fewer mitochondria and less fat deposits but remarkably more glycogen (fig. III.30, III.31).

III.5.4.2. Heart

During development of the fetal guinea pig heart there are many changes in the composition of the myocytes. At 40 days the mitochondria still represent a small percentage of the cell volume while they are also relatively small (fig. III.33). The nuclei are also small but are more numerous than in older hearts (Rolph, Jones and Parry, 1981). At this period of gestation the myofibrillar content is relatively low and consequently the sarcoplasmic volume is high (Rolph, Jones and Parry, 1981). In addition there are numerous glycogen granules (fig. III.33). Towards term the number and size of the mitochondria, the number of myofibrils and the size of the nuclei increases, while the number of nuclei, the sarcoplasmic volume and the number of glycogen granules falls (fig. III.33, III.34, III.36). The adult heart has more and bigger mitochondria, more myofibrils but less nuclei and sarcoplasm than the fetal heart (fig. III.32). The fetal cardiac myocyte mitochondria contain fewer cristae than the mitochondria in the adult heart.

In the heart of the growth retarded fetal guinea pigs the volume of myocyte occupied by mitochondria, cytosol and myofibrils is similar to that of the normal fetal hearts both at 50 and 60 days (fig. III.35, III.37). There is, however, a substantial increase in the volume of myocyte occupied by fat deposits and nuclei in the small compared to the normal fetal hearts at 50 and 60 days (fig. III.35, III.37).

III.5.4.3. Skeletal muscle

The hindlimb muscle of the fetal guinea pig at 40 days consists of both differentiated myotubes and undifferentiated myofibrils (fig. III.39). The myofibrils do not yet contain well developed sarcomeres and have smaller mitochondria (fig. III.39). The myotubes still have many central nuclei; much glycogen is visible in the cells that have a poorly developed sarcoplasmic reticulum (Rolph, 1980) (fig. III.39).

Towards term more well developed myofibrils become visible. At 50 days the nuclei can be found in a subsarcolemmal position while the mitochondria contain more cristae and are situated in an interfibrillar position (fig. III.40). Many vacuoles and focal glycogen deposits are seen. The sarcoplasmic reticulum is more developed (fig. III.40).

In the 60 days fetal skeletal muscle the myofibre has very extensive myofibrils with adjacent Z bands in register (fig. III.42). The mitochondria are fairly numerous and oriented longitudinally in the interfibrillar location (fig. III.42). There are still large focal deposits of glycogen. At this stage the sarcoplasmic reticulum is well developed (Rolph, 1980).

Fig. III.38 shows a longitudinal section trough adult upper hind limb muscle. As at 60 days of gestation the myofibrils dominate the cell with adjacent Z bands in register. In contrast to the late fetal muscle the myofibrils of the adult are clearly demarcated by a well defined interfibrillar space. There is also less glycogen than in the fetal skeletal muscle (fig. III.38).

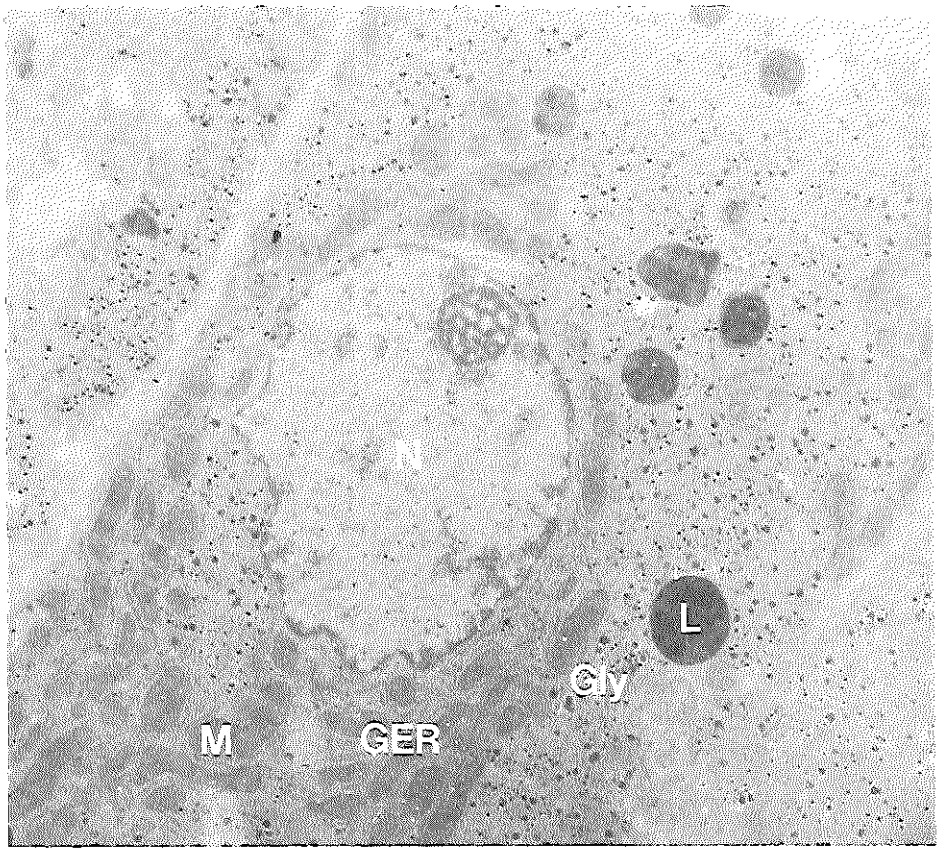

Fig. III.21. Electron micrograph (x 7200) of an adult guinea pig hepatocyte N: nucleus, M: mitochondrion, L: lysosome, G.E.R.: granular endoplasmic reticulum, Gly: glycogen.

In the skeletal muscle section from 50 days growth retarded fetal guinea pigs less mitochondria can be seen in the myotubes (fig. III.41). There are clearly more fat deposits while still relatively many nuclei can be seen. At 60 days only moderately growth retarded fetuses could be investigated. The electron microscopic picture of the 60 days growth retarded skeletal muscle does not show many differences compared to the normal (fig. III.43). The only apparent difference is a greater nuclear volume in the myotube/myofibre of the moderately growth retarded compared to normal fetus. Both at 50 and 60 days the myofibrils are very often not found in regular strains with adjacent Z bands in register (fig. III.41, III.43).

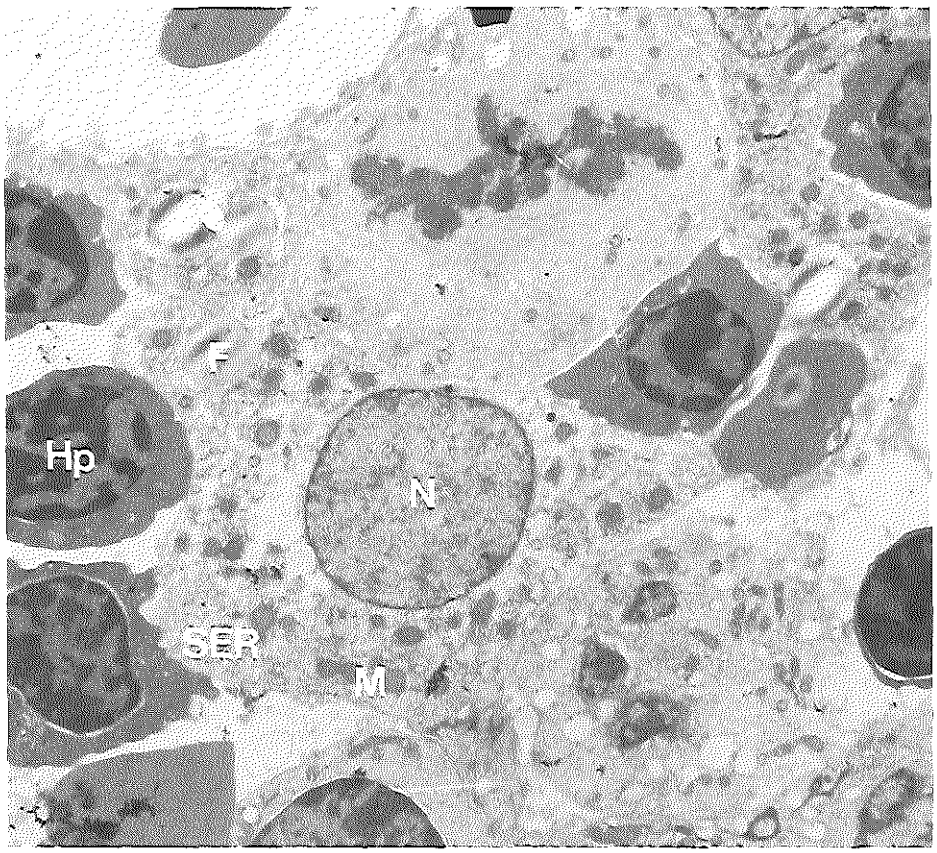

Fig. III.22. Electron micrograph (x 4500) of a fetal guinea pig hepatocyte at 40 days of gestation. N: nucleus, M: mitochondrion, S.E.R.: smooth endoplasmic reticulum, F: fat, Hp: haematopoietic cell.

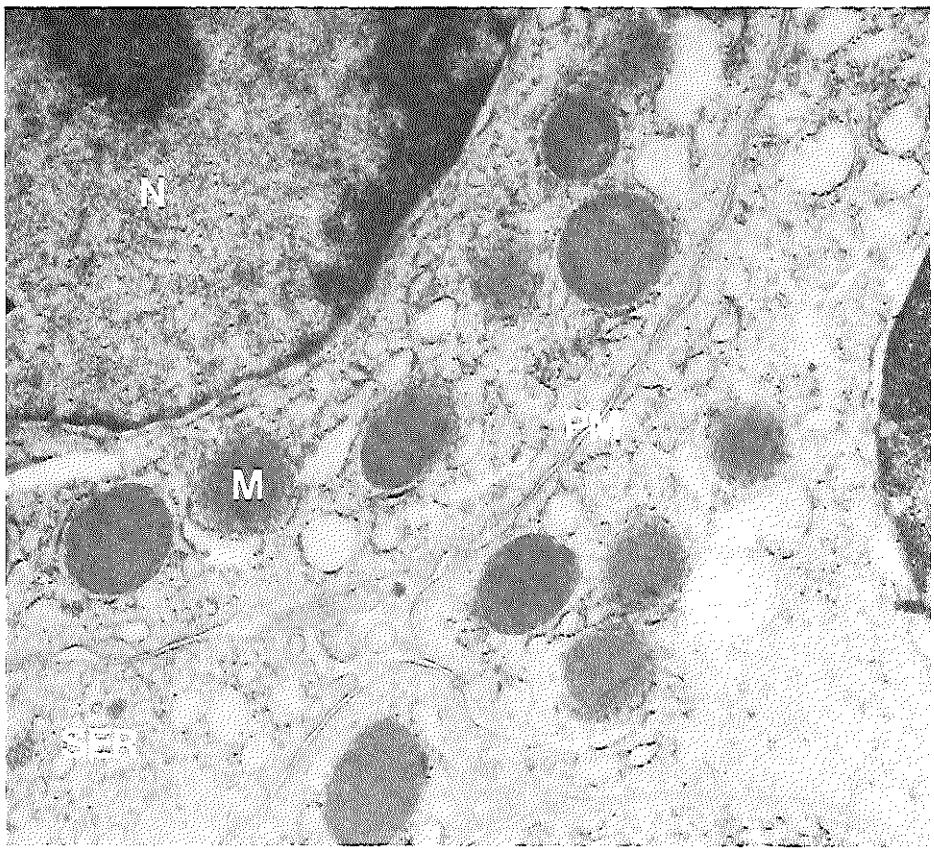

Fig. III.23. Electron micrograph (x 25.000) showing details of a fetal guinea pig hepatocyte at 40 days of gestation. N: nuclues. M: mitochondrion. S.E.R.: smooth endoplasmic reticulum. PM: plasma membrane.

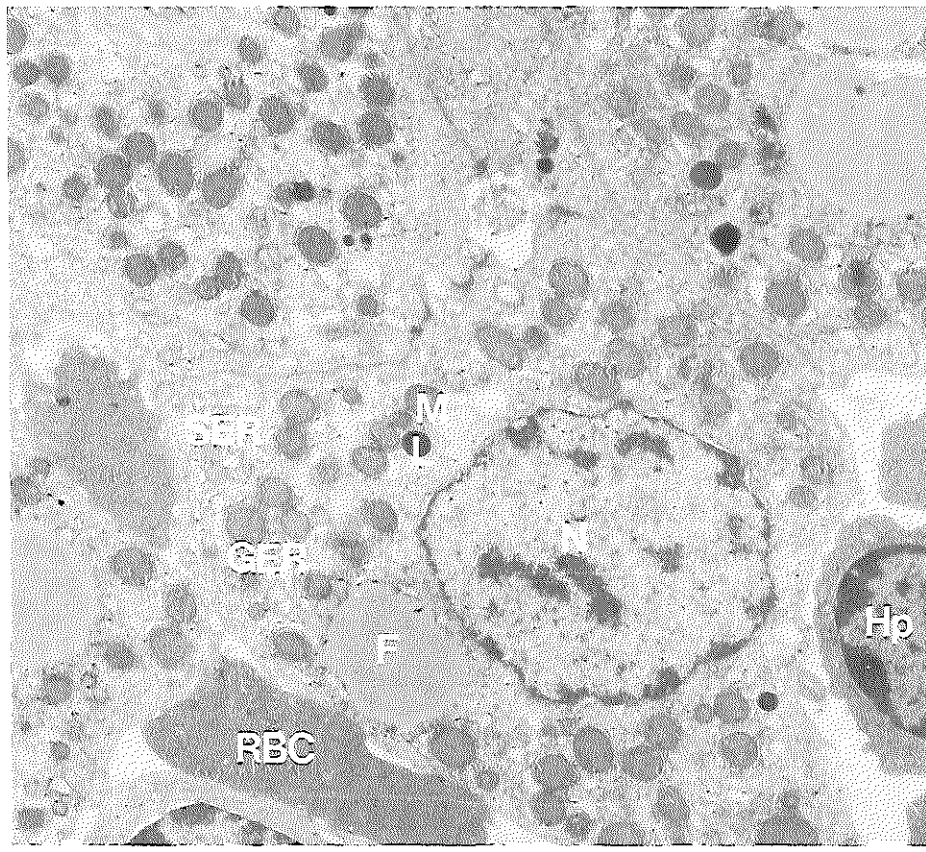

Fig. III.24. Electron micrograph (x 4500) of a fetal guinea pig hepatocyte at 50 days of gestation. N: nucleus, M: mitochondrion, S.E.R.: smooth endoplasmic reticulum, G.E.R.: granular endoplasmic reticulum, L: lysosome, F: fat, Hp: haematopoietic cell, R.B.C.: red blood cell.

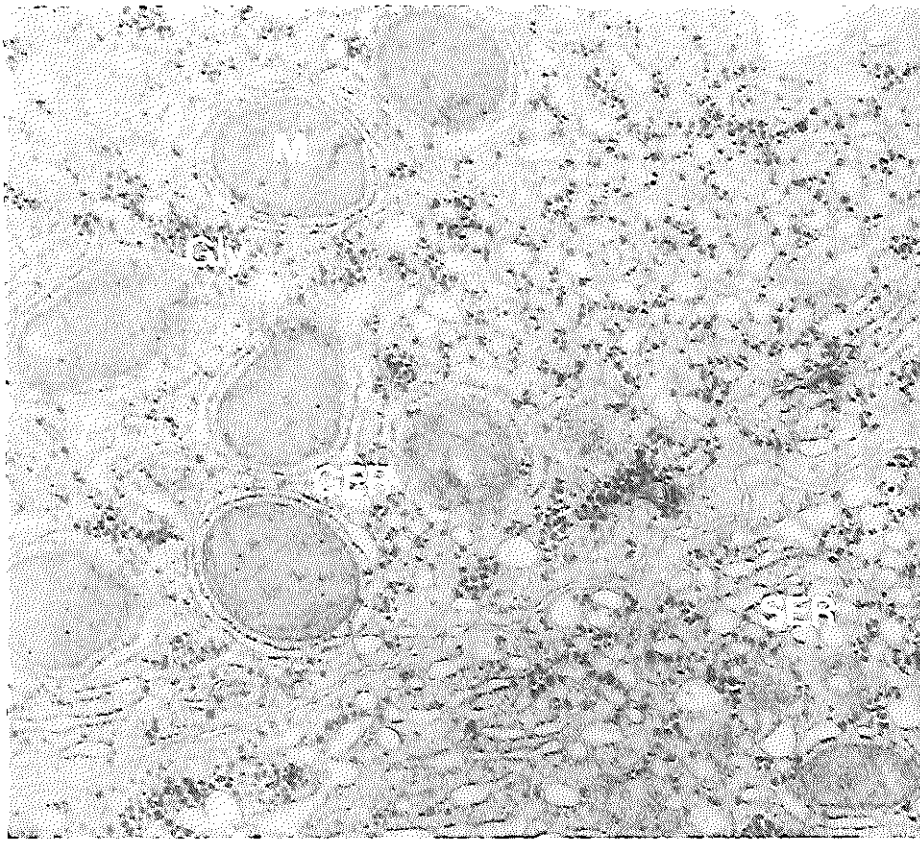

Fig. III.25. Electron micrograph (25.000) showing details of a fetal guinea pig hepatocyte at 51 days of gestation. M: mitochondrion. GER: granular endoplasmic reticulum. SER: smooth endoplasmic reticulum. Gly: glycogen.

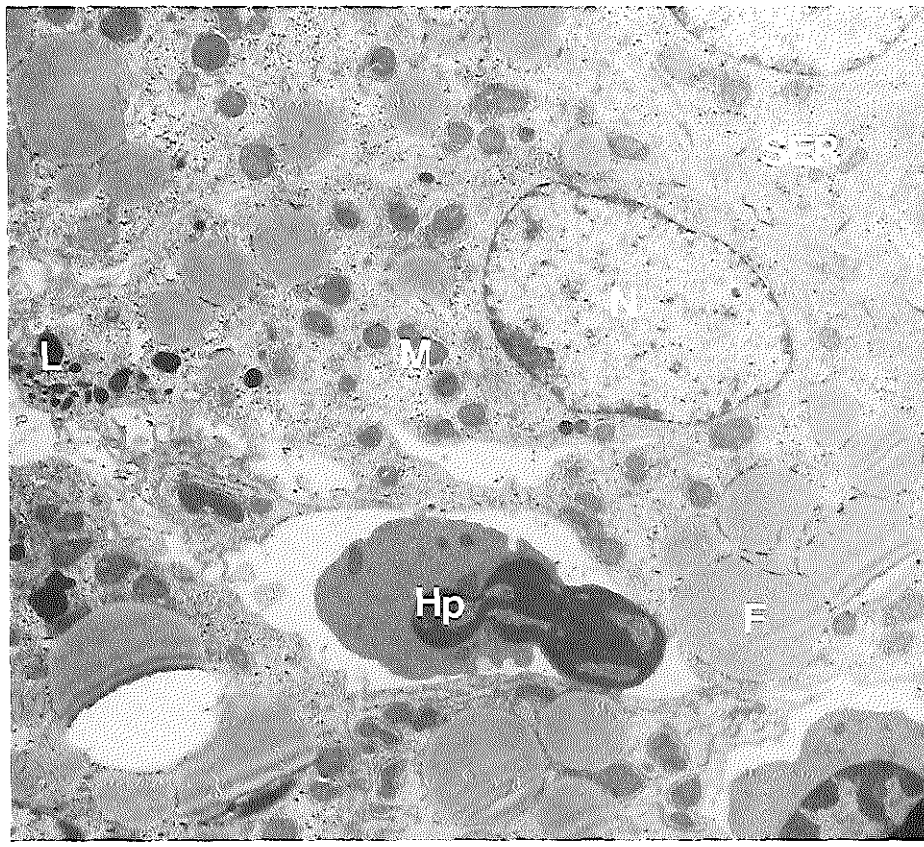

Fig. III.26. Electron micrograph (x 4500) of an intrauterine growth retarded fetal guinea pig hepatocyte at 51 days of gestation. N: nucleus, M: mitochondrion, SER: smooth endoplasmic reticulum, L: lysosome, F: fat, Hp: haematopoietic cell.
Note that only little smooth endoplasmic reticulum is present in comparison with the normal fetal guinea pig liver at this age. There are also fewer mitochondria while many fat droplets are visible.

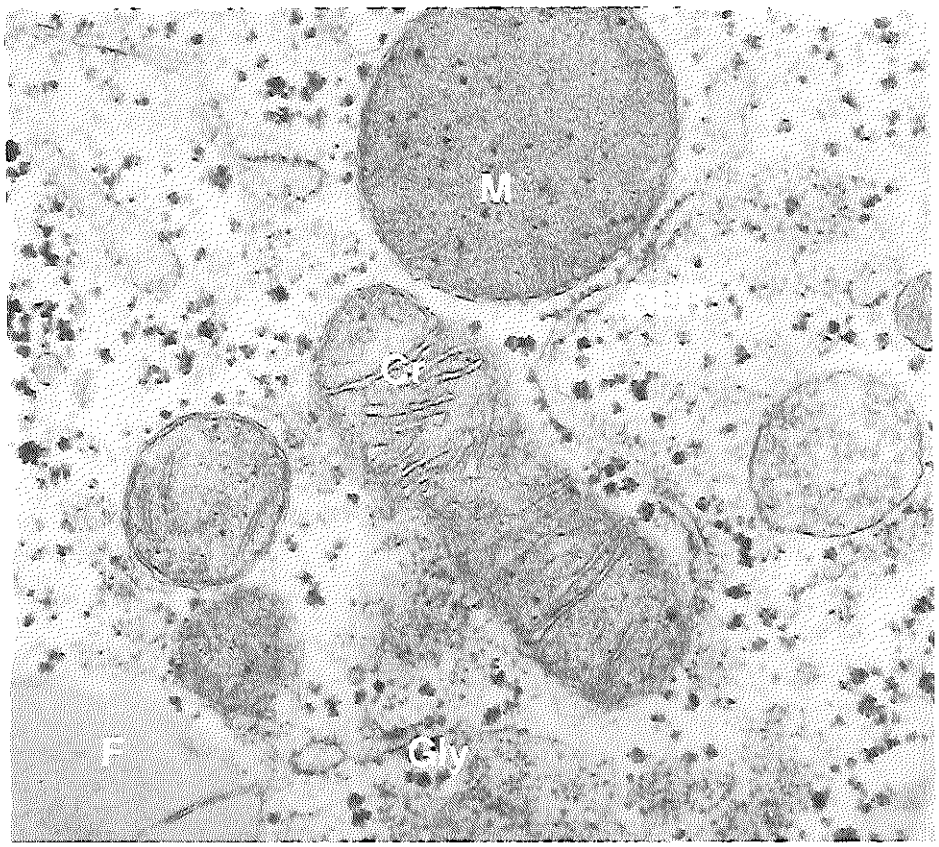

Fig. III.27. Electron micrograph (x 45.500) showing details of an intrauterine growth retarded fetal guinea pig hepatocyte at 51 days of gestation. M: mitochondria, Cr: cristae, GER: granular endoplasmic reticulum, F: fat, Gly: glycogen.
Note that still very little smooth endoplasmic reticulum is visible in comparison with the normal hepatocyte at this age; the granular endoplasmic reticulum is not well organized around the mitochondria.

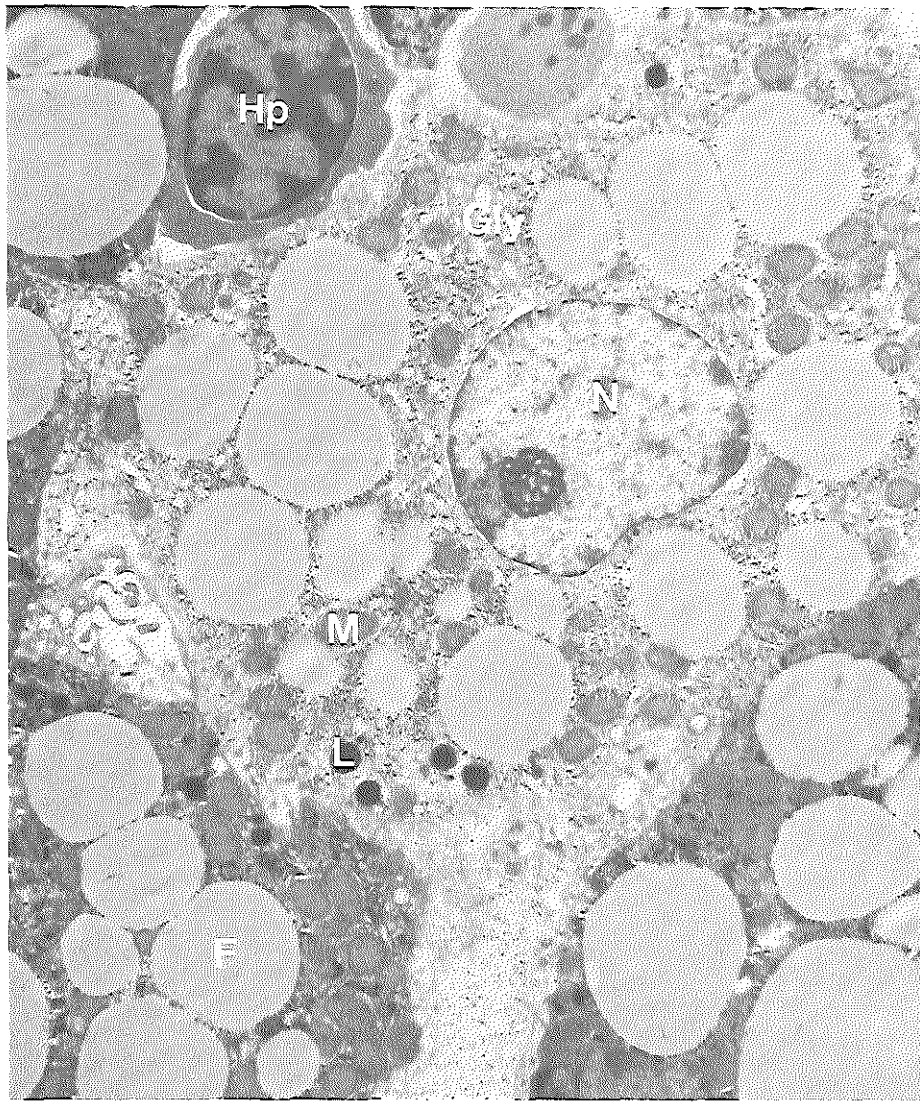

Fig. III.28. Electron micrograph (x 7400) of a fetal guinea pig hepatocyte at 61 days of gestation. N: nucleus, M: mitochondrion, L: lysosome, F: fat, Gly: glycogen, Hp: haematopoietic cell.

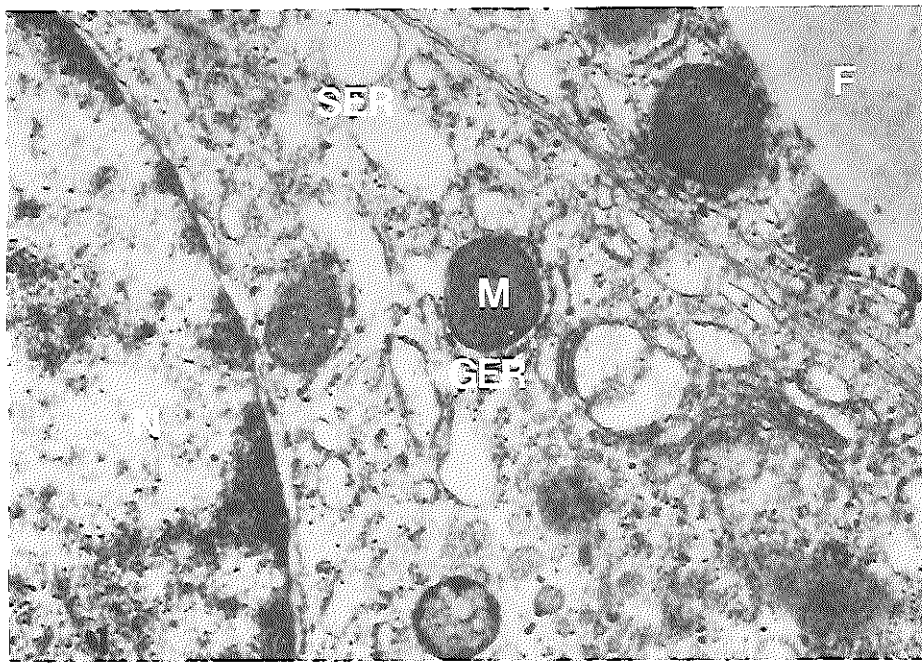

Fig. III.29. Electron micrograph (x 27.800) showing details of a fetal guinea pig hepatocyte at 61 days of gestation. N: nucleus, M: mitochondrion, SER: smooth endoplasmic reticulum, GER: granular endoplasmic reticulum, F: fat.

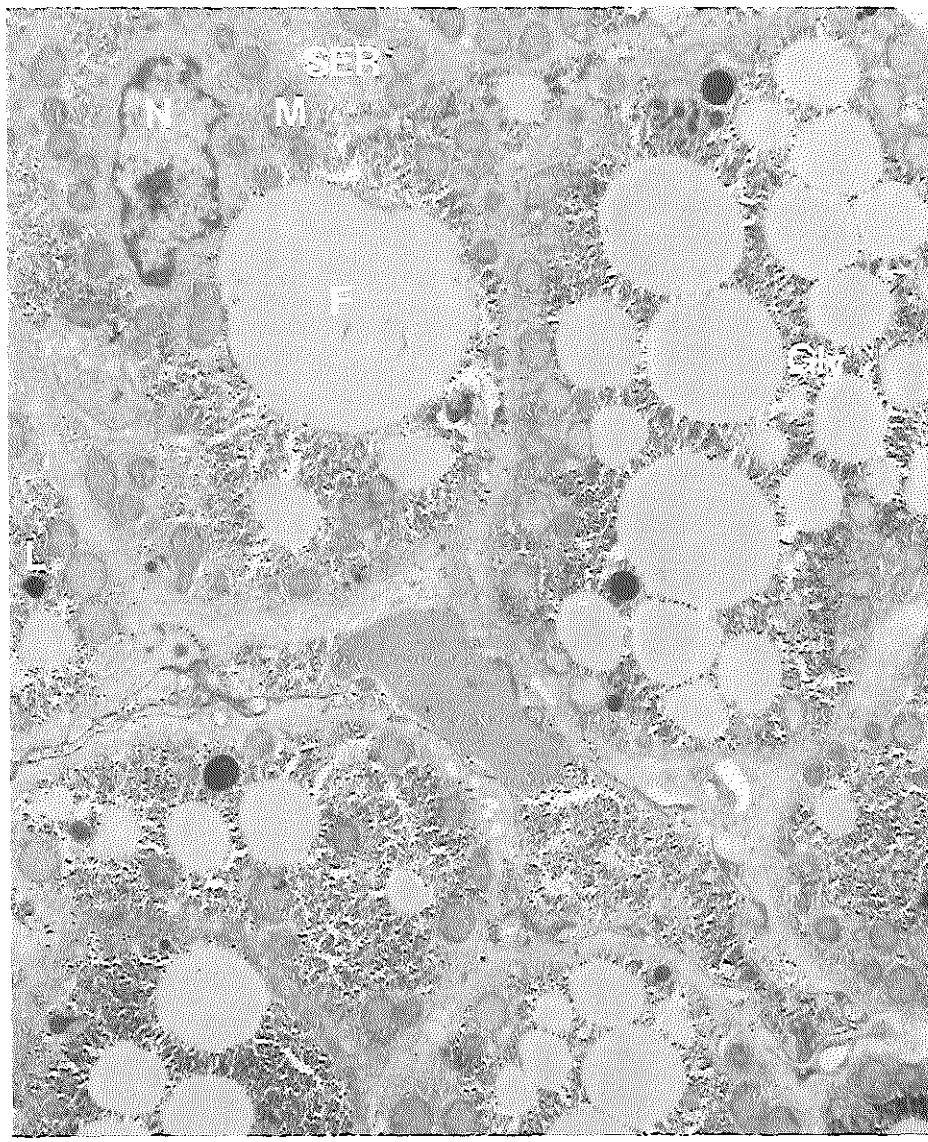

Fig. III.30. Electron micrograph (x 7400) of an intrauterine growth retarded fetal guinea pig hepatocyte at 61 days of gestation. N: nucleus, M: mitochondrion, L: lysosome, SER: smooth endoplasmic reticulum, F: fat, Gly: glycogen.
Note that the hepatocyte of this small fetus has more glycogen and less fat, mitochondria and cytoplasma.

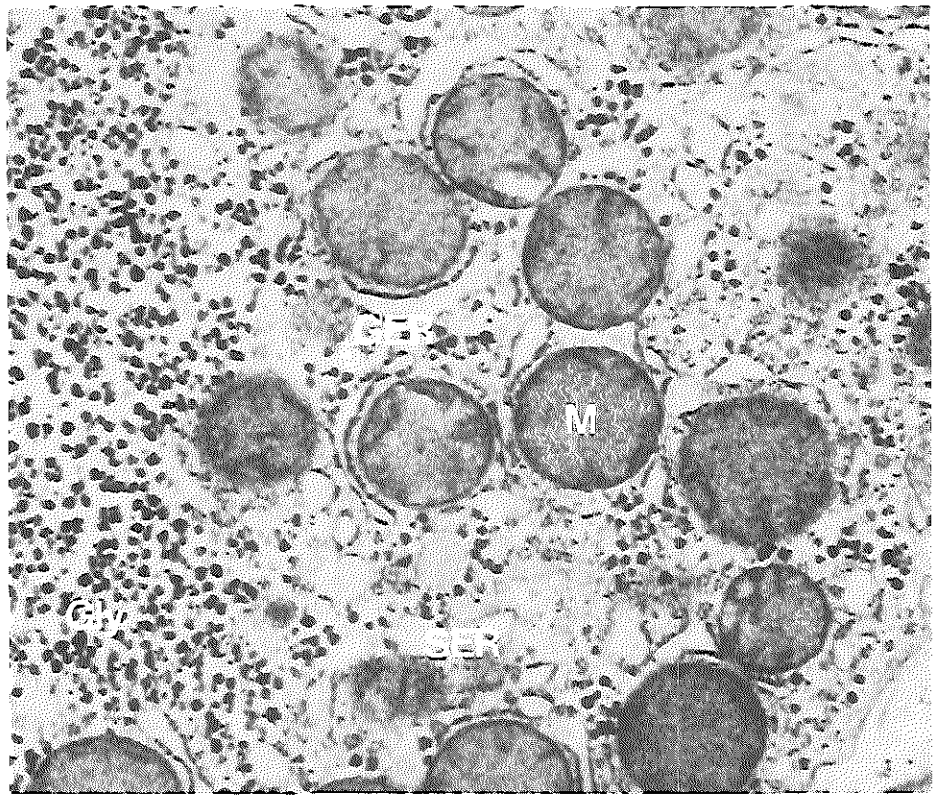

Fig. III.31. Electron micrograph (x 33.600) showing details of an intrauterine growth retarded fetal guinea pig hepatocyte at 61 days of gestation. M: mitochondrion, SER: smooth endoplasmic reticulum, GER: granular endoplasmic reticulum, Gly: glycogen.
Note that mitochondria and endoplasmic reticulum are less well developed in comparison with that in the normal hepatocyte at this age. There is more glycogen and less fat in the cytoplasma.

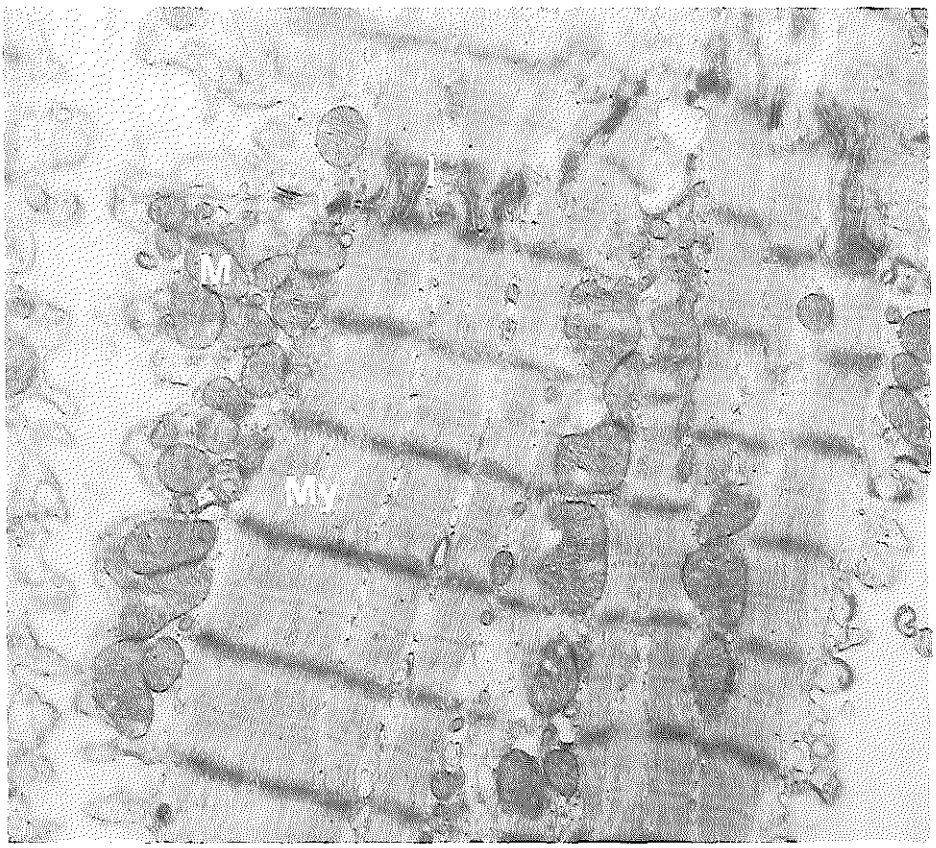

Fig. III.32. Electron micrograph (x 15.000) of the adult guinea pig heart. My:myofibril, M: mitochondrion, I: intercalated disk.

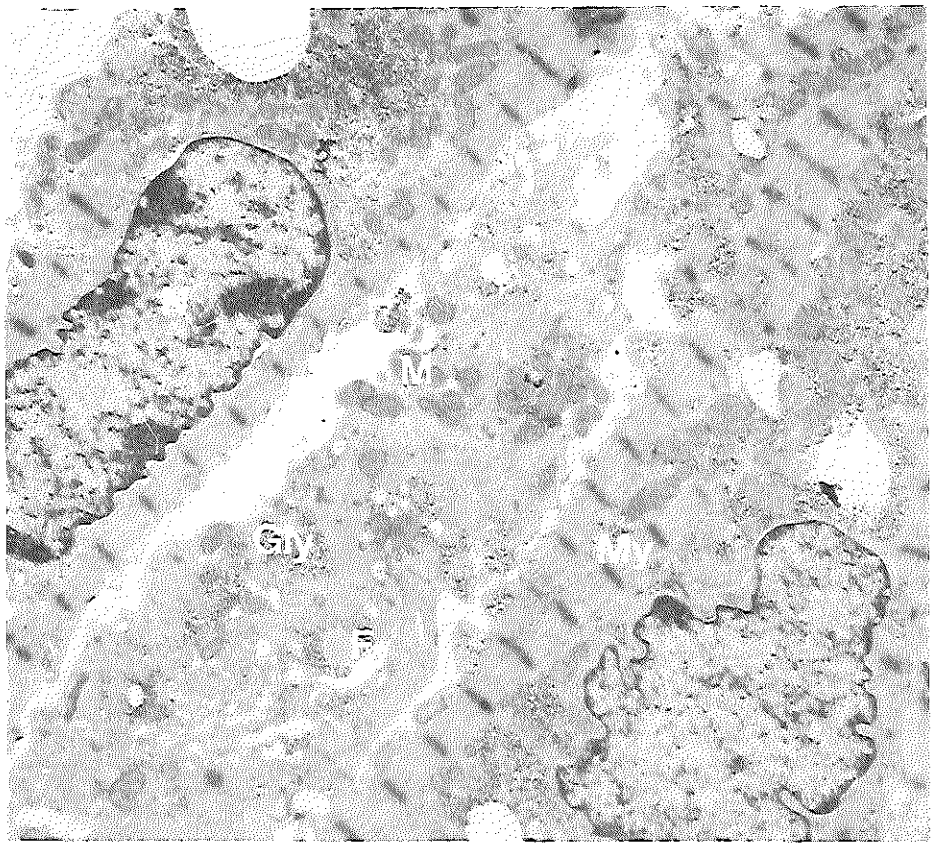

Fig. III.33. Electron micrograph (x 30.000) of the heart of a fetal guinea pig at 40 days of gestation. N: nucleus, M: mitochondrion, Gly: glycogen, F: fat, My: myofibril.
Note the central nucleus; few mitochondria and myofibrils are visible while there is no development of sarcolemma.

Fig. III.34. Electron micrograph (x 31.000) showing details of the heart of a fetal guinea pig at 50 days of gestation. M: mitochondrion. Gly: glycogen. My: myofibril.

Fig. III.35. Electron micrograph (x 51.000) of a section from the heart of an intrauterine growth retarded guinea pig at 50 days of gestation. N: nucleus, M: mitochondrion, L: lysosome, My: myofibril. The changes in comparison with the normal myocyte at this age are minimal.

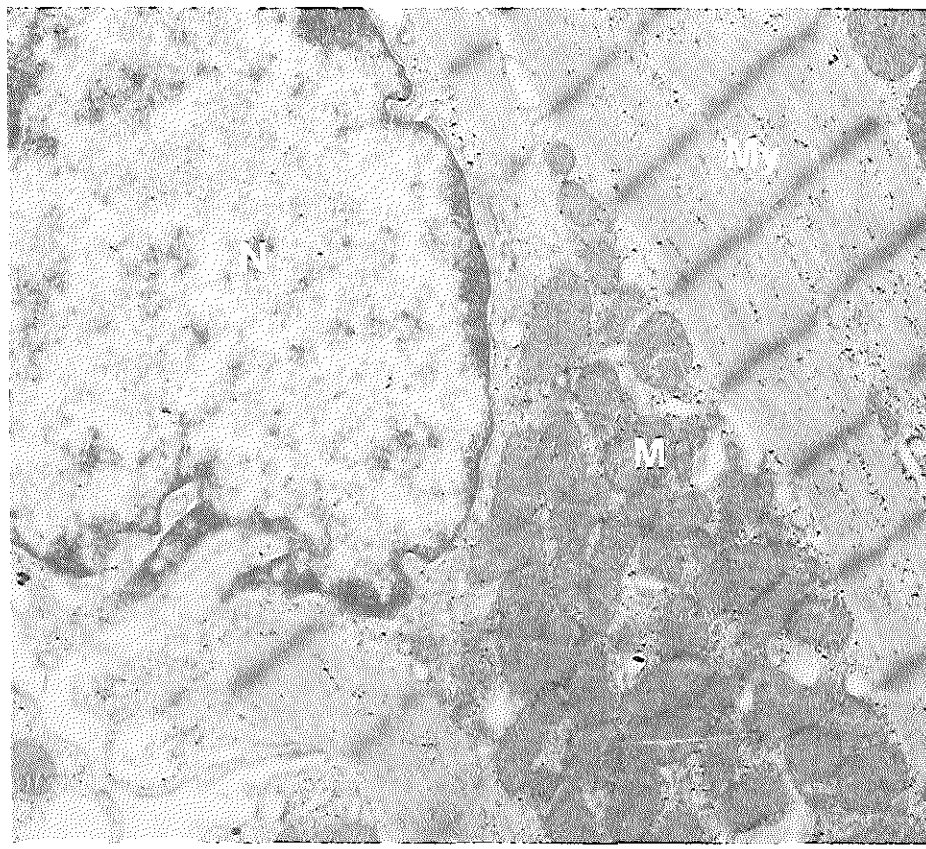

Fig. III.36. Electron micrograph (x 105.000) showing details of a section from the heart of a fetal guinea pig at 60 days of gestation. N: nucleus. M: mitochondrion. My: myofibril.
Note the increase in the number of mitochondria and peripheral location of the nucleus in comparison with the younger fetal heart.

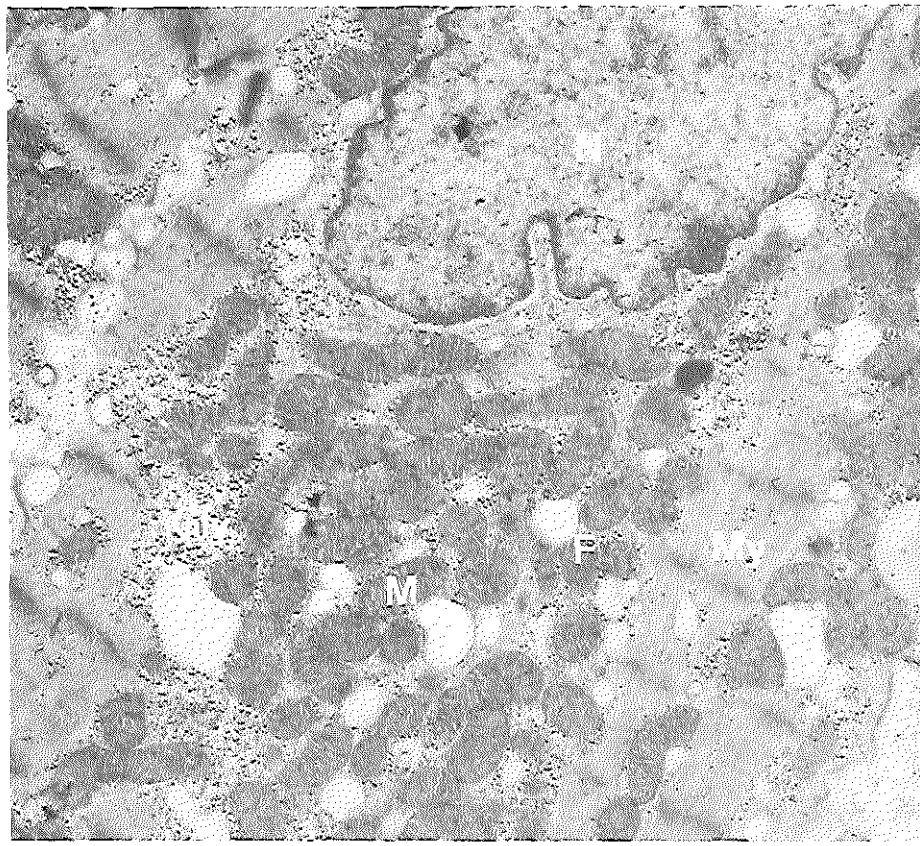

Fig. III.37. Electron micrograph (x 105.000) showing details of a section from the heart of an intrauterine growth retarded fetal guinea pig at 60 days of gestation. N: nucleus, M: mitochondrion Gly: glycogen, F: fat, My: myofibril.
Note the relatively few changes with the normal ultrastructure at this age. There are however more fat droplets in the myocyte while also still many nuclei are visible.

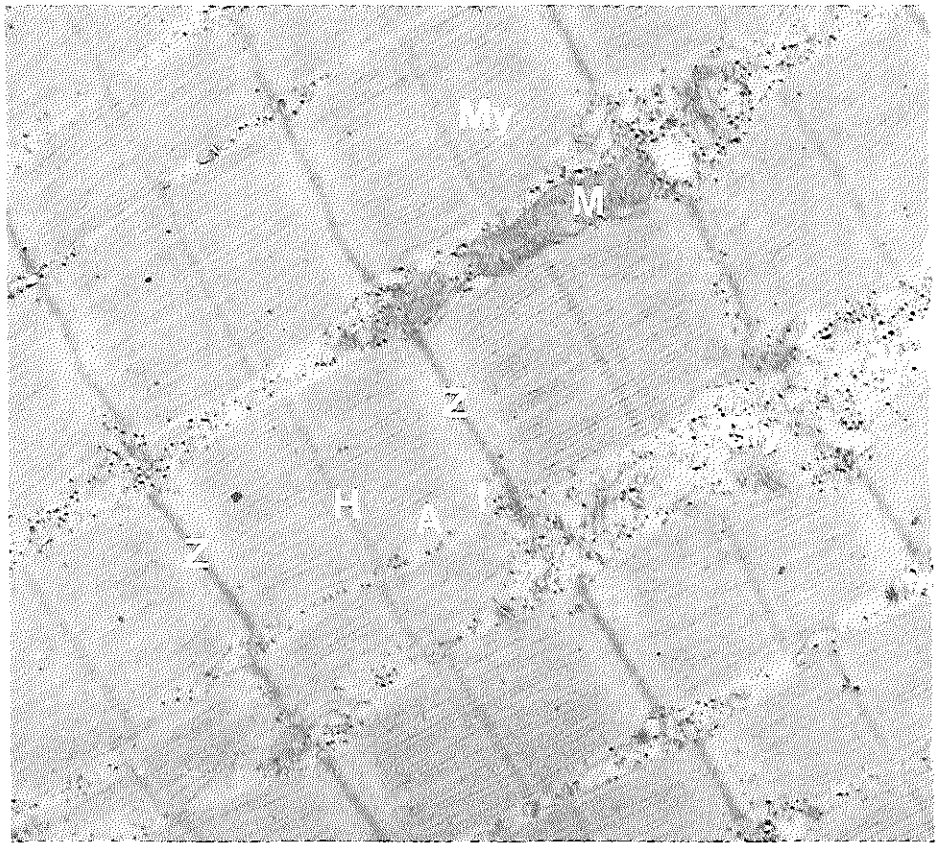

Fig. III.38. Electron micrograph (x 25.000) showing a section from the adult guinea pig skeletal muscle.
M: mitochondrion, Gly: glycogen, My: myofibril.
Sarcomere components: Z: Z-disk, H: H-band, I: I-band, A: A-band.

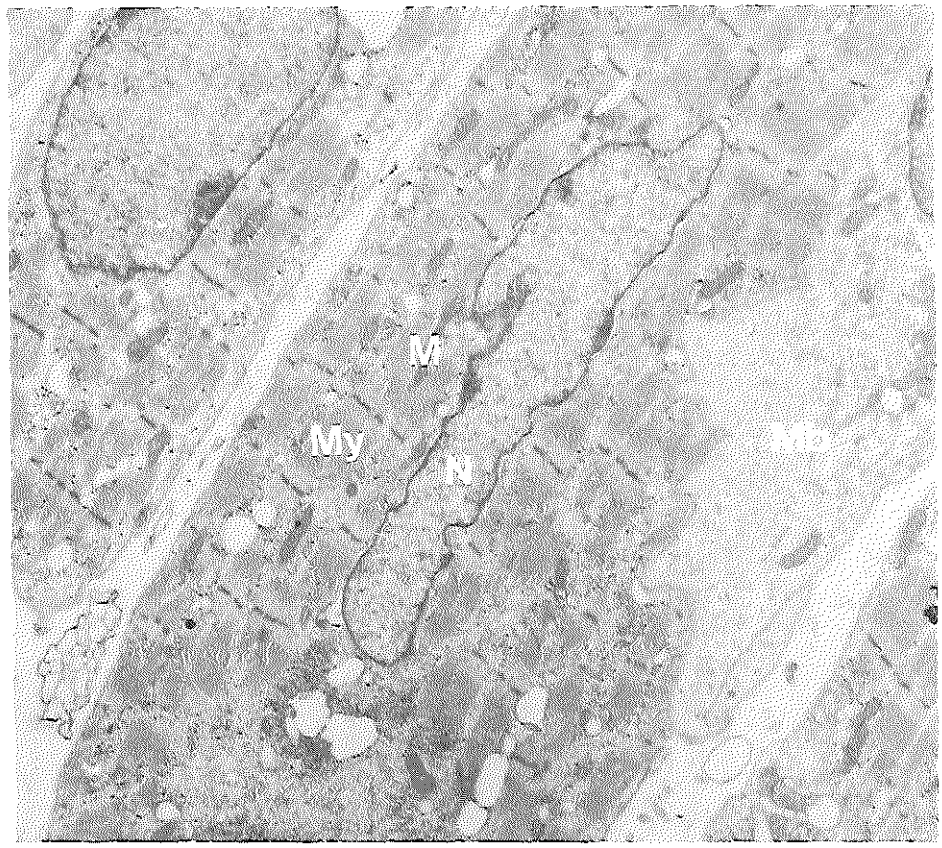

Fig. III.39. Electron micrograph (x 30.000) showing a section from the skeletal muscle of a fetal guinea pig at 40 days of gestation. N: nucleus, M: mitochondrion, My: myofibril, Mb: fusing myoblast.
Note the central nucleus and the relatively few mitochondria and myofibrils.

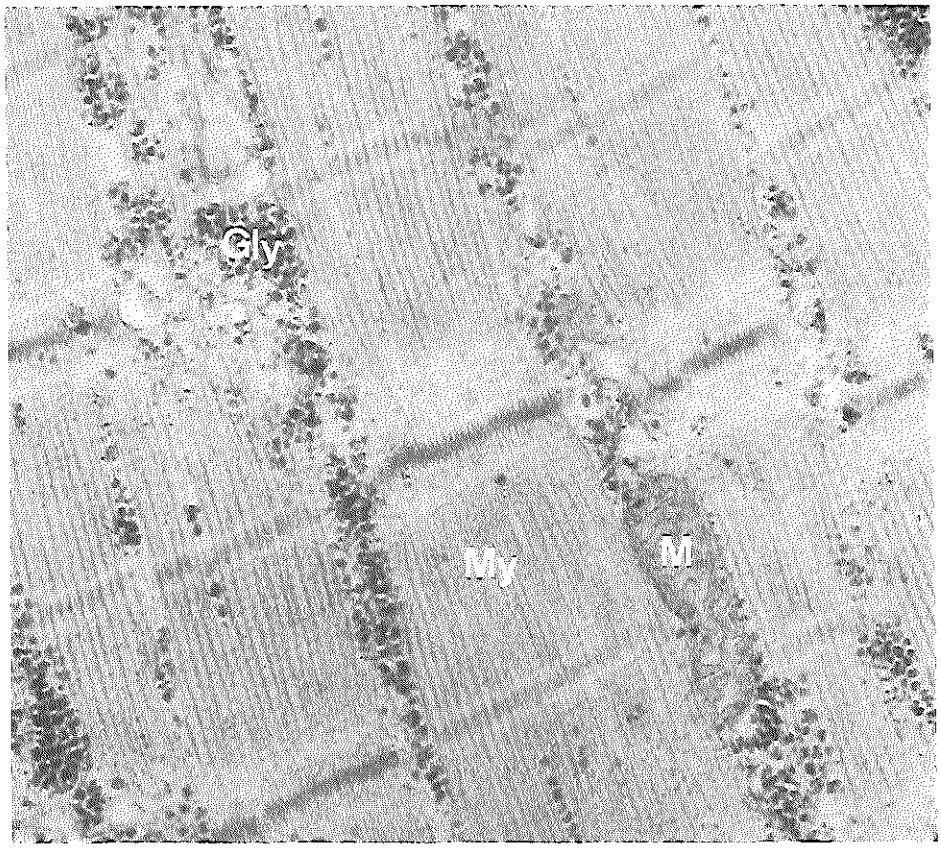

Fig. III.40. Electron micrograph (x 177.000) showing details of the skeletal muscle of a fetal guinea pig at 50 days of gestation. M: mitochondrion, Gly: glycogen, My: myofibril.
Note that the mitochondria contain more cristae and are now in an interfibrillar position.

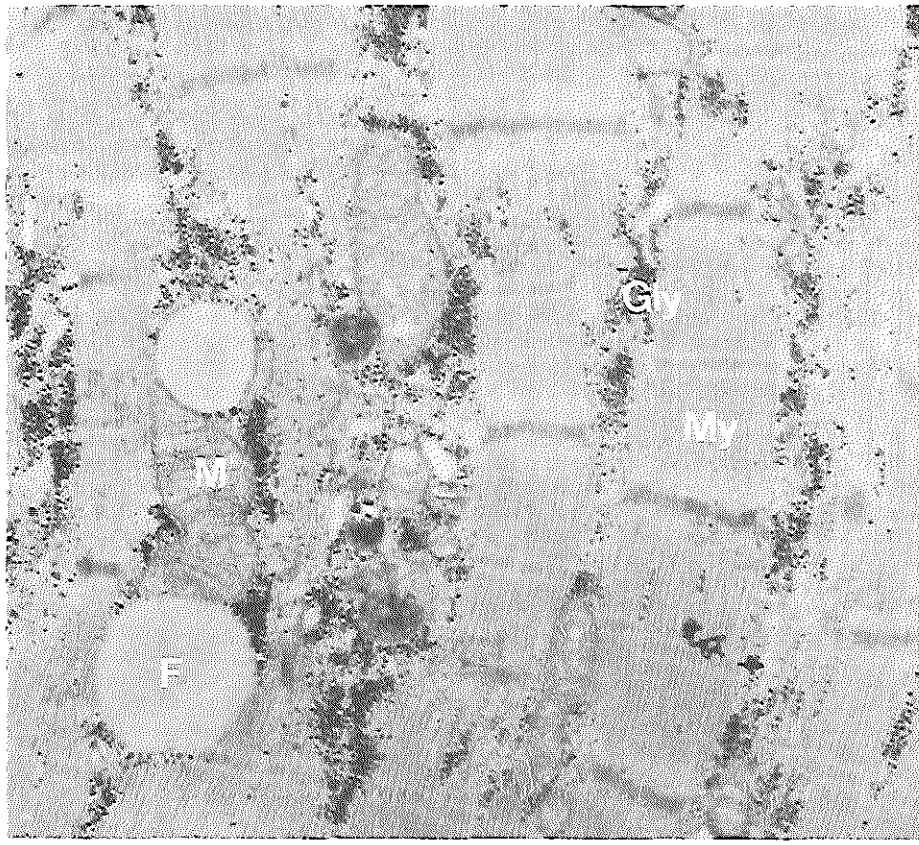

Fig. III.41. Electron micrograph (x 100.000) showing details of the skeletal muscle of an intrauterine growth retarded fetal guinea pig at 51 days of gestation. M: mitochondrion, Gly: glycogen, F: fat, My: myofibril.
Note that many mitochondria contain vacuoles.

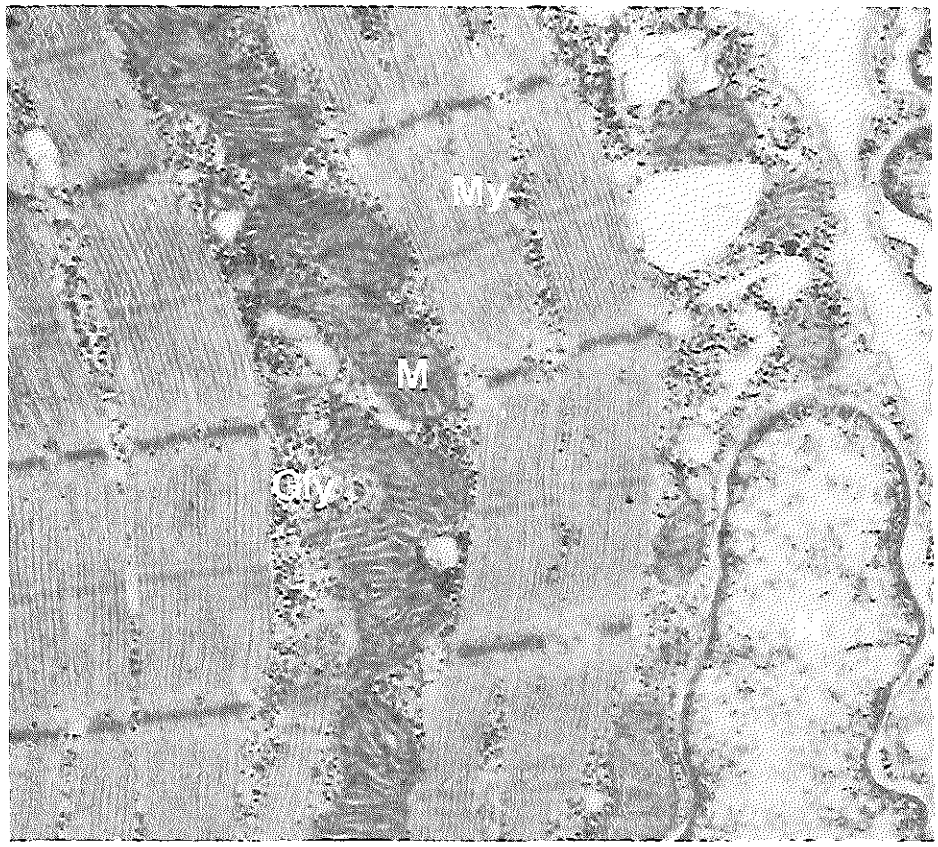

Fig. III.42. Electron micrograph (x 100.000) showing details of the skeletal muscle of a fetal guinea pig at 60 days of gestation. N: nucleus, M: mitochondrion, Gly: glycogen, My: myofibril. Note the more peripheral location of the nucleus while more mitochondria and well developed myofibrils are present in comparison with the myocyte at an earlier gestational age.

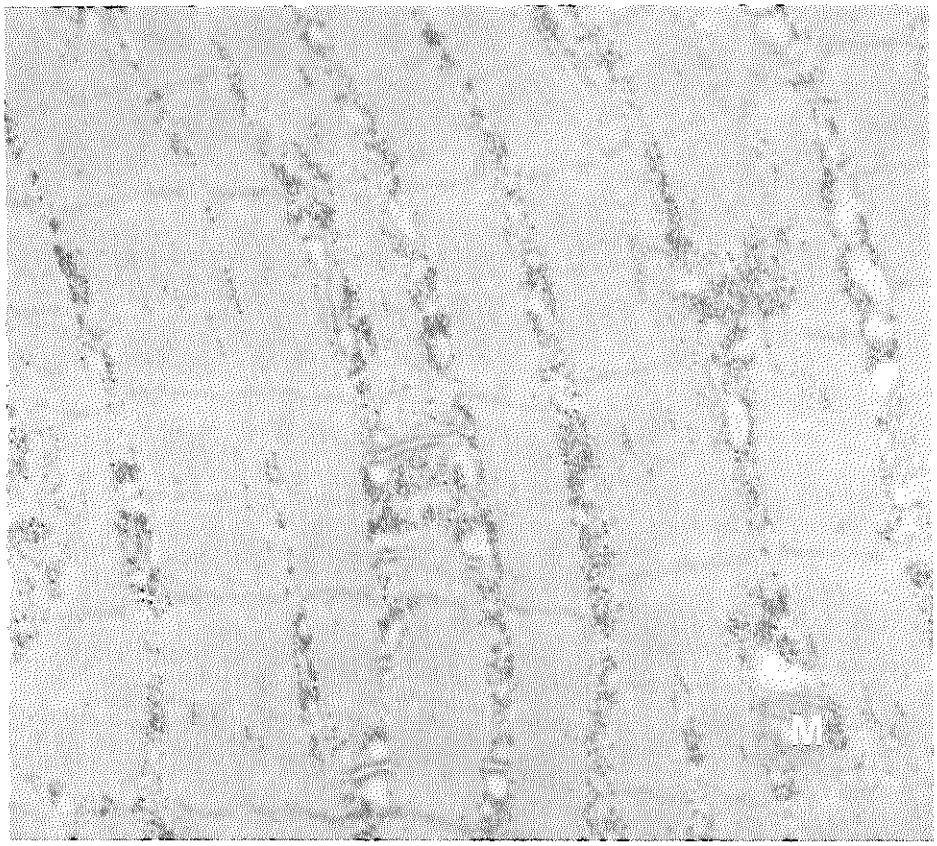

Fig. III.43. Electron micrograph (x 100.000) showing the skeletal muscle of an intrauterine growth retarded fetal guinea pig at 61 days of gestation. Relatively few mitochondria (M) are visible in this section of skeletal muscle.

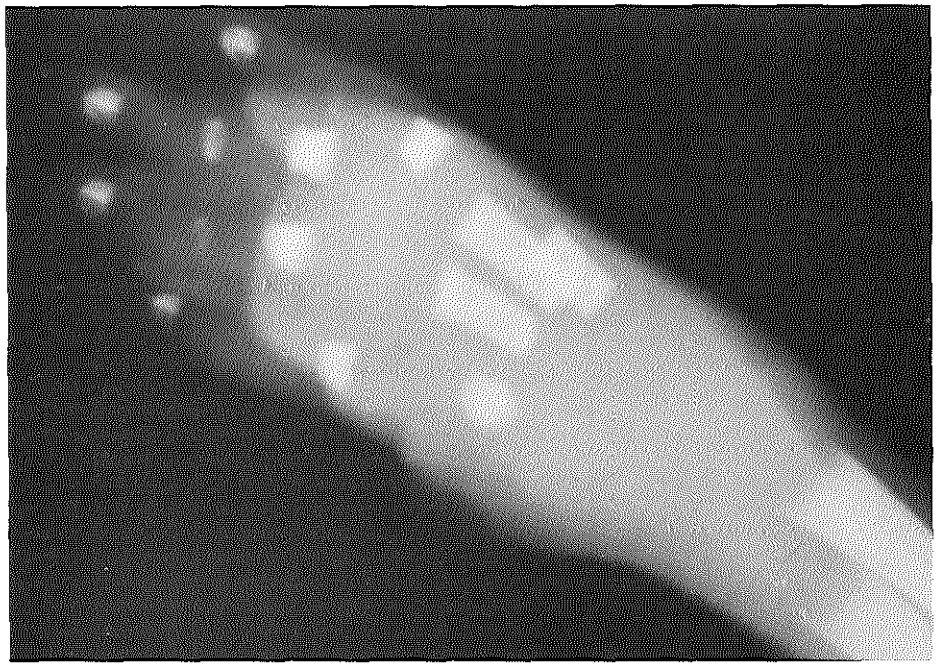

Fig. III.44. X ray photograph of the left upper limb of a fetal guinea pig at 40 days of gestation.

III.5.5. Bone structure

In the normal fetal guinea pig ossification of the skeletal growth nuclei occurs from 40 days to term. The normal fetal guinea pig at 40 days has no central bone formation in the epiphysis of ulna and radius, phalanges, carpals and metacarpals (fig. III.44). At 50 days some ossification can be seen in the normal but not in the small fetus (fig. III.45, III.46). At 60 days normally most ossification of the epiphysial growth nuclei is complete but in the small fetus this process is delayed (fig. III.47, III.48). This delayed ossification is also illustrated in micrography of the os corpi ulnare of the 50 days fetus. In the normal fetus central bone formation is present (fig. III.49) while in the small fetus this structure still consists mostly of chondrocytes (fig. III.50). In the normal fetus at 50 days the distal epiphysis of the radius is well developed and regularly shaped with some bone formation at both sides (fig. III.51). In the small fetus this epiphysis is still poorly developed and irregular with bone formation only on one side (fig. III.52). This delay in skeletal development is also shown by means of a scoring system. Points from 1 to 5 were given for development of central ossification and appearance of the epiphysial discs of lower and upper left limbs. Tables III.12 and III.13 show clearly that the small fetuses scored fewer points.

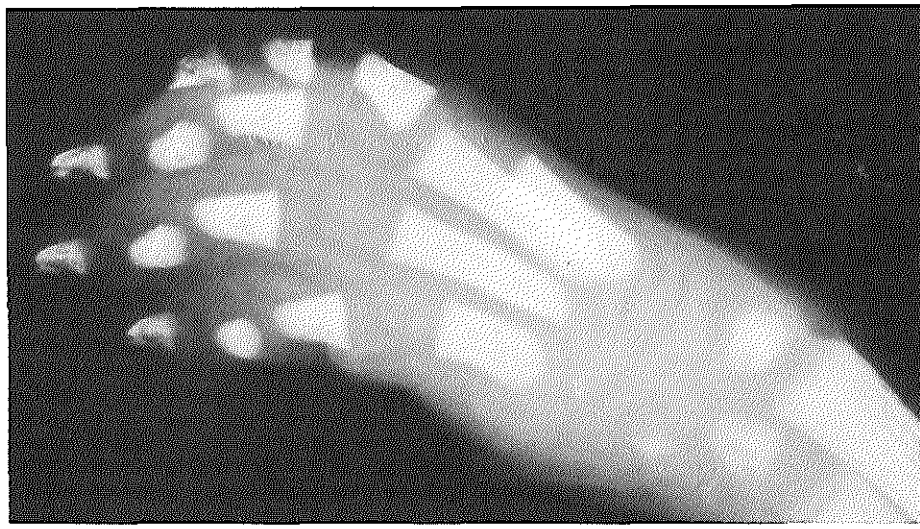

Fig. III. 45. X ray photograph of the left upper limb of a fetal guinea pig at 50 days of gestation.

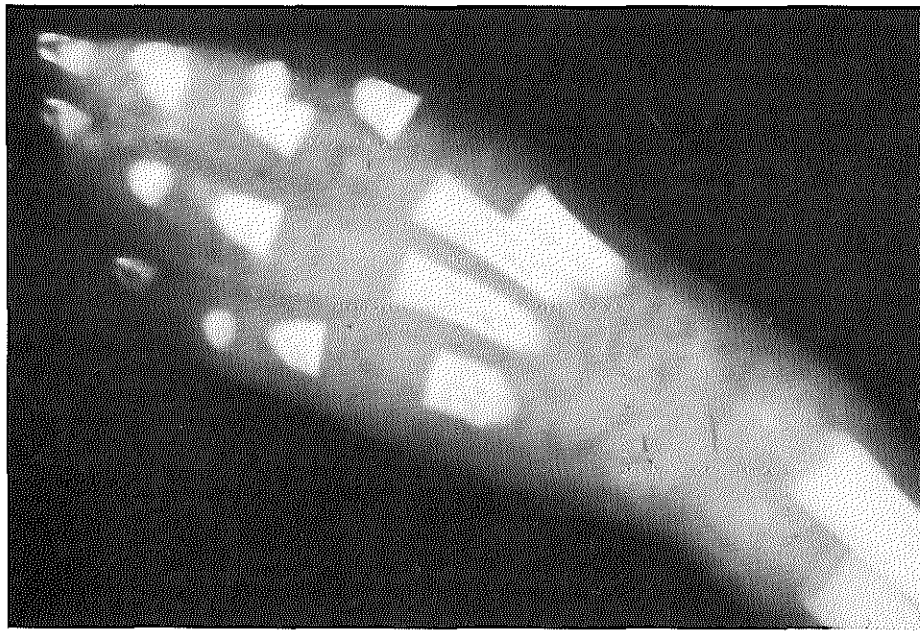

Fig. III. 46. X ray photograph of the left upper limb of an intrauterine growth retarded fetus at 50 days of gestation. This fetus was a littermate of that presented in Fig. III. 45. Their bodyweight and bodylength were 33.0 g. and 19.3 g. and 10.1 cm. and 8.4 cm. Note the delayed ossification in phalanges, carpals, metacarpals, radius and ulna of the small fetus.

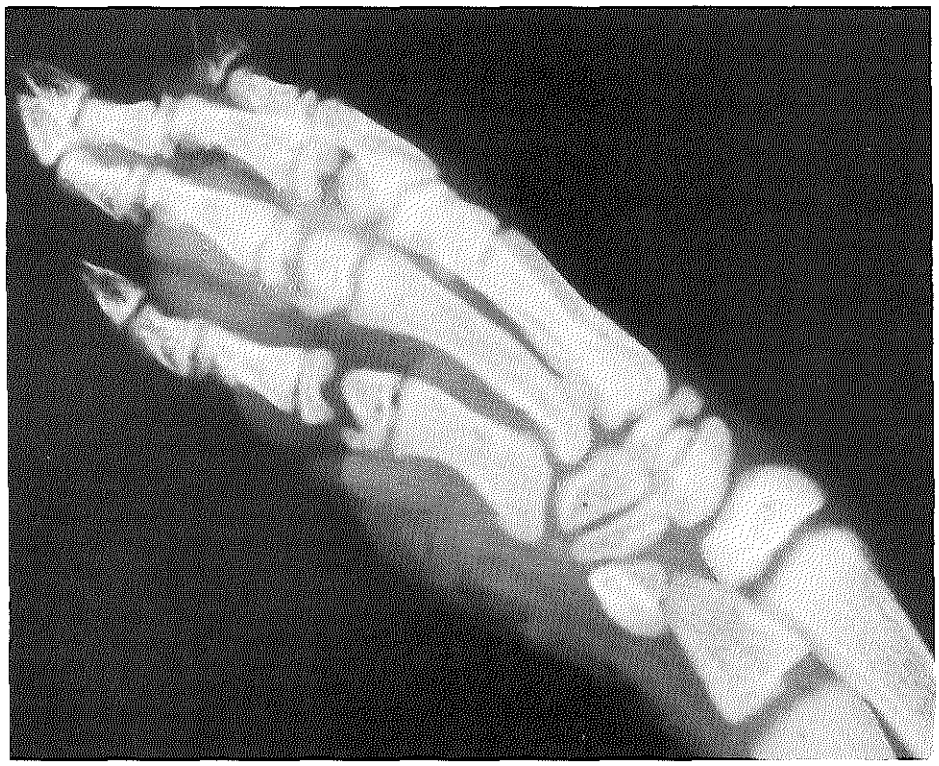

Fig. III. 47. X ray photograph of the left upper limb of a fetal guinea pig at 61 days of gestation.

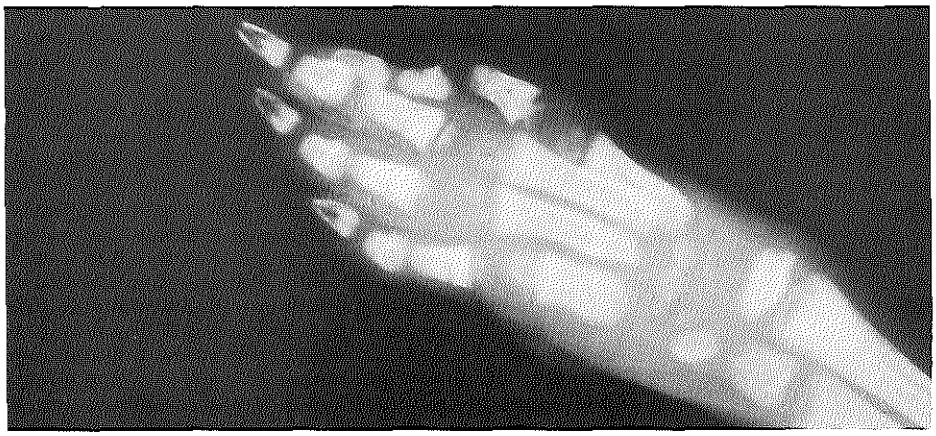

Fig. III. 48. X ray photograph of the left upper limb of an intrauterine growth retarded fetus at 61 days of gestation. This fetus was a littermate of that presented in Fig. III. 47. Their bodyweight and bodylength were 82.2 g. and 23.6 g. and 14.5 cm. and 9.7 cm. Note the delayed ossification in phalanges, carpals, metacarpals, radius and ulna of the small fetus.

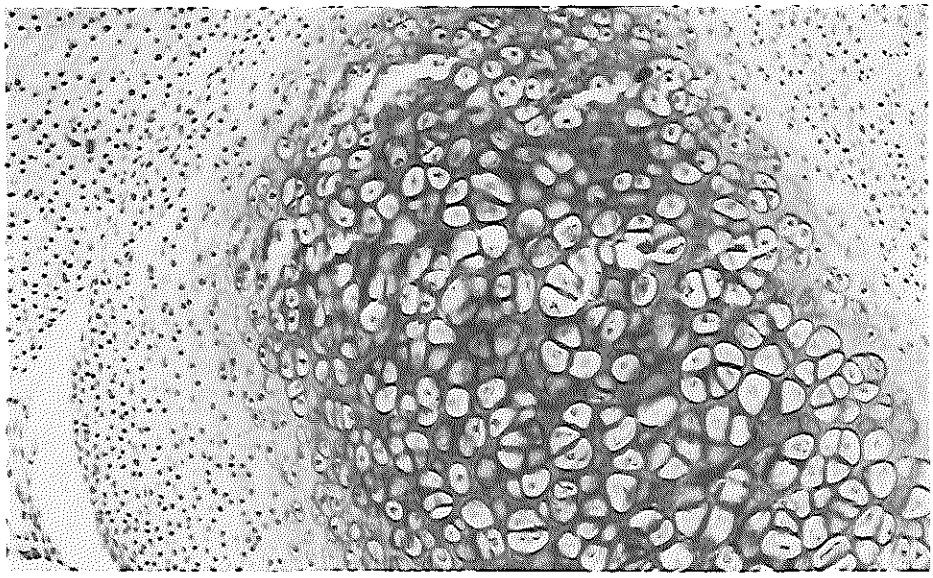

Fig. III. 49. Light micrograph of a central section of the os carpiulnare of a fetal guinea pig at 51 days of gestation (magnification is x 200). The section was stained with haematoxylin and eosin.

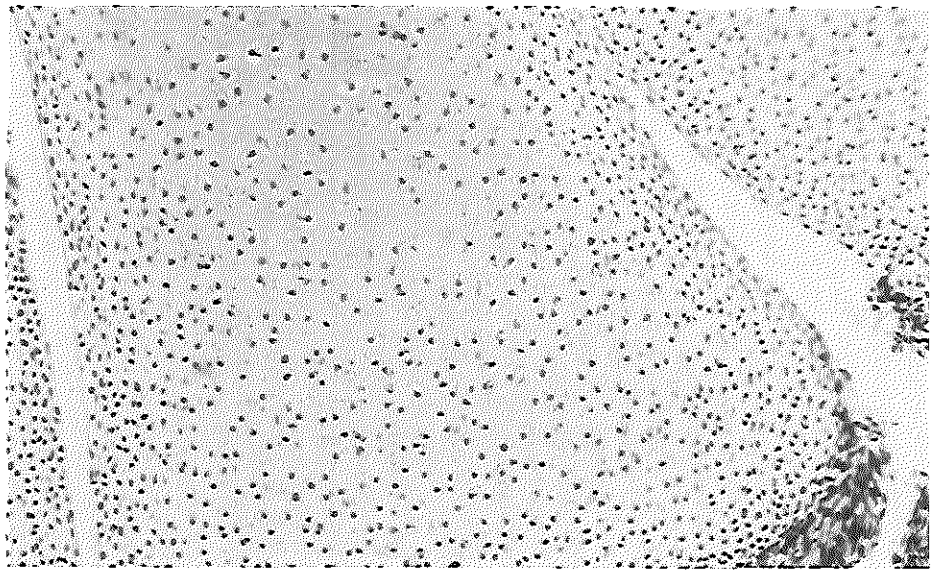

Fig. III. 50. Light micrograph of a central section of the os carpiulnare of an intrauterine growth retarded fetal guinea pig at 51 days of gestation (magnification and staining as in Fig. III. 49). This fetus (bodyweight 20.9. g.) was a littermate of that presented in Fig. III. 49 (bodyweight 42.8 g.). Note the delay in bone formation in the small fetus.

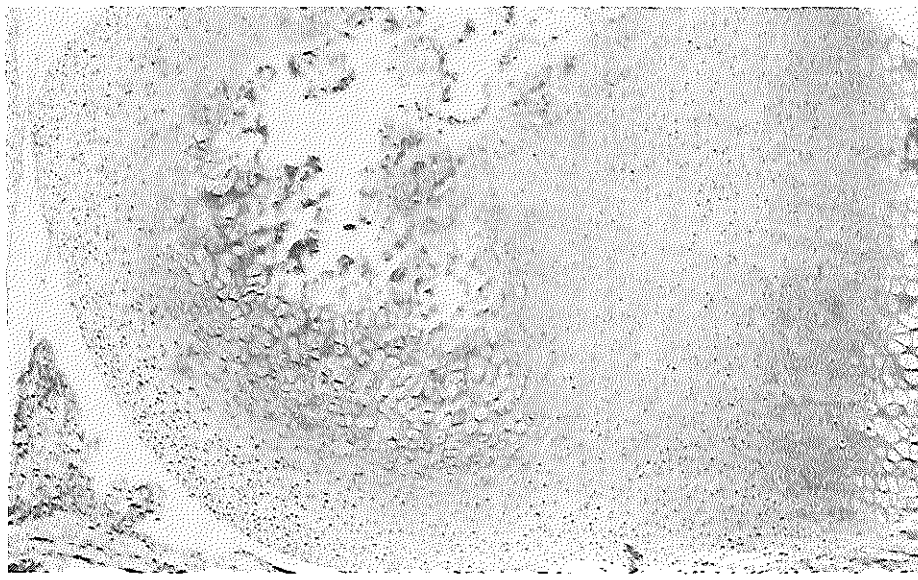

Fig. III. 51. Light micrograph of a central section of the distal epiphysis of os metacarpale 2 in a fetal guinea pig at 51 days of gestation (magnification is x 100). The section was stained with haematoxylin and eosin.

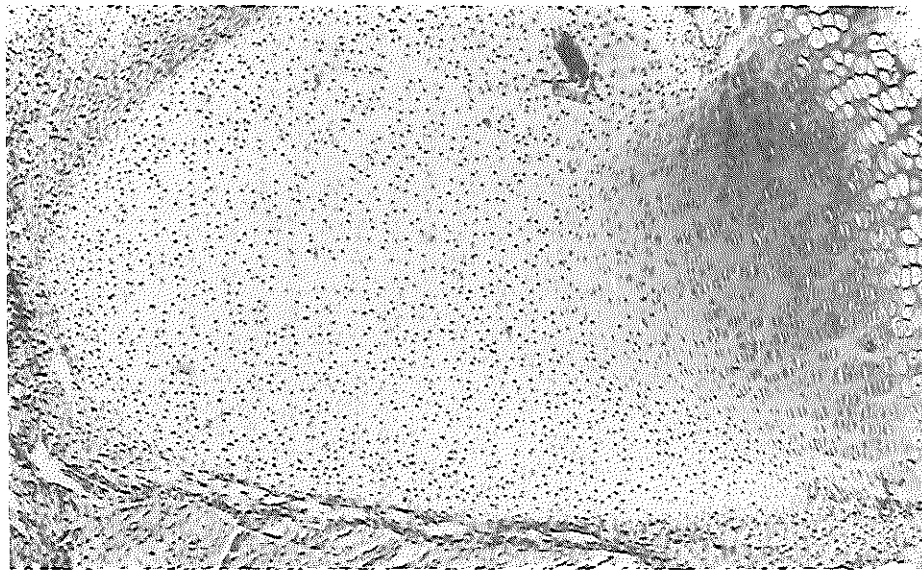

Fig. III. 52. Light micrograph of a central section of the distal epiphysis of os metacarpale 2 in an intrauterine growth retarded fetal guinea pig at 51 days of gestation (magnification and staining as in Fig. III. 51). Normal and small fetuses in Fig. III. 51 and III. 52 were identical to that in Fig. III. 49 and III. 50. Note the delay in central bone formation of the epiphysis of the small fetus.

Table III. 12. Epiphysial development of normal and small fetal guinea pigs.

Points to score for grade of ossification	1: no ossification present 2: irregular traces of central ossification 3: regular traces of central visible ossification 4: irregular but clearly visible ossification 5: regular and clearly visible ossification	A
Appearance of epiphysial disk	1: no disk present 2: disk narrow and irregular border 3: disk narrow and regular border 4: disk wide and irregular border 5: disk wide and regular border	B

	49 – 51 days				60 – 63 days			
	Normal		Small		Normal		Small	
Fetal bodyweight (g.)	40.3 ± 6.6 (3)		20.0 ± 1.4°(3)		77.8 ± 3.1 (8)		35.0 ± 5.7°(8)	
Fetal bodylength (cm.)	11.2 ± 0.8 (3)		9.2 ± 0.7 (3)		14.3 ± 0.4 (8)		11.2 ± 0.7°(8)	
Upper limb	Points	(A+B)	Points	(A+B)	Points	(A+B)	Points	(A+B)
Humerus – proximal	6	(2+4)	2	(1+1)	10	(5+5)	10	(5+5)
Humerus – distal	10	(5+5)	8	(4+4)	8	(5+3)	8	(5+3)
Radius – proximal	7	(4+3)	4	(2+2)	8	(5+3)	6	(4+2)
Radius – distal	10	(5+5)	6	(2+4)	10	(5+5)	10	(5+5)
Ulna – proximal	9	(4+5)	2	(1+1)	10	(5+5)	10	(5+5)
Ulna – distal	10	(5+5)	6	(2+4)	10	(5+5)	10	(5+5)
Metacarpals – distal	9	(4+5)	2	(1+1)	8	(5+3)	7	(5+2)
Proximal phalanges – proximal	3	(1+2)	2	(1+1)	8	(5+3)	7	(5+2)
Proximal phalanges – medial	3	(1+2)	2	(1+1)	8	(5+3)	5	(3+2)
Proximal phalanges – distal	3	(1+2)	2	(1+1)	7	(5+2)	2	(1+1)
Lower limb								
Femur – proximal	6	(2+4)	2	(1+1)	10	(5+5)	8	(4+4)
Femur – distal	7	(4+3)	6	(4+2)	8	(5+3)	7	(5+2)
Tibia – proximal	10	(5+5)	8	(4+4)	10	(5+5)	9	(5+4)
Tibia – distal	10	(5+5)	8	(4+4)	10	(5+5)	9	(5+4)
Fibula – distal	3	(1+2)	2	(1+1)	10	(5+5)	7	(3+4)
Metatarsalia – distal	8	(3+5)	2	(1+1)	8	(5+3)	6	(4+2)
Proximal phalanges – proximal	3	(1+2)	2	(1+1)	8	(5+3)	6	(4+2)
Proximal phalanges – medial	3	(1+2)	2	(1+1)	8	(5+3)	4	(2+2)
Proximal phalanges – distal	3	(1+2)	2	(1+1)	7	(5+2)	2	(1+1)
Total points	123		70		166		133	

°P<0.001

The results of bodyweight and bodylength are expressed as means ± S.D. with the number of observations in parentheses.

Table III. 13. Ossification of carpal and tarsal bones in normal and small fetal guinea pigs.

Points to score	1: no ossification present 2: irregular traces of central ossification 3: regular traces of central ossification 4: irregular but clearly visible ossification 5: regular but clearly visible ossification

	49 - 51 days		60 - 63 days	
	Normal	Small	Normal	Small
Fetal bodyweight (g.)	40.3 ± 6.6 (3)	20.0 ± 1.4°(3)	77.8 ± 3.1 (8)	35.0 ± 5.7°(8)
Fetal bodylength (cm.)	11.2 ± 0.8 (3)	9.2 ± 0.7 (3)	14.3 ± 0.4 (8)	11.2 ± 0.7°(8)
Carpus.	Points	Points	Points	Points
Os calciforme	1	1	3	1
Os carpiradiale	1	1	5	5
Os carpiulnare	2	2	5	5
Os pisiforme	1	1	3	1
Os carpale I	2	1	5	4
Os carpale II	3	1	5	4
Os carpale III	2	1	5	4
Os carpicentrale	3	1	5	4
Os carpale IV	2	1	5	4
Tarsus				
Talus caput	2	1	5	4
Talus corpus	5	3	5	5
Talus collum	2	1	5	4
Calcaneus corpus	5	4	5	5
Calcaneus tuber	2	1	5	4
Os carpitibiale	2	1	5	4
Os tarsicentrale	1	1	5	4
Os cuboideum	1	1	5	4
Os tarsale I	2	1	5	4
Os tarsale II	2	1	5	4
Os tarsale III	2	1	5	4
Sesamoid tarsale I	1	1	5	1
Sesamoid tarsale III	1	1	5	1
Total points	45	28	106	80

° $P < 0.001$

The results of bodyweight are expressed as means ± S.D. with the number of observations in parentheses.

III.6. Discussion

III.6.1. Surgery

As indicated in table III.1, uterine artery ligation caused intrauterine growth restriction in about 60% of all operations. However half of these fetuses had died or were already reabsorbed. This was probably related to the extent of reduction of the blood supply to the placenta as death was frequently observed when the ligatures were very close to the main radial arteries. (fig. II.1)

Another cause was the presence of a large number of fetuses in the operated horn where the ovarian artery supply was probably unable to sufficiently compensate for the ligation of the uterine artery. However the cause of death was not clear in all cases.

Moll and Künzel (1971), using pressure measurements, estimate that the uterine artery supplies 60% and the ovarian artery about 40% of the arterial blood flow of one uterine horn. The present data are comparable to that of Wigglesworth (1964) who was using the rat. He also found a variable degree of growth retardation in the ligated horn but the fetuses closest the ligation always died.

Widdowson (1974) has found a significant difference in birth weight of male and female guinea pigs. The mean male birth weight was heavier than the female birth weight. In our experiments we were not able to establish such difference (table III.2). There were also no significant differences in mean fetal length between males and females (table III.2).

III.6.2. Fetal and neonatal growth

Mainly for practical reasons only a limited number of neonatal guinea pigs was studied. Operated pregnant guinea pigs were allowed to deliver and when this resulted in the birth of at least one significantly small newborn, the litter in each case was restricted to one normal and one small newborn kept at the same temperature. But in the 8 cases in which this was succesfull 6 newborns died within one month without obvious reasons. It should also be noted that it is not clear whether or not the ability of the growth retarded newborn guinea pig to feed itself is as good as that of its normal littermate. The study of neonates has therefore been restricted to weight and length measurements.

In the fetal guinea pig just before birth the growth rate decreases but during the first 20 postnatal days there is a sharp increase (fig. III.2, III.4). This is very similar to that found in many species including man (Tanner, 1962, 1978). After birth the normal infant grows at a rate greater than at any other time in postnatal life. This rapid growth decreases from birth onwards over the first 4 years of life and than the rate falls to become comparatively steady until the beginning of the puberty growth spurt (Tanner, 1962, 1978).

In the guinea pig the normal postnatal growth rate at 10.2 g./day (between 10 and 20 days after birth) is higher than that of 4.2 g./day between 50 and 60 days

gestational age (fig. III.5). As far as body length is concerned the pre- and postnatal growth velocity in the normal guinea pig remain unchanged between 50 and 60 days of gestation and between 10 and 20 days after birth (fig. III.6). In the growth retarded guinea pig however the growth velocity increases from 0.22 cm/day to 0.30 cm./day over the periods mentioned above (fig. III.6). After the first 20 postnatal days the rate of increase in weight and length are approximately similar in normal and small guinea pigs. Whether or not the small animals still continue to grow at a high rate while the normal growth rate becomes steady at approximately 150 days after birth and whether or not they may eventually "catch up" is not certain.

The human "follow up" studies (Ounsted and Taylor, 1971; Fitzhardinge and Steven, 1972; Beck and Van den Berg, 1975; Fancourt *et al.*, 1976; Commey and Fitzhardinge, 1979) indicate that although some small for dates infants grow at higher rates than normal infants during the first six months after birth, most of them remain smaller for the rest of their life. While "catch up growth" in length rarely leads to normal size later in life (i.e. to reach the 10th centile of the growth curve for bodyheight) (Fitzhardinge and Steven, 1972), "catch up growth" in head-circumference is often noticed to lead to normal head size at one year of age (i.e. the 50th centile of headcircumference) (Babson, 1970, 1974; Van Gils, 1971; Fancourt *et al.*, 1976).

III.6.3. Asymmetric organ growth

A striking feature of intrauterine growth retardation in man found at autopsies is the asymmetric growth of the various organs (Gruenwald, 1963; Naye, 1965). The same was found in the growth retarded primate (Hill, 1974) and other species (fig. III.53).

When analysing the present data in the growth retarded guinea pig the same effect on organ growth was found. Body length was proportionally less affected than body weight (table III.2, fig. III.4, III.10). Similarly the fall in brain weight, although significant, is proportionally less in the small fetus (tables III.5, III.6, fig. III.53).

As a percentage of body weight the brain weight is increased (fig. III.7). This increase is even more pronounced in the severely growth retarded guinea pig especially at 50 days (fig. III.7). Measurements of subdivided organs like cerebrum, cerebellum and medulla oblongata (tables III.5, III.6, fig. III.7) showed in general the same results as for the whole brain. Expressed as a percentage of normal the medulla oblongata seems less affected (fig. III.10). Many organs are reduced in weight in the small fetal guinea pigs in proportion to body weight. Examples of these are the heart, the kidney and the lungs (tables III.5, III.6, fig. III.8, III.9, III.10, III.53). Only in severely retarded fetal guinea pigs at 60 days was the relative lung weight significantly less than normal. No such changes were found for heart and kidney. In general these changes in the guinea pig are consistent with those found in man and rhesus monkey (fig. III.53). The results of some endocrine organs like pancreas and adrenal gland are variable (tables III.5, III.6, fig. III.8, III.9, III.53). The pancreas is reduced in

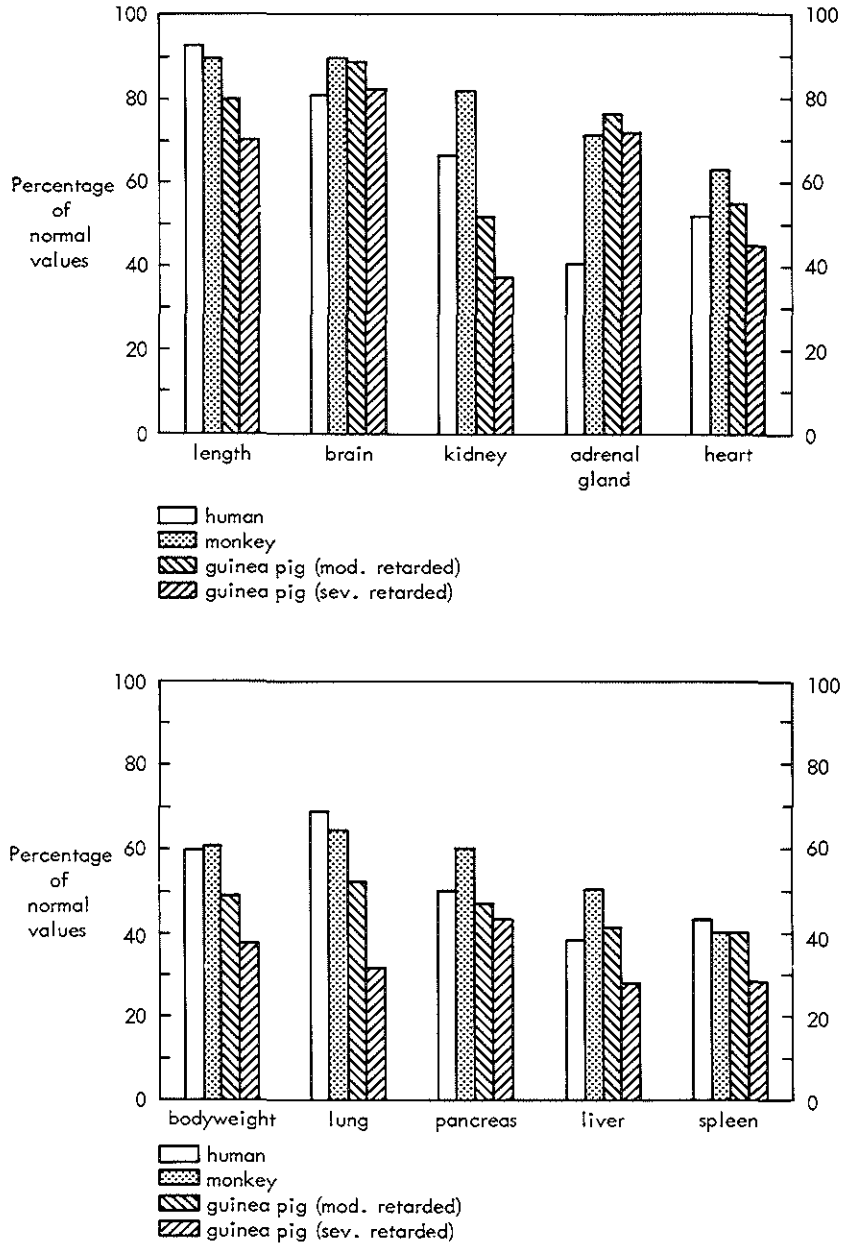

Fig. III. 53. Comparative organ size in intrauterine growth retarded fetuses of man, monkey and guinea pig. The human data is taken from Gruenwald (1963) and Naeye (1965) and that of the monkey from Myers *et al.* (1971) and Hill (1974). The data for the guinea pig refers to 60 - 63 days fetuses studied after 30 days of uterine artery ligation. These fetuses were subdivided into those moderately growth retarded and those severely growth retarded as described in chapter II. (reproduced from Lafeber *et al.*, 1979).

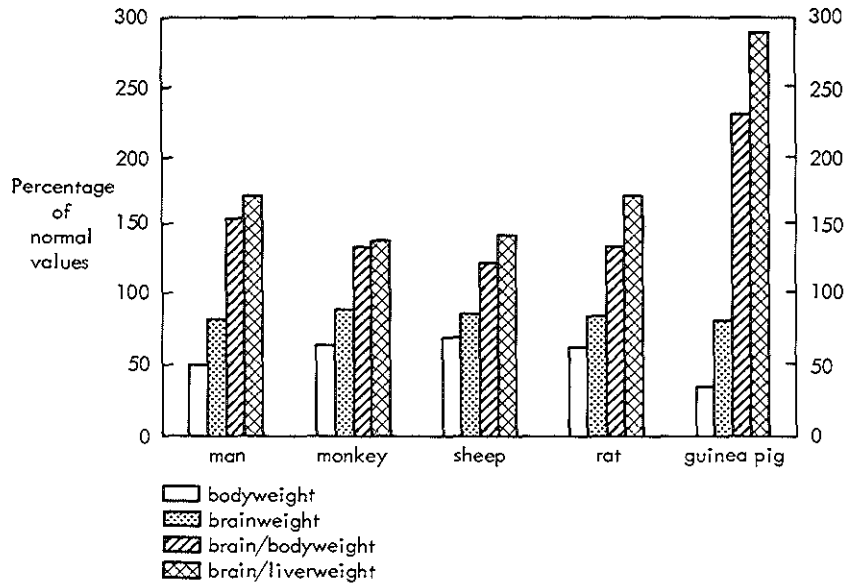

Fig. III. 54. The relative size of the brain in intrauterine growth retarded fetuses of a number of species. The data for men is from Naeye (1965), that for the monkey from Hill *et al.* (1971), Myers *et al.* (1971) and Hill (1974), that for the sheep from Creasy *et al.* (1972) and that for the rat from Roux *et al.* (1970). Data of the guinea pig refers to intrauterine growth retarded fetuses at 60 - 63 days after 30 days of uterine artery ligation (reproduced from Lafeber *et al.*, 1979).

proportion with the body weight (apart from the severely retarded fetal guinea pigs at 50 days), a consistent feature with the findings in man and rhesus monkey (fig. III.53). The fetal adrenal gland / body weight ratio however shows a large increase at both 50 and 60 days, especially in the severely affected guinea pigs (tables III.5, III.6, fig. III.9). Although this change is similar with that found in the monkey it is not consistent with that found in man (fig. III.53).

Some visceral organs like liver and spleen as well as the perirenal fat are characteristically more reduced in proportion to body weight both at 50 and 60 days especially in the severely affected fetuses (tables III.5, III.6, fig. III.8, III.9, III.10, III.53). The ratio of brain to liver weight, representing the organs that are less and most affected in growth by the intrauterine growth restriction,is described as one of the markers of this condition at autopsy (Gruenwald, 1963; Naye, 1965). This ratio normally remains stable at 50 and 60 days of gestational age. A significant increase is seen in the severely affected fetuses at 50 and 60 days. The difference in increase of this ratio between moderately and severely affected fetuses is significant especially at 60 days (tables III.5, III.6, fig. III.54).

III.6.4. The placenta

There are numerous reports of changes in placental structure associated with growth retardation (Gruenwald, 1963; Robertson *et al.*, 1975; Fox, 1975) and cytological changes have been reported for the rat and monkey placentas after uterine artery or placental vessel ligation (Myers and Fujikura, 1968; Metz and Heinrich, 1977). An important question of the growth retarded condition is the extent to which placental function is affected. Is the transport potential of the placenta reduced? Does it make compensatory adjustments in the way it handles nutrients or in its secretion of hormones to the mother and the fetus? The present data (mainly anatomical) indicate possibly a fall in the effective exchange area of the placenta (Robertson, 1975; Fox, 1975).

There are also reports of an increase in the fetal/placental weight ratio which has led to the suggestion that the fetus compensates for the reduction in placental weight (Alexander, 1964; Thomson *et al.*, 1969).

However infarctions are frequently seen in the placenta of the growth retarded fetus (Gruenwald, 1963; Fox, 1975; Gruenwald, 1974; Robertson *et al.*, 1975; Gruenwald, 1975). In the guinea pig for instance these can cover a large part of the placenta (fig. III.11).

In the pregnant guinea pig, uterine artery ligation causes a marked reduction of placental weight at 50 and 60 days, especially in the severely affected fetuses (tables III.5, III.6). The fetal/placental weight ratio is significantly decreased at both age groups (tables III.5, III.6). The reduction in this ratio is remarkably significant in the severely retarded fetal guinea pigs at 60-63 days gestational age (table III.6). Moreover placental weight correlates well with fetal age over the latter third of gestation in the normal but not in the growth retarded fetal guinea pig (fig. III.12). These findings therefore question the value of simple weight measurements in the functional assessment of the placenta. Apart from the anatomical studies in various species, that were already mentioned before, sofar studies on placental function in association with intrauterine growth retardation have only been performed in the rat. Nitzan *et al.* (1979), using the method of Wigglesworth (1964), have infused the maternal rats 2 days after the uterine ligation with [^{3}H] 2-dexoxyglucose and [^{14}C]α-aminoisobutyric acid intravenously. In spite of an increase in fetal to placental weight ratio in the small fetuses less radioactivity derived of both the analogues was found in the small fetuses. Nitzan *et al.* suggested that one of the major causes for intrauterine growth retardation is the diminished supply of nutrients to the fetus via the placenta. It must be remembered however that accumulation of radioactive label in the fetus is not necessarily the same as placental transfer.

Another indication of a poor placental transport function in intrauterine growth retardation is suggested by the lower pO_2 values found in the chronic sheep experiments (Robinson *et al.*, 1979). No significant changes in blood gases and pH were found in the present acute guinea pig experiments (table III.9).

III.6.5. Liver and brain composition

Even though the growth of the brain is maintained in growth retarded fetuses relative to that of other organs, it is clear that there is a significant reduction in its mass (tables III.5, III.6). Moreover it is striking that in the various species in which intrauterine growth retardation has been studied the extent of the reduction in brain mass (i.e. 15-20%) is very similar (fig. III.54). There are examples of restricted brain growth causing a deficit in neuronal and in glial cell numbers and in the extent of myelination (Dobbing and Sands, 1973; Dobbing *et al.*, 1971; Culley and Lineberger, 1968). The extent to which any prenatal deficit can be made up postnatally is unclear and is likely to vary with the species. Brain development is largely postnatal in the rat, is pre- and postnatal in man and is mainly prenatal in the guinea pig (Dobbing and Sands, 1970) although even here some postnatal neurogenesis occurs (Altman and Das, 1967). In the guinea pig brain the major phase of neurogenesis occurs between days 40-45 of gestation (Peters and Flexner, 1950; La Velle and Windle, 1951). Thus the smaller brain, with less total DNA, of the 50 days growth retarded fetal guinea pig may contain fewer neurones than normal (tables III.5, III.7). Between days 55-65 of gestation there is marked water loss, glial cell formation, appearance of continuous electrical activity and maximal rates of myelination (Wender and Hierowski, 1960; Wender and Waligora, 1961; Rosen and McLaughlin, 1966; Agrawal *et al.*, 1968; Dobbing and Sands, 1970; Fellows and Jones, 1981). The smaller brain of the growth retarded fetus at 60-63 days and its lower DNA concentration and content suggests that these processes are delayed (tables III.6, III.7).

Indeed there are reduced rates of myelin formation in the brain of small fetuses (Fellows and Jones, 1981). Much cerebellar growth is prenatal in the guinea pig and unlike the cerebrum and mid-brain centres it is almost complete at birth (Altman and Das, 1967). Thus in contrast to the cerebrum, as the major effects of growth retardation on cerebellar growth were seen only by 60-63 days (table III.6), the sensitive phases of cerebellar development probably occur between 50 and 63 days. These relatively small effects on the guinea pig cerebellum contrast with the greater sensitivity of the cerebellum than cerebrum to malnutrition in the fetal guinea pig (Chase *et al.*, 1971) and the newborn rat (Fish and Winick, 1969). As a whole the reduction in brain mass associated with a fall in DNA, RNA and protein is consistent in the various species investigated (table III.15). But whether or not these deficits can be made up postnatally is not clear since no reliable data are present. In man follow up studies of small for gestational age fetuses, determined by ultrasound techniques, suggest that if the growth restriction occurs after 26 weeks, at which time much neurogenesis and some myelination has occurred (Dobbing, 1973), the biparietal distance is reduced but there is postnatal compensation probably to produce a normal headcircumference. Growth restriction before 26 weeks causes permanently reduced headcircumference and neurological damage (Fancourt *et al.*, 1976; Wallis *et al.*, 1977).

In the present study the effects on liver growth were the most striking of any of the

Table III. 14. Comparison of energy stores of intrauterine growth retarded fetuses of several species and human small for dates neonates.

PERCENTAGE OF NORMAL VALUES

Species	Man	Monkey	Rat	Guinea pig
Fetal bodyweight	30 - 60°	65°	66°	38°
Liver				
Glycogen				
(wt./g. liver)	35	100	43 - 81°	164$^{\square}$
(wt. liver/g. bwt.)	-	98	45°	125
Triglycerides				
(wt./g. liver)	-	19$^{\triangle}$	-	56$^{\triangle}$
(wt. liver/g. bwt.)	-	21$^{\triangle}$	-	53$^{\triangle}$
Adipose tissue				
Perirenal fat				
(wt./g. bwt.)	-	-	-	90°
Interscapular fat				
(wt./g. bwt.)	-	-	62$^{\square}$	-
Total fat				
(wt./g. bwt.)	reduced	-	73$^{\square}$	-

$\triangle P<0.05$ $\square P<0.01$ $\circ P<0.001$

References
Man: Dawkins, 1964, Shelley and Neligan, 1966; Brans *et al.*, 1974; Dauncey *et al.*, 1977; Mettau *et al.*, 1977, 1978.
Monkey: Myers *et al.*, 1971; Hill *et al.*, 1971; Hill, 1974.
Rat: Hohenauer and Oh, 1969; Roux *et al.*, 1970; Nitzan and Groffman, 1970, 1971; Oh *et al.*, 1970; Chanez *et al.*, 1971; Cogneville *et al.*, 1975.
Guinea pig: Data of severely growth retarded fetal guinea pigs at 60 - 63 days of gestation (bodyweight < 35 g.) after 30 days of uterine artery ligation.

Data refers to intrauterine growth retarded fetuses near term and where appropriate to human "small for dates" neonates shortly after birth (reproduced from Lafeber *et al.*, (1979).

organs and there were large changes in chemical and cellular composition in addition to those in size. Previous investigations with the growth retarded fetal rat and rhesus monkey have shown that, while in general the total mass of hepatic constituents is reduced, the composition of the liver is relatively unchanged (Hohenauer and Oh, 1969; Roux *et al.*, 1970; Oh and Guy, 1971; Hill *et al.*, 1971). The present observations indicate a much more complex picture. Normally during fetal

Table III. 15. Changes in brain composition of intrauterine growth retarded fetuses of several species.

	PERCENTAGE OF NORMAL VALUES					
Species	Man	Monkey	Rat	Rabbit	Guinea pig	
Fetal bodyweight	61$^{\square}$	65°	67	85°	48$^{\square}$	38°
Cerebral weight	79$^{\triangle}$	90$^{\triangle}$	91	87°	86$^{\square}$	79°
Total cerebral DNA	81	87	99	88°	93$^{\triangle}$	72$^{\square}$
Total cerebral RNA	-	91$^{\triangle}$	104	-	71$^{\square}$	69$^{\square}$
Total cerebral protein	89	74	95	85°	81	74

$\triangle P<0.05$ $\square P<0.01$ $\circ P<0.001$

References: Man: Chase *et al.*, 1972.
Monkey: Hill, 1974.
Rat: Winick *et al.*, 1972; Winick, 1976.
Rabbit: Van Martens *et al.*, 1975.
Guinea pig: Data of moderately and severely growth retarded fetal guinea pigs at 60 - 63 days of gestation after 30 days of uterine artery ligation.

Data refers to intrauterine retarded fetuses near term; the human data refers to intrauterine growth retarded fetuses from 34 weeks to term (reproduced from Lafeber *et al.*, 1979).

development in the guinea pig, and in other species that have been investigated, there is an increase of liver-weight which during the first half of gestation is mainly caused by an increase in cell number and during the last half by an increase in cell volume and deposition of glycogen and of fat stores (Faulkner and Jones, 1978). In the fetal guinea pig liver there is an increase in the number of hepatocytes between day 30 and 50 of gestation (fig. III.13, Faulkner and Jones, 1978). At this time the cell volume remains stable (fig. III.13). Between 50 days and term the cell number decreases while the cell volume increases (fig. III.13). Measurements of the same parameters in the control 50 and 60 days fetuses show the same results (table III.8). The results of the protein and nucleic acids measurements are in accordance with these findings (table III.7). In the small, 50 days fetal guinea pig a significantly lower cell number was found in comparison with the normal number found at this age while the cell volume and the fractional volume were also significantly lower (table III.8). At 60 days however the cell number in small fetuses was significantly larger while cell volume and fractional volume were still lower, thus suggesting a delay of the normal development as indicated in fig. III.13. At 60 days no striking differences were found between moderately and severely retarded fetuses as far as this delay is concerned (table III.8).

The differences in the protein and nucleic acids concentration between normal and

small fetuses both at 50 and 60 days were more or less consistent with the differences found in cell number and volume.

The number of haematopoietic cells in the fetal guinea pig liver is much higher between 30 and 50 days of gestational age than the number of hepatocytes (fig. III.13, III.14, III.15, table III.8) but decreases towards term (fig. III.15, III.17) especially from day 50 onwards. The volume of these cells showed no changes during fetal development (fig. III.14). In the growth retarded fetuses both at 50 and 60 days the haematopoietic cell number was larger while the cell volumes were equal to that found in normal fetuses (table III.8, fig. III.15, III.16, III.17, III.18).
This delay in the normal decline in haematopoietic cell content of the fetal liver is consistent with the polycythaemia that was also found in the small fetuses (table III.9) at 50 days and even more at 60 days. An increase in hepatic haematopoietic cell content has been previously reported for small for gestational age newborn infants (Gruenwald 1963; Naeye, 1965) and is strikingly observed in fetuses during maternal anaemia (Dietzmann and Lessel, 1976). It would be of interest to know the origin of the signal to maintain hepatic erythopoiesis. It appears to be under erythropoietin control in the fetus (Zanjani *et al.*, 1973; Peschle *et al.*, 1975) and elevated erythropoietin levels have been reported in cord blood of small newborns (Finne, 1966) together with polycythaemia and a rise in hemoglobin (Lugo and Cassady, 1971; Haworth *et al.*, 1967; Humbert *et al.*, 1969). The fetal kidney is not the site of erythropoietin production (Zanjani *et al.*, 1973; Peschle *et al.*, 1975) and surprisingly the liver has been implicated as the major source (Peschle *et al.*, 1975; Peschle and Condorelli, 1976). Towards term the fetal liver of the guinea pig shows a large increase in glycogen and triacylglycerol content as indicated in fig. III.19 and III.20. These changes especially take place between 50 days and term. These deposits become important energy stores at and immediately after birth when they are rapidly mobilized. The same has been reported for glycogen deposition in the human newborn liver (Shelley and Neligan, 1966). In the small for dates newborn infant it has been suggested that the hepatic glycogen stores are less than normal (table III.14) and that this may therefore contribute to the neonatal hypoglycaemia. Unfortunately in these studies it is not possible to exclude the effects of the poor neonatal condition prior to death. Animal studies have shown no consistent picture. The hepatic glycogen concentration of the growth retarded fetus is less than normal in the rat (Hohenauer and Oh, 1969; Roux *et al.*, 1970; Nitzan and Groffman, 1970; Oh *et al.*, 1970; Hohenauer, 1971; Chanez *et al.*, 1971; Nitzan and Groffman, 1971) and unaffected in the monkey (Hill, 1974; Myers *et al.*, 1971) (table III.14).

In the fetal guinea pig however we find a larger glycogen concentration both at 50 and 60 days. In spite of the relatively low liver weight at 60 days the growth retarded fetus still has the same amount of liver glycogen available per gram body weight (table III.10). The triglyceride stores in adipose tissue and particularly in the liver are reduced by intrauterine growth retardation in man and various species (table III.14). In the guinea pig the picture seems rather complicated. At 50 days there is a small but significant increase in liver triglyceride concentration to that extent that, per gram

body weight, the same amount of liver triacylglycerol is available in both normal and small fetuses. (table III.11). Approximately the same difference although not significant was found between normal and moderately retarded fetuses. Severely retarded fetuses however have less triglyceride in their hepatocytes than normals (table III.11). This is comparable to the situation in the rhesus monkey (table III.14). In addition to the chemical measurements, hepatocytes of growth retarded fetal guinea pigs show more glycogen in their cytoplasma especially at 60 days (as described in III.6.6.). These findings are consistent with the results of the measurements of cell number and volume and of protein and nucleic acids in the small fetal liver. The higher glycogen concentration without the associated large increase in cell volume reduces the cytosolic volume of the cell and hence lowers the protein concentration. On the other hand in spite of the marked differences in liver fat content between 60 days moderately and severely retarded fetuses, their cell volumes are comparable and this is therefore suggesting that the changes in hepatocyte cell volume are not necessarily closely tied to changes in lipid content. The changes in hepatic glycogen and fat concentration will also be discussed in chapter V with respect to the metabolism of the fetus. No changes were found in the glycogen content of heart and skeletal muscle. The triacylglycerol content of the heart at 50 and 60 days was larger in the small fetuses. The reason for this is not clear.

III.6.6. Electron microscopic structure

In general the electron microscopic study of the normal development of the fetal guinea pig liver offers a good addition to the data on cellular and metabolite content that is discussed elsewhere in this chapter. Good examples of this are the deposition of glycogen and fat in the fetal liver towards term and the increase in cell number at 50 days followed by a decrease towards term. Cell volume of the hepatocyte rises between 50 and 60 days (tables III.7, III.8, III.10, III.11; fig. III.22-III.31).

As far as intrauterine growth retardation is concerned there are clearly parallel changes in structure and cellular and metabolite composition. Examples of the latter are for instance in the fetal liver the delay of normal development indicated at 50 days by a smaller amount of cytoplasma with fewer mitochondria that are less dense and with less endoplasmic reticulum (fig. III.24-III.27). At 60 days there is relatively more smooth endoplasmic reticulum, possibly also indicating a delay in normal development (fig. III.27, III.31). The high amount of glycogen found in the growth retarded liver (table II.10) was also noticed in the electron microscopic structure (fig. III.30) while in the same liver less fat was seen (table III.11, fig. III.30).

The general picture that emerges from the electron microscopic analysis of normal development and of growth retardation in the fetal guinea pig heart and skeletal muscle is that of a delay of normal development by growth retardation. It is notable that the fetal heart is less affected by growth retardation than the skeletal muscle (fig. III.12, III.43), a fact that is reflected also by the reduction in size of these two organs (tables III.5, III.6). The delay of the normal development of the skeletal muscle as

indicated by the poor development of the myofibrils may have significant causes for its functioning especially at and after birth. The relative sparing of the heart on the other hand, with maintenance of the glycogen storage could prove to be essential for survival.

Many aspects concerning structure of the fetal guinea pig liver, heart and skeletal muscle will be discussed in more detail in chapters V and VI.

III.6.7. Skeletal development

One of the first reports describing poor skeletal development in small for dates newborns was that of Scott and Usher (1964). Studying X-rays of the knee of human newborns that were made within 51 days after birth they found a significant delay in epiphysial development in small for dates newborn infants. The same was found by Wilson *et al.*, (1967) while these also performed a prospective follow up study of small for dates infants in which they found the same neonatal fibula growth rate for both these and normal infants until 3 months after birth. Because of the heterogeneity of the causes and the time of onset of intrauterine growth retardation in man, the delay of skeletal maturation at birth is a poor parameter for prediction of future growth (Philip, 1978).

In the small fetal guinea pigs at 50 and 60 days, there are also clear indications of poor skeletal development (tables III.12, III.13, fig. III.44-III.52) although there is a wide variability between individual fetuses. No neonatal data on skeletal development are present at this moment.

CHAPTER IV INTRA-UTERINE GROWTH RETARDATION, NUTRIENT SUPPLY AND ENDOCRINE DEVELOPMENT

IV.1. Introduction

In the preceding chapter it has been shown that uterine artery ligation affects fetal growth. This procedure not only results in large changes in relative organ size but also affects organ composition. It is likely that there is an effect on the vascular supply to the placenta in this condition, which could result in an alteration of the transfer of nutrients to the fetus. Therefore changes in circulating levels of nutrients in the growth retarded fetus may be expected. In order to investigate this the concentrations of some important metabolites have been measured in normal and growth retarded fetal guinea pigs at two different periods of gestation.

In the following chapters it will be shown that during intrauterine growth retardation there are marked changes in the functional development of various fetal organs. The underlying causes of these are not clear. There is evidence that the normal development of many fetal organs is under hormonal control; therefore it may be expected that in the growth retarded fetus the circulating levels of hormones are altered. To investigate this also the concentrations of a variety of hormones was measured in the plasma of normal and growth retarded fetal guinea pigs at the two periods of gestation.

This chapter will describe the results of these measurements in relation to that of the circulating nutrients. The possible implications on the functional development and growth of various fetal organs will be discussed.

IV.2. Results

IV.2.1. Plasma metabolite concentrations

Reduction of the rate of fetal growth because of uterine artery ligation was associated with low concentrations of plasma glucose (tables IV.1, IV.2) both at 49-51 days and at 60-63 days of gestation. Low concentrations of glucose in amniotic fluid coincide with the low concentrations in plasma (table IV.3) but there was no significant relationship between the two (fig. IV.1).

At 49-51 and 60-63 days the plasma concentrations of lactate and pyruvate were apparently unaffected by growth retardation (tables IV.1, IV.2). However the ratio of lactate to pyruvate was significantly higher at both age groups (tables IV.1, IV.2). The

Table IV. 1. Metabolite concentrations in plasma of normal and intrauterine growth retarded fetal guinea pigs at 49 - 51 days of gestation (concentrations in μmol./ml.).

	49 - 51 days		
	Maternal	Normal fetus	Small fetus
Bodyweight (g.)		39.8 ± 4.2 (6)	21.9 ± 2.6° (6)
Glucose	10.7 ±3.1 (6)	4.5 ± 0.6 (6)	1.6 ± 0.9° (6)
Lactate	8.0 ±2.4 (6)	8.9 ± 2.3 (6)	10.2 ± 2.9 (6)
Pyruvate	0.33 ± 0.10 (6)	0.28 ± 0.12 (5)	0.20 ± 0.07 (5)
Lactate/Pyruvate	24.2 ±9.4 (6)	32.4 ±11.2 (5)	51.6 ±12.7$^{\triangle}$ (5)
Citrate	0.20 ± 0.09 (6)	0.15 ± 0.06 (5)	0.25 ± 0.06$^{\triangle}$ (5)
Alanine	0.08 ± 0.03 (6)	0.10 ± 0.04 (6)	0.42 ± 0.25$^{\triangle}$ (6)
Serine	0.09 ± 0.03 (6)	0.13 ± 0.04 (6)	0.31 ± 0.07° (6)
Threonine	0.07 ± 0.04 (6)	0.09 ± 0.03 (6)	0.24 ± 0.06° (6)
Glutamate	0.05 ± 0.03 (6)	0.06 ± 0.03 (6)	0.05 ± 0.04 (6)
Glutamine	0.21 ± 0.06 (6)	0.41 ± 0.11 (6)	0.23 ± 0.12$^{\triangle}$ (6)
α aminonitrogen	16.4 ±2.6 (6)	13.1 ± 2.1 (6)	6.7 ± 2.2° (6)
Ammonia	0.15 ± 0.06 (6)	< 0.04 (6)	0.35 ± 0.04° (6)

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are means ± S.D. with the number of observations in parentheses.

plasma citrate concentration was approximately double the normal value in the growth retarded fetuses. The plasma acetate concentration, an important substrate for fatty acid synthesis in the fetal liver, showed no marked differences.

During the last two weeks before birth fatty acids, transported from the maternal circulation become an important source for triglyceride synthesis in the fetal guinea pig (Jones, 1976). It was therefore of note that the plasma free fatty acid concentration was related to the degree of growth retardation at 60-63 days. Fetuses that were 40% of normal weight or heavier had concentrations that were equal to those found in normal fetuses while those that were about 30% of normal weight had plasma free fatty acid concentrations that were 50% of that of their normal littermate controls (table IV.2).

In the plasma of the small 49-51 days and 60-63 days fetal guinea pigs the alanine, serine and threonine concentrations were much higher than normal (tables IV.1, IV.2). In the small fetuses the concentration of glutamine was lower at 49-51 days but much higher at 60-63 days. Plasma glutamate concentration was low both in normal and small fetuses (tables IV.1, IV.2).

The total α-amino nitrogen concentration was lower in the plasma of small fetuses at both ages (tables IV.1, IV.2). A very marked rise in plasma ammonia

Table IV. 2. Metabolite concentrations in plasma of normal and intrauterine growth retarded fetal guinea pigs at 60 - 63 days of gestation (concentrations in μmol./ml.).

	60 - 63 days		
	Maternal	Normal fetus	Small fetus
Bodyweight (g.)		86.8 ± 11.8 (12)	33.9 ± 9.4° (12)
Glucose	7.8 ± 2.3 (12)	5.7 ± 1.2 (12)	3.5 ± 1.0° (12)
Lactate	5.1 ± 2.2 (12)	6.4 ± 2.6 (12)	8.4 ± 2.9 (12)
Pyruvate	0.23 ± 0.06 (8)	0.19 ± 0.06 (8)	0.17 ± 0.07 (8)
Lactate/Pyruvate	22.7 ± 8.4 (8)	34.3 ± 7.8 (8)	48.4 ± 10.1□ (8)
Citrate	0.19 ± 0.07 (8)	0.16 ± 0.04 (8)	0.34 ± 0.10□ (8)
Acetate	0.89 ± 0.27 (6)	0.60 ± 0.15 (6)	0.71 ± 0.18 (6)
Alanine	0.07 ± 0.03 (12)	0.23 ± 0.21 (12)	0.54 ± 0.44△ (12)
Serine	0.08 ± 0.02 (8)	0.26 ± 0.09 (10)	0.47 ± 0.13° (10)
Threonine	0.08 ± 0.02 (8)	0.19 ± 0.05 (10)	0.39 ± 0.07° (10)
Glutamate	0.04 + 0.02 (8)	0.05 ± 0.02 (10)	0.15 ± 0.04° (10)
Glutamine	0.15 ± 0.07 (8)	0.32 ± 0.07 (8)	0.56 ± 0.14□ (8)
αaminonitrogen	14.0 ± 4.0 (12)	15.7 ± 4.0 (12)	12.8 ± 4.1 (12)
Ammonia	0.09 ± 0.03 (6)	< 0.04 (6)	0.37 ± 0.08° (6)

	Maternal	Normal	35–50 g.	< 35 g.
Bodyweight (g.)		83.5 ± 12.7 (13)	39.4 ± 3.6° (8)	28.3 ± 7.8° (5)
Free fatty acids	0.7 ± 0.1 (13)	0.59 ± 0.23 (13)	0.66 ± 0.14 (8)	0.30 ± 0.11□ (5)

△P<0.05 □P<0.01 °P<0.001

The results are means ± S.D. with the number of observations in parentheses. For the determination of free fatty acids small fetuses were subdivided into those that were moderately growth retarded (35 - 50 g.) and those that were severely growth retarded (<35 g.).

was found in growth retarded fetuses at both age groups (tables IV.1, IV.2). The concentration of amino acids in the amniotic fluid of normal and small fetuses was lower than that in plasma and showed comparable changes (table IV.3). The concentration of ammonia in amniotic fluid was much higher than in plasma. It was significantly higher in the small fetal guinea pigs (table IV.3)

Table IV. 3. Metabolite concentrations in the amniotic fluid of normal and intrauterine growth retarded fetal guinea pigs at 49 - 51 and 60 - 63 days of gestation. (concentrations in μmol./ml.).

	49 - 51 days	
	Normal	Small
Bodyweight (g.)	39.8 ± 4.2 (6)	21.9 ±2.6° (6)
Glucose	4.0 ± 0.8 (6)	3.1 ±0.9 (6)
Lactate	4.3 ± 1.2 (6)	5.5 ±1.6 (6)
Alanine	0.04 ± 0.02 (6)	0.13 ± 0.04$^{\triangle}$ (4)
Serine	0.09 ± 0.03 (5)	0.14 ± 0.06 (5)
Threonine	0.05 ± 0.02 (5)	0.05 ± 0.02 (5)
α aminonitrogen	3.6 ± 0.6 (6)	2.6 ±0.8$^{\triangle}$ (6)
Ammonia	0.33 ± 0.04 (4)	0.60 ± 0.04° (6)
	60 - 63 days	
	Normal	Small
Bodyweight (g.)	86.8 ±11.8 (12)	33.9 ±9.4° (12)
Glucose	3.1 ± 0.3 (12)	2.1 ±0.5$^{\square}$ (12)
Lactate	2.3 ± 1.4 (12)	2.5 ±1.9 (12)
Citrate	0.19 ± 0.04 (8)	0.34 ± 0.07$^{\square}$ (8)
Acetate	0.38 ± 0.03 (6)	0.30 ± 0.03 (6)
Alanine	0.05 ± 0.02 (12)	0.08 ± 0.05 (8)
Serine	0.12 ± 0.04 (6)	0.18 ± 0.04$^{\triangle}$ (6)
Threonine	0.06 ± 0.02 (6)	0.10 ± 0.03$^{\triangle}$ (6)
α aminonitrogen	2.8 ± 0.9 (12)	2.2 ±0.8 (12)
Ammonia	0.64 ± 0.10 (6)	1.25 ± 0.35° (6)

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are means ± S.D. with the number of observations in parentheses.

IV.2.2. Plasma hormone concentrations

IV.2.2.1. Pituitary hormones

The fetal plasma ACTH concentration in the guinea pig normally rises towards term (Jones and Roebuck, 1980). A similar rise was found in the plasma concentration in both normal and small fetuses between 50 and 60 days. The

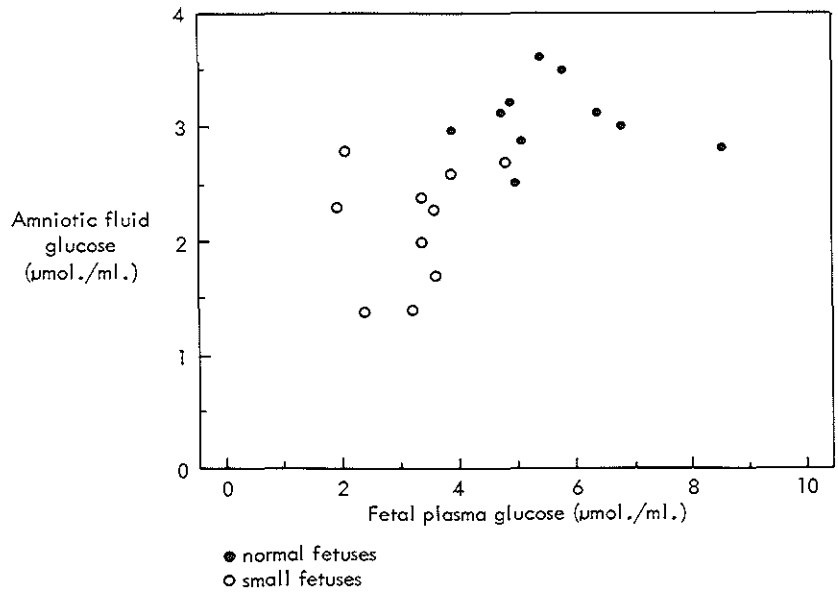

Fig. IV. 1. Relationship between the glucose concentrations in amniotic fluid and plasma of normal and small fetal guinea pigs at 60 - 63 days of gestation.

concentration in the small fetuses however was at both ages only half that of the normal littermates (table IV.4). The concentrations of growth hormone in plasma fell between 50 to 60 days. No significant differences were found between the values in normal and small fetuses at both age groups (table IV.4).

IV.2.2.2. Adrenal steroid hormones

The changes in plasma cortisol concentration during the last two weeks of gestation in the fetal guinea pig are closely related to those of ACTH (Jones and Roebuck, 1980). There was a five fold increase between 50 and 60 days. A similar rise occurred in the small fetus but plasma cortisol remained lower throughout the last 25 days of gestation (table IV.4). The changes in plasma androstenedione concentrations at 60-63 days were not apparently related to those of ACTH, in spite of the fact that ACTH stimulates androstenedione production from the fetal adrenal of the guinea pig (Jones and Roebuck, 1980). At 60-63 days the plasma androstenedione concentration in the small fetuses was 100% higher than normal (table IV.4).

IV.2.2.3. Pancreatic hormones

During normal development of the fetal guinea pig there is a rise in plasma insulin concentration between 40 and 50 days and there is a decrease towards term

Table IV. 4. Plasma hormone concentrations in normal and intrauterine growth retarded fetal guinea pigs (concentrations in ng./ml.).

	Normal	Small
49 - 51 days		
Bodyweight (g.)	40.3 ± 3.8 (14)	22.0 ± 2.7° (14)
ACTH	0.19 ± 0.06 (5)	0.10 ± 0.03 (5)
Cortisol	41 ± 10 (6)	14 ± 15△ (6)
Insulin	12.8 ± 3.2 (14)	4.9 ± 1.3° (7)
Growth hormone	121 ± 36 (6)	89 ± 42 (6)
Thyroxine (T_4)	46.3 ± 11.4 (7)	20.7 ± 4.2° (7)
Triiodothyronine (T_3)	1.4 ± 0.4 (7)	0.6 ± 0.2° (7)
Rev. triiodothyronine (rT_3)	3.4 ± 0.9 (7)	1.6 ± 0.6□ (7)
60 - 63 days		
Bodyweight (g.)	93.1 ± 13.1 (14)	41.5 ± 4.9° (14)
ACTH	0.54 ± 0.15 (7)	0.27 ± 0.07 (7)
Cortisol	207 ± 102 (10)	117 ± 62△ (10)
Androstenedione	0.41 ± 0.11 (8)	0.85 ± 0.49△ (8)
Insulin	5.9 ± 1.9 (13)	2.7 ± 1.6 (12)
Glucagon	0.025 ± 0.007 (14)	0.120 ± 0.040° (9)
Growth hormone	63 ± 31 (8)	52 ± 34 (8)
Thyroxine (T_4)	37.3 ± 7.6 (8)	33.7 ± 4.2 (8)
Triiodothyronine (T_3)	1.1 ± 0.4 (8)	0.7 ± 0.3 (8)
Rev. Triiodothyronine (rT_3)	2.6 ± 0.7 (8)	2.1 ± 0.2 (8)

△ $P<0.05$ □ $P<0.01$ ° $P<0.001$

The results are means ± S.D. with the number of observations in parentheses.

(fig. IV.2). The values in the plasma of small fetuses were much lower than normal (table IV.4, fig. IV.2).

Plasma glucagon concentrations decrease towards term (fig. IV.3) in the normal fetal guinea pig to very low levels shortly before birth.

As a result of these normal developmental changes in insuline and glucagon concentration the ratio between insulin and glucagon has risen especially during the last week before birth (fig. IV.4). The plasma glucagon concentration of the growth retarded fetal guinea pigs at 60 days was much higher than normal (table IV.4, fig.

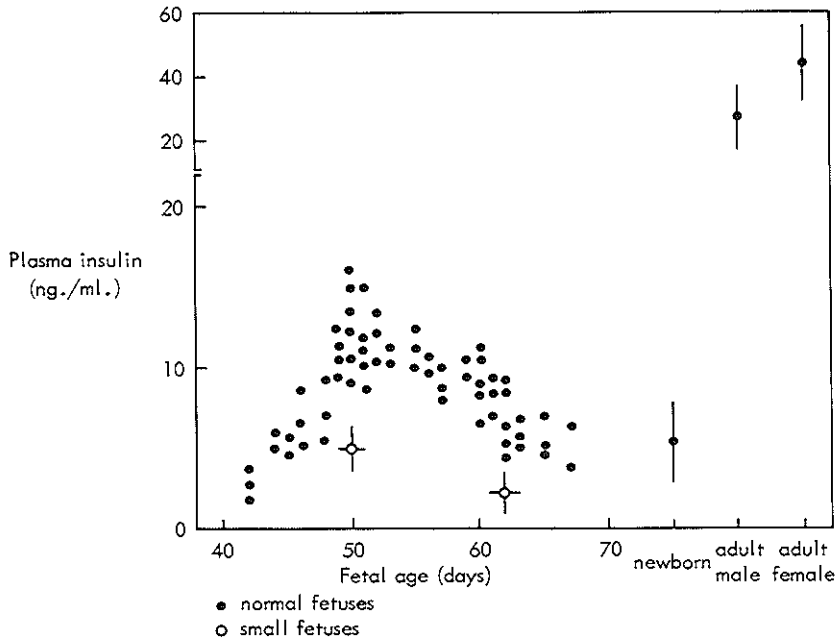

Fig. IV. 2. Plasma insulin concentrations of intrauterine growth retarded fetal guinea pigs at 49 - 51 and 60 - 63 days of gestation in comparison with the normal developmental profile in the fetal guinea pig (data of normal plasma insulin concentrations in the fetal guinea pig are courtesy of Dr. C.T. Jones). Vertical bars represent 2 S.D.

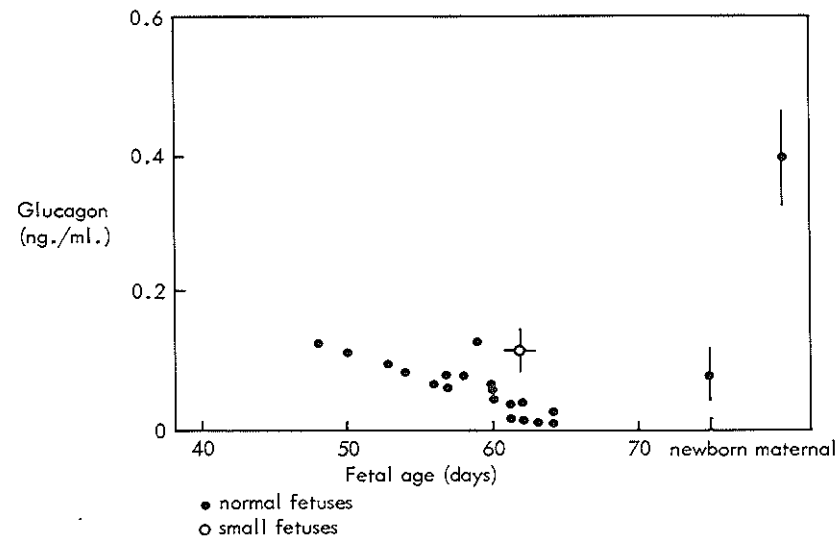

Fig. IV. 3. Plasma glucagon concentration of intrauterine growth retarded fetal guinea pigs at 60 - 63 days of gestation in comparison with the normal developmental profile in the fetal guinea pig (data of normal plasma glucagon concentrations in the fetal guinea pig are courtesy of Drs.C.T. Jones and J.M. Bassett). Vertical bars represent 2 S.D.

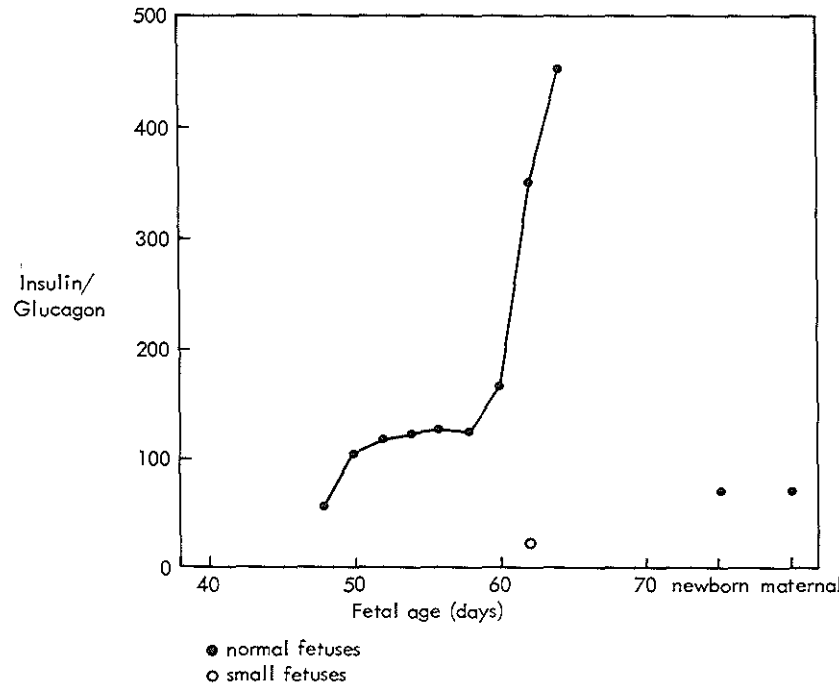

Fig. IV. 4. Plasma insulin/glucagon ratio of intrauterine growth retarded fetal guinea pigs at 60 - 63 days of gestation in comparison with the normal developmental profile in the fetal guinea pig. (data of normal plasma insulin/glucagon ratio's in the fetal guinea pig are courtesy of Drs. C.T. Jones and J.M. Bassett).

IV.3) and as a result the insulin/glucagon ratio at 60 days was much lower than normal (fig. IV.4).

IV.2.2.4. Thyroid hormones

During the gestational period that was studied no significant changes were found between values of the thyroid hormones thyroxine, triiodothyronine and reverse triiodothyronine in normal fetuses at the two age groups. At 49-51 days significantly lower values of all three thyroid hormones were found in the small fetuses while the values at 60-63 days were not different in normal and small fetuses (table IV.4).

IV.2.2.5. Plasma sulphation promoting activity

The ability of fetal guinea pig plasma to stimulate sulphate incorporation in porcine cartilage was assayed in 60-63 days normal and small fetuses. As shown in fig. IV.5 both normal and small fetal guinea pig plasma caused less ^{35}S-sulphate incorporation in porcine cartilage than adult male guinea pig plasma that was used as a standard. Small fetal guinea pig plasma however stimulated less ^{35}S uptake than

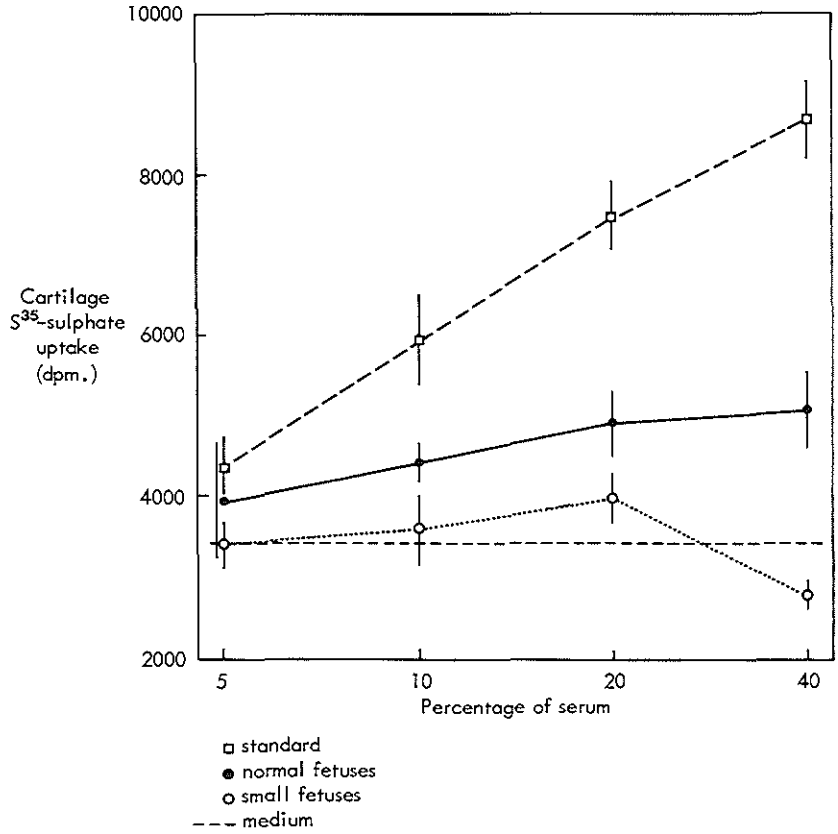

Fig. IV. 5. Porcine cartilage S^{35}-sulphate uptake of normal and intrauterine growth retarded fetal guinea pigs at 60 - 63 days of gestation. As a standard adult male guinea pig plasma was used. Vertical bars represent 2 S.D.

normal fetal plasma. When the values were expressed as a percentage of paired controls, using plasma at 20% dilution, significant differences were found for the plasma sulphation promoting activity (fig. IV.6). In the severely retarded group this activity was the lowest but even in the moderately retarded group sulphate incorporation was reduced to values below that given by the medium alone.

Plasma samples taken from hypophysectomized young male guinea pigs also showed less sulphation promoting activity than that of normal adult animals.

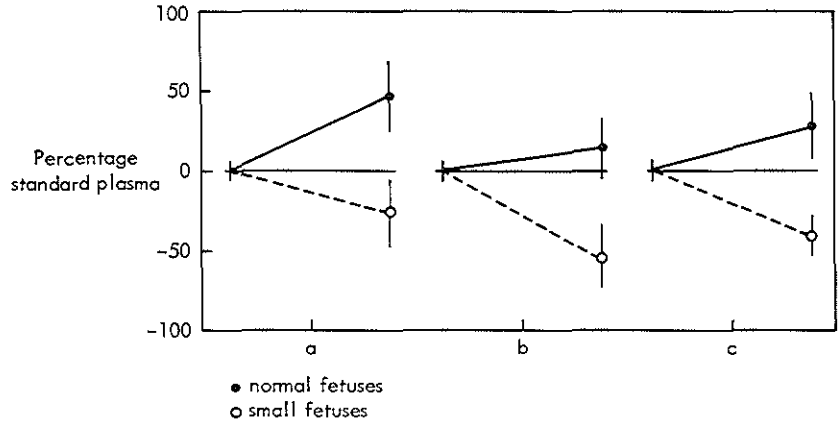

Fig. IV. 6. Plasma sulphation promoting activity of normal and small fetal guinea pigs at 60 - 63 days of gestation. a: comparison of normal and moderately intrauterine growth retarded fetal guinea pigs (bodyweight 35 - 50 g.) ($n = 5$). $P < 0.05$. b: comparison of normal and severely growth retarded fetal guinea pigs (bodyweight < 35 g.) ($n = 5$). $P < 0.01$. c: comparison of normal and small fetal guinea pigs (bodyweight < 50 g.) ($n = 10$) $P < 0.001$. Vertical bars represent 2 S.D.

IV.3. Discussion

IV.3.1. Plasma metabolites

The present results demonstrate that the endocrine and metabolic state of the growth retarded fetus differs substantially from that of the normal fetus. The hypoglycaemia correlates well with that reported for small fetuses or newborns of species such as the rat, sheep and man (table IV.5). As glucose concentration and uptake are closely related in the fetus, a low blood glucose concentration in the growth retarded fetus is indicative (particular in view of the hypoinsulinaemia) of reduced glucose consumption. This implies that there is an impaired transport of nutrients across the placenta after uterine artery ligation. It is likely that the lower plasma glucose concentration has an important effect on fetal growth and development since it is considered to be one of the most important nutrients for the fetus (Dawes and Shelley, 1968).

Low plasma free fatty acid concentrations were also seen, similar to that reported in small fetuses of rat and sheep (table IV.5). In the guinea pig some of the free fatty acids circulating in the fetus are of maternal origin (Jones, 1976) and therefore the lower level of free fatty acids in the growth retarded fetus would be consistent with a reduced transport via the placenta.

However if the lower blood glucose and free fatty acid concentrations are indicative of altered placental transport, the effects on growth retardation are not simple. An interpretation of the results, for instance, is that in moderately retarded fetuses

Table IV. 5. Relative plasma metabolite and hormone concentrations in intrauterine growth retarded fetuses of several species and human "small for dates" newborns.

PERCENTAGE OF NORMAL VALUES

	Man	Sheep	Rat	Guinea pig
Fetal bodyweight (g.)	65°	75$^{\square}$	66°	38°
Glucose	80$^{\triangle}$	55$^{\square}$	70°	61°
Alanine	184$^{\triangle}$	70$^{\square}$	151$^{\square}$	234$^{\square}$
Ammonia	137$^{\triangle}$	-	-	925°
Free fatty acids	100-125*)	84$^{\triangle}$	77$^{\square}$	56°
Insulin	100-140*)	47$^{\square}$	46$^{\triangle}$	39°
Glucagon	153	-	129	480°

$\triangle P<0.05$ $\square P<0.01$ $\circ P<0.001$

References
Man: Melichor *et al.*, 1965; Blum *et al.*, 1969; Rubaltelli *et al.*, 1979; Haymond *et al.*, 1974; Williams *et al.*, 1975; De Leeuw and De Vries, 1976.
Sheep: Robinson *et al.*, 1979; Jones and Robinson, 1979.
Rat: Roux *et al.*, 1970; Girard *et al.*, 1976; Maniello *et al.*, 1977.
Guinea pig: Data of severely intrauterine growth retarded fetal guinea pigs at 60 - 63 days of gestation after 30 days of uterine artery ligation.

*) Data refers to plasma samples taken 2 - 24 hours after birth; all other data in this table refers to venous cord blood. (reproduced from Lafeber *et al.*, 1979).

glucose transport is affected while the transport of free fatty acids is not. The absence of any significant change of free fatty acid concentration in the more moderately retarded fetuses at 60-63 days is consistent with the unaltered hepatic triglyceride concentrations of normal and moderately retarded fetal guinea pigs (chapter III). In the severely retarded fetuses plasma free fatty acids and hepatic triglyceride concentrations at 60-63 days are less than normal. At present there is no quantitative data on glucose and fatty acid transport across the placenta of the growth retarded fetal guinea pig.

The maintained concentrations of plasma acetate indicate that this substrate is in adequate supply as a metabolic fuel and a precursor for fatty acid synthesis by the fetal liver. The importance of the increased citrate level in the small 60-63 days fetal guinea pig is not clear. Fenton *et al.* (1976) have demonstrated that it is secreted by the guinea pig placenta.

The absence of any significant difference in lactate concentration may simply reflect the fact that plasma values were generally high probably because of the method of animal handling. For instance, in a species such as the sheep with a low placenta permeability to lactate the normal plasma concentration *in utero* is about 1-2

nmol/l. On the other hand the higher plasma cellular volume and the increased hepatic haematopoietic cell content found in the growth retarded guinea pig fetuses (chapter III) are indicative of chronic hypoxaemia. The same was found in the growth retarded fetal sheep (Robinson *et al.*, 1979). In these studies chronic low values of PaO_2 were also demonstrated, measured by indwelling catheters (Robinson *et al.*, 1979). In this species inspite of the lower values for plasma lactate also a higher lactate to pyruvate ratio was demonstrated (Robinson *et al.*, 1979).

The higher amino acid concentrations, particularly that of alanine, serine and threonine, found in the plasma of the growth retarded fetal guinea pig, are comparable, although much more pronounced, to that found in the growth retarded fetal rat (Manniello *et al.*, 1977) and that found in newborn infants of very low birth weight (Haymond *et al.*, 1974) (table IV.5). This result argues against the view of an impairment of placental transport in fetal growth retardation.

The fetus normally has a high rate of amino acid consumption and the plasma amino acid concentration in the fetus is much lower than that which the placenta is capable of maintaining on the fetal side in the steady state situation (Young, 1976). Thus the increased amino acid concentration seen in the intrauterine growth retarded fetuses could reflect major changes in tissue consumption. The very low levels of some of the key aminotransferases in some of the fetal tissues (see the following chapters) is consistent with the view that amino acid consumption is reduced. Clearly in this situation it is possible to have both an impaired placental transfer and peripheral consumption of amino acids leading to an increase in plasma amino acids. If peripheral consumption is substantially reduced, at least for those amino acids studied, it is surprising that the ammonia concentration was much higher than normal.

Hyperammonaemia has also been reported in human "small for dates" newborns (Rubaltelli *et al*, 1970) (table IV.5). An explanation for this finding in the growth retarded fetal guinea pig could be the reduced ability of the liver of the small fetus to trap ammonia and produce urea as will be discussed in the following chapter. The results of metabolite measurements in amniotic fluid show that in general in this species their concentrations have little predictive value unless the plasma concentration is much less than normal, like is shown for instance in the concentration of glucose (fig. IV.1).

In conclusion it can be said that the metabolite concentrations found in plasma of growth retarded fetal guinea pigs are consistent with the view of impaired placental transfer in this condition although possible changes in peripheral consumption of carbohydrate, free fatty acids and amino acids complicate the simple interpretation of these changes in plasma concentrations.

IV.3.2. Plasma hormones

A substantial body of evidence has accumulated to suggest that hormones play an important role in influencing fetal growth and maturation. Since the placenta in

those species that have been studied, including man, seems to be impermeable to most hormones (apart from cortisol and possibly thyroxine) (Nathanielsz 1976), they are produced by the fetus itself or the placenta. Early in pregnancy production of androgens by the fetal gonad play an important role in sexual differentiation (Jost, 1947, 1953). The classical decapitation experiments in many species have shown the importance of many pituitary hormones for instance on adrenal function in rabbits (Jost, 1966) and monkeys (Chez *et al.*, 1970) and on thyroid function in sheep (Hopkins and Thorburn, 1972). On the other hand it raised questions on the functioning of hormones that are considered important in the adult, like growth hormone, since no inhibition of fetal growth was seen after decapitation (Liggins, 1974; Jost, 1966). The fetal thyroid gland has an important function with regard to bone development and neurological functioning (Thorburn, 1974, Nathanielsz, 1976). The importance of the fetal adrenal gland has been demonstrated in many studies. The steroid hormones produced by this gland have been shown to influence many other fetal organs, like for instance the effect of cortisol on liver glycogen deposition and lipid metabolism (Jost and Picon, 1970) while recent studies have demonstrated the function of the fetal adrenal on the initiation of parturition (Liggins, 1974).

The importance of the fetal pancreas is clearly shown in animal experiments in which diabetic pregnancy was induced by streptozotocin (Van Assche and Aerts, 1975).

Thus it is not surprising that in intrauterine growth retardation changes in plasma hormone concentration were found. The low levels of plasma ACTH at both 49-51 and 60-63 days in the growth retarded fetal guinea pig were similarly demonstrated in the growth retarded fetal rat (Chatelain, 1977) and in the growth retarded fetal lamb (Robinson *et al.*, 1979). This is surprising as hypoxaemia increased plasma ACTH concentration (Jones *et al.*, 1977). However the results for the fetal guinea pig and sheep suggest that chronic hypoxia has a different effect. It is possible that the lower ACTH concentration in the growth retarded fetus is caused by a poor development of the pituitary. Normally the ACTH concentration increases between 50 and 60 days of gestational age (Jones and Roebuck, 1980). This change is also seen in small fetuses (table IV.4). The cortisol concentration follows the changes in ACTH fairly closely. The very low concentration in the growth retarded fetuses at 49-51 days may also be caused by a restriction of transport across the placenta from the maternal circulation. At this time the fetal adrenal is relatively unresponsive to ACTH (Jones and Roebuck, 1980) and much of the cortisol in the fetal circulation is likely to be of maternal origin (Jones, 1974). A similar situation exists in other species (Liggins *et al.*, 1973). At 60-63 days it is likely that much of the cortisol is of fetal origin (Jones, 1974) and its concentration at this age correlates closely with the ACTH concentration. In contrast, despite the fact that androstenedione production from the adrenal gland of the fetal guinea pig is also under ACTH control (Jones and Roebuck, 1980) the concentration of androstenedione in the plasma of the small fetus was about twice the normal concentration at 60-63 days (table IV.4). This could

be counted for by changes in the concentration of other hormones that influence adrenal performance or by changes in the cellular constitution of the adrenal cortex. It clearly indicates that ACTH alone does not determine adrenal maturation.

Insulin produced by the fetal pancreas together with glucagon is thought to be an important growth promoting hormone in the fetus. It is therefore not surprising that retardation of fetal growth coincides with low insulin levels in the fetal guinea pig. Not only is there a close relation to the degree of growth retardation and insulin level but also a close correlation exists between the plasma glucose and insulin level (fig. IV.7). A similar situation exists in the growth retarded fetal lamb (Robinson *et al.*, 1979) and rat (Girard *et al.*, 1976). In man Van Assche *et al.* (1977) found at autopsy that the pancreas of severely growth retarded newborn infants contained less β-cells within 48 hours after birth.

Glucose is an important substrate for the fetus and in sheep for instance it has been demonstrated that fetal blood glucose levels are controlled by plasma insulin (James *et al.*, 1972, Jones *et al.*, 1981). Moreover the fetal consumption of glucose is influenced by the A/V difference of glucose across the placenta and hence by the umbilical vein glucose concentration. Thus glucose consumption by the fetus is likely to be directly related to the circulating insulin concentration which in turn will at least indirectly determine the rate of fetal growth. Evidence to support this comes from studies on diabetic pregnancy or experimental destruction of the pancreas with streptozotocin (Van Assche and Aerts, 1975). In addition to the role of insulin determining fetal growth by regulating peripheral glucose consumption, studies on its importance on controlling growth and proliferation of hepatocytes (Leffert and Koch, 1977; Starzl *et al.*, 1973) indicate that it could be of primary importance in determining organ maturation. The present observations are entirely consistent with this view and the poor fetal growth is probably partly the result of reduced tissue availability of glucose being caused by reduced placental transfer and accompanying low insulin concentrations.

It is possible that the asymmetric effect on organ growth is partly explained by the more pronounced effect on insuline sensitive organs.

Similar high glucagon levels compared to low insulin levels were reported in the growth retarded fetal rat, (Girard, 1976) and sheep (Robinson *et al.*, 1979). Compared to the other species however the fetal guinea pig shows very high insulin to glucagon ratios probably because of the fact that fetal guinea pig insulin has a relatively low biological activity (Zimmerman *et al.*, 1974). Normally the sharp increase in the insulin to glucagon ratio coincides with the metabolic changes in fetal organs like liver and skeletal muscle. The possible effects of the low insulin to glucagon ratio on these organs in intrauterine growth retarded fetal guinea pigs will be discussed in the following chapters.

The unaltered concentration of growth hormone in the growth retarded fetal guinea pig is consistent with that found in the growth retarded fetal sheep (Robinson *et al.*, 1979) and confirms the findings of the classical decapitation experiments in rabbits (Jost, 1966), hypophysectomy in monkeys (Chez *et al.*, 1970) and

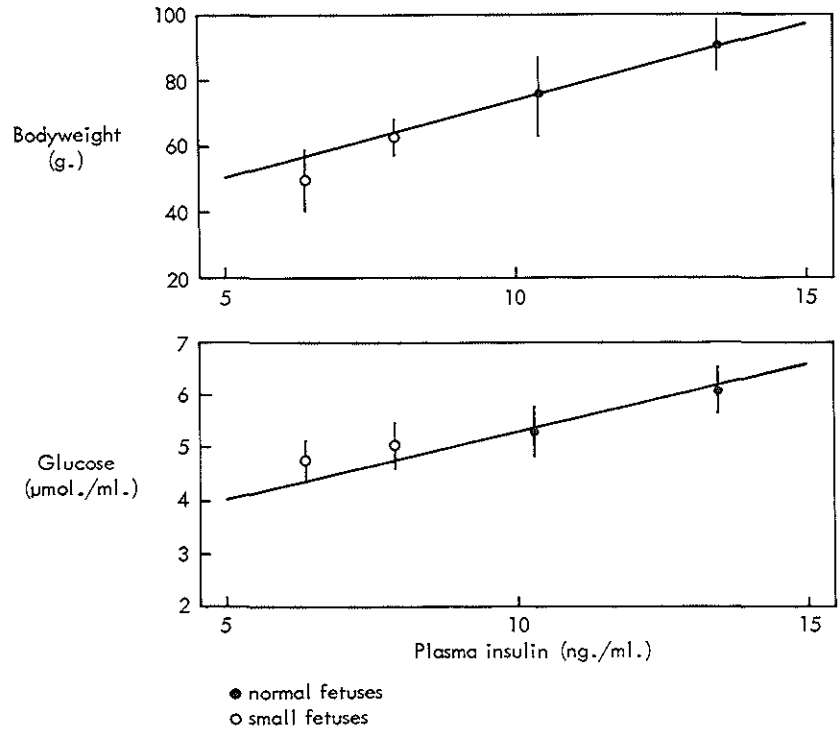

Fig. IV. 7. Relationship between bodyweight, plasma glucose and plasma insulin concentration in normal and small fetal guinea pigs. (data are obtained from normal and intrauterine growth retarded fetal guinea pigs at 49 - 51 and 60 - 63 days of gestation). Vertical bars represent 2 S.D.

anencephaly in man (Honnebier and Swaab, 1973, Liggins, 1974). These studies suggest that growth hormone plays no obvious role in the regulation of fetal growth.

The importance of the thyroid gland in fetal development was clearly shown in the thyroidectomy experiments in sheep (Hopkins and Thorburn, 1972) which if performed at half term caused reduction of fetal growth to a third of normal. Especially bone maturation was retarded. This last phenomena was also found in rat and rabbit and in congenital hypothyroidism in man although no growth retardation was found in these species (Nathanielsz, 1976).

Intrauterine growth retardation by uterine artery ligation caused low values of thyroxine, triiodothyronine and 3,3', 5'-triiodothyronine (or reverse T_3) at 49-51 days but not near term at 60-63 days (table IV.4). This could therefore be explained as a delay of normal functional development of the thyroid gland that however manages to catch up at least at 60-63 days. The very low activity of factors promoting sulphate incorporation into pig cartilage could be an explanation for the impairment of skeletal maturation. At present however it is not yet clear whether these results are due to low somatomedin levels in the plasma of the growth retarded fetuses or

the presence of factors that inhibit the mechanism of sulphate incorporation into cartilage. Recent data indicates a higher somatomedin A and C concentration measured by radioimmunoassay (Hall *et al.*, 1979) in the plasma of growth retarded fetal guinea pigs (Jones *et al.*, 1981).

CHAPTER V THE INFLUENCE OF INTRA-UTERINE GROWTH RETARDATION ON THE DEVELOPMENT OF LIVER METABOLISM

V.1. Introduction

In general the developmental changes in metabolic capacity of the fetal liver are qualitatively similar in different species. These changes are associated with particular biosynthetic phases of the liver metabolism. For instance there is usually a high rate of fatty acid synthesis associated first with membrane synthesis and later with triglyceride storage. The latter process occurs over much of the last half of gestation but declines towards term. Another example of the changes in metabolic capacity of the liver during development is the synthesis of glycogen that for much of gestation is low but sharply increases during the last days before birth in all species that were investigated (Dawes and Shelley, 1968). It is very likely that these changes are related with changes of substrate supply and/or changes in hormone concentrations to the fetal liver. A good example of the latter is the rise in plasma cortisol concentration that coincides with the period of glycogen deposition in the fetal liver (Jones, 1976).

In the preceding chapters it was shown that the growth of the visceral tissues was very much affected by intrauterine growth retardation, not only in weight but also in composition. Moreover some of these changes may at least in part be ascribed to the reduction in nutrient supply to and of the altered endocrine state in the fetus. Therefore it might be expected that the restriction of fetal growth modifies the normal programme of metabolic development of the fetal liver. Some evidence for this was found in studies on the influence of fetal growth retardation in the rat liver (Chanez *et al.*, 1971). However in these studies no differentiation was made between different degrees of growth retardation. It was also difficult to separate the effects of the actual surgery from those of the reduction of uterine arterial blood flow to the placenta because of the short period of gestation of the rat.

The objective of the studies described in this chapter was to demonstrate that the liver of the growth retarded fetus is not only structurally different from the normal fetus but also has a substantially altered metabolism.

V.2. Carbohydrate metabolism

V.2.1. Glycolysis

The activities of some enzymes of glycolysis were measured in the livers of growth

Table V. 1. The activities of enzymes associated with glucose metabolism in the livers of normal and small fetal guinea pigs. (μmol./min. per gram wet weight).

	49 - 51 days	
	Normal	Small
Bodyweight (g.)	36.2 ± 3.9 (8)	22.6 ± 3.3 (8)$^{\circ}$
Hexokinase	1.0 ± 0.2 (6)	1.6 ± 0.3 (6)$^{\square}$
Phosphofructokinase	5.7 ± 1.6 (5)	2.3 ± 1.0 (5)$^{\square}$
Aldolase	5.1 ± 1.4 (5)	2.6 ± 0.7 (5)$^{\triangle}$
Glycerol 3-phosphate dehydrogenase	23.4 ± 9.1 (6)	10.3 ± 1.7 (6)$^{\triangle}$
Enolase	19.7 ± 6.2 (6)	9.7 ± 2.3 (6)$^{\square}$
Pyruvate kinase	16.7 ± 3.6 (5)	8.3 ± 4.2 (5)$^{\square}$

	60 - 63 days		
	Normal	35-50 g.	< 35 g.
Bodyweight (g.)	85.2 ± 4.1 (12)	44.1 ± 6.1 (8)$^{\circ}$	31.9 ± 3.1 (7)$^{\circ}$
Hexokinase	0.5 ± 0.1 (10)	0.7 ± 0.1 (6)$^{\circ}$	0.9 ± 0.1 (5)$^{\square}$
Phosphofructokinase	4.2 ± 0.7 (6)	2.7 ± 0.6 (6)$^{\square}$	1.6 ± 0.5 (6)$^{\circ}$
Aldolase	4.9 ± 1.6 (6)	4.0 ± 1.0 (6)	1.6 ± 0.8 (6)$^{\square}$
Glycerol 3-phosphate dehydrogenase	36.4 ± 5.6 (10)	24.2 ± 4.6 (6)$^{\circ}$	9.4 ± 2.6 (5)$^{\circ}$
Enolase	25.4 ± 1.3 (10)	21.2 ± 3.2 (6)$^{\triangle}$	10.6 ± 0.4 (5)$^{\circ}$
Pyruvate kinase	12.8 ± 2.5 (6)	14.6 ± 2.3 (6)	18.2 ± 3.2 (6)$^{\triangle}$

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are means ± S.D. with the number of observations in parentheses. Small fetal guinea pigs at 60 - 63 days of gestation are subdivided into those moderately growth retarded (35 - 50 g.) and those severely growth retarded (< 35 g.).

retarded fetuses at 49-51 days and 60-63 days together with the activities in the livers of the normal littermates from the unoperated horn (table V.1.). At 49-51 days the small fetuses had much lower activities of phosphofructokinase, aldolase, glycerol-3-phosphate dehydrogenase, enolase and pyruvate kinase than normal. The activity of hexokinase was 60% higher than normal (table V.1.) At 60-63 days the results were generally similar except for an increase in pyruvate kinase activity in the livers of the small fetuses. In this age group the magnitude of the changes was largest in those fetuses that were below 35 gram.

Table V. 2. The activities of enzymes associated with gluconeogenesis in the livers of normal and small fetal guinea pigs (μmol./min. per gram wet weight).

49 - 51 days

	Normal	Small
Bodyweight (g.)	35.6 ± 4.7 (6)	22.1 ± 3.4°(6)
Pyruvate carboxylase	<0.05	<0.02
Phosphoenolpyruvate carboxykinase	2.9 ± 0.8 (6)	0.9 ± 0.2°(6)
Fructose 1,6-diphosphatase	5.3 ± 1.2 (6)	0.5 ± 0.1°(6)
Glucose 6-phosphatase	2.2 ± 0.7 (6)	0.1 ± 0.1°(6)

60 - 63 days

	Normal	35-50 g.	< 35 g.
Bodyweight (g.)	84.3 ± 3.6 (10)	45.6 ± 8.0°(6)	31.2 ± 3.4°(5)
Pyruvate carboxylase	1.5 ± 0.2 (10)	1.0 ± 0.4△(6)	0.3 ± 0.1°(5)
Phosphoenolpyruvate carboxykinase	6.4 ± 0.5 (10)	4.1 ± 0.9°(6)	1.1 ± 0.3°(5)
Fructose 1,6-diphosphatase	7.5 ± 1.0 (10)	13.7 ± 2.1°(6)	9.6 ± 1.9 (5)
Glucose 6-phosphatase	3.4 ± 0.5 (10)	6.9 ± 1.4□(6)	5.3 ± 2.1 (5)

△P<0.05 □P<0.01 °P<0.001

The results are means ± S.D. with the number of observations in parentheses. Small fetal guinea pigs at 60 - 63 days of gestation are subdivided into those moderately growth retarded (35 - 50 g.) and those severely growth retarded (< 35 g.).

V.2.2. Gluconeogenesis

In the fetal guinea pig gluconeogenic enzyme activities are present in the liver during the last half of pregnancy but gluconeogenesis and glyconeogenesis were not detected until 7-9 days before birth (Jones and Ashton, 1976b). Synthesis of glyceride-glycerol from 3-carbon compounds, in which pathway some gluconeogenic enzymes participate, can be detected from 35-40 days onwards (Jones and Ashton, 1976b). At 49-51 days phosphoenolpyruvate carboxykinase activity was lower in the growth retarded fetal liver, while the activities of this enzyme and of pyruvate carboxylase were also lower at 60-63 days (table V.2).

In the severely retarded animals (< 35 g) the activities of these enzymes were only 15-20% of normal. At 49-51 days the activities of fructose 1, 6 diphosphatase and glucose 6-phosphatase were lower than in the liver of their normal littermates while

Table V. 3. The rates of glucose uptake, glycogen synthesis and respiration by liver slices from normal and small fetal guinea pigs.

	49 - 51 days		60 - 63 days	
	Normal	Small	Normal	Small
Bodyweight (g.)	38.2 ± 3.5	22.0 ± 1.9°	88.1 ± 10.3	33.4 ± 5.2°
(nmol./min./g.)				
O_2-consumption	570 ± 40	460 ± 50$^{\square}$	490 ± 40	390 ± 30$^{\square}$
Glucose uptake	150 ± 56	82 ± 30	-	-
^3H_2O-production	173 ± 42	75 ± 20	100 ± 25	90 ± 23
Glycogen incorporation	7.3 ± 2.0	18 ± 5$^{\square}$	14 ± 3	23 ± 6$^{\square}$
$^{14}CO_2$-production (nmol. precursor/min./g.)				
10 mM(U-^{14}C) glucose	14 ± 3	4 ± 1$^{\square}$	2.4 ± 1.0	0.5 ± 0.2$^{\square}$
10 mM(1-^{14}C) pyruvate	67 ± 24	42 ± 14$^{\square}$	110 ± 14$^{\square}$	51 ± 23$^{\square}$
10 mM(2-^{14}C) pyruvate	43 ± 16	23 ± 9$^{\triangle}$	57 ± 16	21 ± 7$^{\square}$

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are the means ± S.D. of 6 determinations.

at 60-63 days of pregnancy the activities in the liver of the severely retarded fetuses were similar to those in the normal. The activities of fructose 1,6-diphosphatase and glucose 6-phosphatase in the moderately retarded fetal livers were higher than normal (table V.2.).

V.2.3. Carbohydrate metabolism in liver slices

Liver slices from small fetuses consumed less oxygen and took up less glucose than those from control fetuses (table V.3.). At 49-51 days glucose phosphorylation rate as indicated by ^3H_2O production from [2-^{3}H] glucose was also less in the livers of growth retarded fetuses but incorporation of glucose into glycogen was higher than normal (table V.3.). The rate of production of $^{14}CO_2$ from glucose was also much lower in liver slices from growth retarded fetuses than in those from the controls (table V.3.). This reduced rate of CO_2 production was also found with pyruvate as substrate (table V.3.).

The lower activities of the gluconeogenic enzymes were associated with a much lower rate of glucose synthesis by slices of liver from the growth retarded fetuses (table V.4.). This was demonstrated when incorporation from pyruvate or alanine was measured while also glycerol incorporation was depressed (table V.4). The effect of intrauterine growth retardation on incorporation of [^{14}C] pyruvate, [^{14}C] alanine and [^{14}C] glycerol into glycogen was similarly affected (table V.4). Between 50 and 60

Table V. 4. The synthesis of glucose and glycogen from pyruvate, alanine and glycerol by liver slices of normal and small fetal guinea pigs. (incorporation in μmol./h. per gram wet weight).

	49 - 51 days		60 - 63 days	
	Normal	Small	Normal	Small
Bodyweight (g.)	34.7 ±3.1 (5)	21.9 ±2.9(5)$^{○}$	87.6 ±5.2 (6)	32.8 ±3.7 (6)$^{○}$
Glucose synthesis				
10 mM(3-^{14}C)pyruvate	0.50 ± 0.20 (5)	<0.1$^{□}$	2.73 ± 0.90 (6)	0.30 ± 0.15 (6)$^{□}$
10 mM(U-^{14}C)alanine	0.24 ± 0.10 (5)	<0.05$^{□}$	1.26 ± 0.30 (6)	0.17 ± 0.05 (6)$^{□}$
2 mM(U-^{14}C)glycerol	0.75 ± 0.20 (5)	<0.1$^{□}$	3.72 ± 1.23 (6)	1.00 ± 0.40 (6)$^{○}$
Glycogen synthesis				
10 mM(3-^{14}C)pyruvate	<0.02	<0.02	0.73 ± 0.20 (6)	0.11 ± 0.07 (6)$^{□}$
10 mM(U-^{14}C)alanine	<0.02	<0.02	0.20 ± 0.07 (6)	<0.05$^{□}$
2 mM(U-^{14}C)glycerol	<0.02	<0.02	0.90 ± 0.30 (6)	0.31 ± 0.10 (6)$^{○}$

$^{□}$P<0.01 $^{○}$P<0.001

The results are means ± S.D. with the number of observations in parentheses.

Table V. 5. The synthesis of fatty acids and glyceride-glycerol by liver slices of normal and small fetal guinea pigs (incorporation in μmol./h. per gram wet weight).

	49 - 51 days		60 - 63 days	
	Normal	Small	Normal	Small
Bodyweight (g.)	37.6 ±3.2	24.7 ±2.6$^{○}$	86.3 ±7.6	33.9 ±2.7$^{○}$
Glyceride-glycerol				
10 mM(U-^{14}C)glucose	0.67 ± 0.19	0.46 ± 0.21	0.71 ± 0.23	0.20 ± 0.10$^{□}$
4 mM(2-^{14}C)propionate	0.21 ± 0.05	0.03 ± 0.02$^{○}$	0.13 ± 0.04	0.05 ± 0.02$^{□}$
10 mM(2-^{14}C)pyruvate	0.05 ± 0.01	0.02 ± 0.01$^{□}$	1.31 ± 0.32	0.43 ± 0.16$^{□}$
Fatty acid				
10 mM(U-^{14}C)glucose	0.18 ± 0.04	0.10 ± 0.03$^{□}$	0.30 ± 0.06	0.16 ± 0.10$^{△}$
10 mM(2-^{14}C)pyruvate	0.76 ± 0.19	0.70 ± 0.22	0.79 ± 0.08	0.94 ± 0.29

$^{△}$P<0.05 $^{□}$P<0.01 $^{○}$P<0.001

The results are means ± S.D. of 6 determinations.

Table V. 6. The activities of enzymes associated with fatty acid synthesis in the livers of normal and small fetal guinea pigs. (μmol./min. per gram wet weight).

	49 - 51 days	
	Normal	Small
Bodyweight (g.)	35.6 ±4.7	22.1 ±3.4°
Fatty acid synthetase	0.21 ± 0.07	0.20 ± 0.10
ATP-citrate lyase	2.01 ± 0.23	0.42 ± 0.13°
Glucose 6-phosphate dehydrogenase	2.00 ± 0.50	1.40 ± 0.30$^{\triangle}$
6-Phosphogluconate dehydrogenase	1.84 ± 0.30	1.00 ± 0.16°
Isocitrate dehydrogenase (-NADP+)	20.60 ± 3.40	11.40 ± 0.90°

	60 - 63 days		
	Normal	Small	
		35-50 g.	<35 g.
Bodyweight (g.)	84.3 ±3.6	45.6 ±8.0°	31.2 ±3.4°
Fatty acid synthetase	0.12 ± 0.02	0.25 ± 0.20	0.29 ± 0.10$^{\triangle}$
ATP-citrate lyase	0.50 ± 0.16	-	1.30 ± 0.30°
Glucose 6-phosphate dehydrogenase	1.10 ± 0.12	2.20 ± 0.40°	2.40 ± 0.40°
6-Phosphogluconate dehydrogenase	1.80 ± 0.30	1.60 ± 0.30	1.50 ± 0.40
Malic enzyme	0.85 ± 0.32	-	0.16 ± 0.11$^{\square}$

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are means ± S.D. of 5 - 10 determinations.

days there was an increase in the rate of fatty acid synthesis from glucose in slices of liver from the normal and the small fetal guinea pigs (table V.5).
The rate of incorporation of pyruvate into fatty acid was higher than that of glucose; it was unaffected by gestational age and the range studied was not lower in the liver of the small fetus (table V.5).

Table V. 7. The synthesis of fatty acids by liver slices of normal and small fetal fuinea pigs (incorporation in μmol./h. per gram wet weight).

	49 - 51 days		60 - 63 days	
	Normal	Small	Normal	Small
Bodyweight (g.)	37.6 ±3.2	24.7 ±2.6°	86.3 ±7.6	33.9 ±2.7°
Precursor				
$^{3}H_2O$	2.40 ± 0.47	1.80 ± 0.32$^{\triangle}$	0.71 ± 0.16	1.50 ± 0.32°
(1-^{14}C)acetate	1.53 ± 0.34	0.94 ± 0.26$^{\square}$	0.47 ± 0.10	1.00 ± 0.26$^{\square}$
(2-^{14}C)propionate	1.20 ± 0.36	1.00 ± 0.25	0.32 ± 0.07	0.62 ± 0.14$^{\square}$

$\triangle P<0.05$ $\square P<0.01$ $\circ P<0.001$

The results are means ± S.D. of 6 determinations.

V.3. Lipid biosynthesis in the fetal liver

V.3.1. Liver enzyme activities

Acetyl CoA carboxylase activity is barely detectable in the liver of the fetal guinea pig (Jones and Ashton, 1976a) and similarly little activity could be detected in that of the growth retarded fetus. Fatty acid synthetase activity in the livers of the control fetuses was similar to that previously reported (Jones and Ashton, 1976a) (table V.6). In the livers of the growth retarded fetuses at 60-63 days values were higher than normal while at 49-51 days values were equal to that found in normal fetal livers at this period of gestation (table V.6). There were biphasic changes caused by intrauterine growth retardation on the activities of ATP-citrate lyase and of the enzymes of NADPH production, with values lower than normal at 49-51 days and equal to, or higher than controls at 60-63 days except for malic enzyme (table V.6).

V.3.2. Rates of fatty acid synthesis

The same biphasic changes because of growth retardation were found in the experiments measuring fatty acid synthesis *de novo* in fetal liver slices from $^{3}H_2O$, acetate and propionate.

Normally fatty acid synthesis *de novo* is the highest in the liver of the fetal guinea pig at 45-50 days and then declines towards term (Jones, 1973; Jones and Ashton, 1976a). In the livers of growth retarded fetuses the rates of synthesis were lower than or similar to the normal values at 49-51 days and higher than usual at 60-63 days (table V.7).

The high rates of lipid synthesis of the fetal liver at 45-50 days coincides with the

Table V. 8. The incorporation of (1-^{14}C) acetate into lipid fatty acids by liver slices of normal and small fetal guinea pigs (incorporation in μmol./min. per gram wet weight).

49 - 51 days

	Normal	Small
Bodyweight (g.)	38.2 ±2.8	23.1 ±3.4^{o}
Triglyceride	11.30 ± 2.60	9.10 ± 3.20
Diglyceride	0.36 ± 0.10	0.27 ± 0.09
Monoglyceride	0.27 ± 0.08	0.19 ± 0.01
Cholesterol	1.30 ± 0.31	2.10 ± 0.45$^{\Delta}$
Cholesterol ester	0.24 ± 0.10	0.14 ± 0.06
Phosphatidylethanolamine	0.58 ± 0.12	0.15 ± 0.06^{o}
Phosphatidylcholine	0.89 ± 0.20	0.42 ± 0.13$^{\square}$
Phosphatidylserine	0.34 ± 0.14	0.13 ± 0.07$^{\Delta}$
Sphingomyelin	0.11 ± 0.04	0.09 ± 0.06
Cerebroside	0.20 ± 0.05	0.10 ± 0.04$^{\square}$
Hydrocarbon	0.15 ± 0.06	0.12 ± 0.04

60 - 63 days

	Normal	Small
Bodyweight (g.)	88.4 ±7.9	33.3 ±3.9^{o}
Triglyceride	5.10 ± 1.20	8.70 ± 2.30$^{\Delta}$
Diglyceride	0.15 ± 0.09	0.28 ± 0.08
Monoglyceride	0.13 ± 0.10	0.24 ± 0.07
Cholesterol	0.78 ± 0.14	1.40 ± 0.40$^{\Delta}$
Cholesterol ester	0.08 ± 0.04	0.25 ± 0.08$^{\square}$
Phosphatidylethanolamine	0.02 ± 0.01	0.37 ± 0.12^{o}
Phosphatidylcholine	0.02 ± 0.01	0.59 ± 0.20^{o}
Phosphatidylserine	0.02 ± 0.01	0.26 ± 0.10^{o}
Sphingomyelin	0.01 ± 0.01	0.10 ± 0.04$^{\square}$
Cerebroside	0.28 ± 0.06	0.20 ± 0.07
Hydrocarbon	0.31 ± 0.21	0.14 ± 0.05

$^{\Delta}P<0.05$ $^{\square}P<0.01$ $^{o}P<0.001$

The results are means ± S.D. of 5 determinations.

appearance of large amounts of smooth endoplasmic reticulum in the hepatocyte (fig. IV.25). As the rate of fatty acid synthesis falls over the 15-20 days before birth so does the amount of smooth endoplasmic reticulum in the hepatocyte (fig. IV.29). In the livers of the growth retarded fetuses the quantity of smooth endoplasmic reticulum correlates with the changes in fatty acid synthesis. As shown in fig. IV.27 there is less than the normal amount present at 49-51 days and more than normal at 60-63 days (fig. IV.29, IV.31). Not only did the rates of lipid synthesis change in response to intrauterine growth retardation but also the nature of the lipids as to which the fatty acids were incorporated. At 49-51 days the normal predominant incorporation of acetate into triglycerides was maintained (table V.8) but that into cholesterol was increased. The incorporation into phospholipids however was substantially reduced (table V.8). At 60-63 days the acetate incorporation in the growth retarded fetal liver into triglycerides and cholesterol and particularly into phospholipid was higher than in controls (table V.8).

V.4. Amino acid metabolism

The amino acids that the placenta delivers to the umbilical circulation are used by the fetus in part to build new tissue and in part as fuels for catabolic processes. Amino acid oxidation has been proven to exist predominantly in the liver of the fetuses of many species (Battaglia and Meschia, 1978). Also data are available to suggest that a moderate rate of urea production is present late in gestation especially in sheep.

To study the influence of uterine artery ligation both amino acid transamination and urea production were studied in normal and small fetal guinea pig liver.

V.4.1. Liver aminotransferase activities

Most liver aminotransferase activities become detectable in progressive activity towards term. In the normal fetal guinea pig liver at 49-51 days alanine aminotransferase is not consistently detectable although it is by 60-63 days (table V.9). The activity of this enzyme and particular of its cytosolic form was much less in the liver of the small fetus. The extent of the reduction in activity was greater the smaller the fetus (table V.9). Aspartate aminotransferase activity changes little in the last 20 days of the development of the fetal guinea pig liver. At 49-51 days the small fetus had lower activities of this enzyme (table V.9) whilst at 60-63 days the activity in fetuses of 35-50 g was similar to the normal values and that in fetuses below 35 g was comparatively low (table V.9).

Tyrosine aminotransferase activity in the liver of the fetal guinea pig progressively rises over the last 20 days of gestation. In the small 49-51 day fetus the activity was much less than normal (table V.9) but at 60-63 days this effect was much less pronounced and in fetuses of 35-50 g the activity was significantly higher than normal (table V.9).

Comparatively similar affects were noted for glutamate dehydrogenase (table V.9).

Table V. 9. The activity of some enzymes associated with the metabolism of amino acids in the liver of normal and small fetal guinea pigs. (μmol./min. per gram wet weight).

49 - 51 days

	Normal	Small
Bodyweight (g.)	35.6 ± 4.7 (6)	22.1 ±3.4° (6)
Aspartate aminotransferase		
- cytosolic	48.3 ±10.2 (5)	30.4 ±4.2$^{\triangle}$ (5)
- particulate	18.4 ± 3.2 (5)	9.6 ±2.7$^{\square}$ (5)
Tyrosine aminotransferase	0.70 ± 0.12 (6)	0.15 ± 0.03° (6)
Glutamate dehydrogenase	41.2 ± 6.4 (5)	9.4 ±3.9° (6)

60 - 63 days

	Normal	Small	
		35-50 g.	< 35 g.
Bodyweight (g.)	84.3 ±3.6 (10)	45.6 ±8.0 (6)	31.2 ±3.4° (5)
Alanine aminotransferase			
- cytosolic	3.4 ±0.6 (10)	1.9 ±0.4° (6)	0.4 ±0.2° (5)
- particulate	2.9 ±0.7 (10)	2.4 ±0.9° (6)	1.2 ±0.5° (5)
Aspartate aminotransferase			
- cytosolic	56.4 ±6.2 (10)	52.4 ±5.8 (6)	43.4 ±11.2$^{\triangle}$ (5)
- particulate	21.2 ±1.6 (10)	25.0 ±5.2 (6)	8.6 ±2.3° (5)
Tyrosine aminotransferase	1.50 ± 0.28 (10)	2.90 ± 0.60$^{\square}$ (6)	0.90 ± 0.10° (5)
Valine aminotransferase			
- cytosolic	0.06 ± 0.01 (7)	0.07 ± 0.02 (4)	0.08 ± 0.01$^{\triangle}$ (4)
- particulate	0.15 ± 0.10 (7)	0.13 ± 0.10 (4)	0.13 ± 0.10 (4)
Glutamate dehydrogenase	51.4 ±5.4 (10)	74.5 ±6.1° (6)	13.9 ±1.9° (5)

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are means ± S.D. with the number of observations in parentheses.

Intrauterine growth retardation had little effect on the activity of branched chain aminotransferases. The value in both normal and small fetuses of the activity of valine aminotransferase was similar at 60-63 days (table V.9).

Table V. 10. The metabolism of amino acids by liver slices of normal and small fetal guinea pigs incorporation in μmol. precursor/h. per gram wet weight).

	49 - 51 days		60 - 63 days	
	Normal	Small	Normal	Small
Bodyweight (g.)	37.9 ± 3.1 (12)	23.2 ± 2.5°(12)	86.7 ± 7.9 (11)	33.4 ± 3.1°(11)
$^{14}CO_2$-production				
10mM(U-^{14}C)alanine	540 ± 180 (6)	<60°	840 ± 180 (6)	180 ± 78°(6)
10mM(U-^{14}C)leucine	360 ± 120 (6)	138 ± 42□(6)	180 ± 78 (6)	60 ± 18△(6)
10mM(U-^{14}C)serine	222 ± 60 (6)	40 ± 18°(6)	1080 ± 180 (6)	438 ± 126°(6)
Incorporation into fatty acids				
10mM(U-^{14}C)alanine	550 ± 120 (6)	<20°(6)	200 ± 40 (6)	90 ± 30°(6)
10mM(U-^{14}C)leucine	40 ± 10 (6)	<10°(6)	80 ± 20 (6)	<10°(6)

△P<0.05 □P<0.01 °P<0.001

The results are means ± S.D. with the number of observations in parentheses.

V.4.2. Amino acid metabolism in fetal liver slices

Normally there is a progressive increase in the ability of fetal guinea pig liver towards term to oxidize alanine and serine (table V.10). At the same time the capacity to oxidize leucine, one of the branched chain amino acids, falls during this period (table V.10). The liver of the growth retarded guinea pig fetus had a reduced ability to oxidize all the amino acids in particular to oxidize alanine (table V.10). The synthesis of fatty acids from alanine by the fetal guinea pig liver falls after 51 days while that from leucine increases (Jones, 1973; Jones and Ashton, 1976a). Incorporation of both substrates into fatty acids by the liver of the small fetus was much less than normal (table V.10).

V.4.3. Urea synthesis and enzymes of the urea cycle

The ability of the fetal liver of the guinea pig to synthetize urea is comparatively high and increases progressively during development (Raiha and Schwartz, 1973; Jones, 1979). In the liver of the growth retarded fetal guinea pig this ability was much reduced (table V.11). In keeping with this, the activities of some of the urea cycle enzymes were also reduced. Both at 49-51 and 60-63 days the activities of carbamylphosphate synthetase I, ornithine transcarbamylase, argininosuccinate synthetase and arginase, were reduced in small fetal livers compared with the values found in normal fetal livers. In the severely retarded fetuses at 60-63 days values were found that were even lower than the activities normally found at 49-51 days (table V.11).

Table V. 11. The synthesis of urea and the activities of urea cycle enzymes in the liver of normal and small fetal guinea pigs (enzyme activities in μmol./min. per gram wet weight).

	49 - 51 days		60 - 63 days	
	Normal	Small	Normal	Small
Bodyweight (g.)	38.3 ± 4.6 (6)	21.4 ± 2.6^{o} (6)	88.9 ± 9.2 (6)	34.7 ± 2.4^{o} (6)
Urea synthesis (nmol./min./g.)	71 ± 26 (6)	13 ± 5$^{\square}$ (6)	136 ± 54 (6)	53 ± 20$^{\square}$ (6)

49 - 51 days

	Normal	Small
Bodyweight (g.)	37.1 ± 3.9 (5)	21.2 ± 4.3^{o} (5)
Carbamylphosphate synthetase I	1.1 ± 0.7 (5)	0.3 ± 0.1$^{\triangle}$ (5)
Ornithine transcarbamylase	43 ± 11 (5)	17 ± 8$^{\square}$ (5)
Argininosuccinate synthetase	0.5 ± 0.3 (5)	0.10 ± 0.04$^{\triangle}$ (5)
Arginase	250 ± 82 (5)	116 ± 32$^{\triangle}$ (5)

60 - 63 days

	Normal	35 - 50 g.	<35 g.
Bodyweight (g.)	86.9 ± 8.4 (6)	47.3 ± 8.4^{o} (6)	31.6 ± 4.7^{o} (6)
Carbamylphosphate synthetase I	2.6 ± 1.1 (6)	1.7 ± 0.4 (6)	0.5 ± 0.2$^{\square}$ (6)
Ornithine transcarbamylase	72 ± 23 (6)	37 ± 11$^{\triangle}$ (6)	21 ± 9$^{\square}$ (6)
Argininosuccinate-synthetase	1.2 ± 0.4 (6)	0.70 ± 0.05$^{\triangle}$ (6)	0.30 ± 0.01$^{\square}$ (6)
Arginase	422 ± 110 (6)	329 ± 81 (6)	106 ± 23^{o} (6)

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{o}P<0.001$

The results are means ± S.D. with the number of observations in parentheses. Small fetal guinea pigs at 60 - 63 days of gestation are subdivided into those moderately growth retarded (35 - 50 g.) and those severely growth retarded (< 35 g.).

V.5. Liver metabolite concentrations

During development intracellular [K^+] rises in the liver of the fetal guinea pig while intracellular [Na^+] falls (Faulkner and Jones, 1976b). In the small fetuses at both 49-51 and 60-63 days the [Na^+] was higher and the [K^+] was lower in the livers

Table V. 12. Hepatic sodium and potassium concentrations in normal and small fetal guinea pigs (ion concentration in intracellular water in μmol./ml.).

49 - 51 days

	Normal	Small
Bodyweight (g.)	42.3 ± 5.7 (6)	18.3 ± 2.1°(6)
Na^+	35.2 ± 2.3 (6)	43.9 ± 3.4°(6)
K^+	82.7 ± 4.4 (6)	69.2 ± 7.3□(6)

60 - 63 days

	Normal	Small	Adult *)
Bodyweight (g.)	87.6 ± 9.4 (6)	40.5 ± 8.2°(6)	–
Na^+	29.4 ± 6.8 (6)	39.8 ± 4.3△(6)	20.9 ± 12.0 (8)
K^+	105.0 ± 10.1 (6)	85.2 ± 7.5□(6)	128.0 ± 25.0 (8)

△P<0.05 □P<0.01 °P<0.001

The results are means ± S.D. with the number of observations in parentheses.
*) Adult data for comparison taken from Faulkner and Jones, 1978.

of small compared with those of normal fetuses (table V.12). Substantial differences in hepatic metabolite concentrations of normal and small fetuses at 49-51 days are demonstrated in table V.13. While the hexosemonophosphates were similar, the concentrations of fructose 1, 6-diphosphate, ribulose 5-phosphate and most of the triose phosphates were no more than half the values in the livers of normal fetuses (table V.13). Also the concentrations of α-ketoglutarate, aspartate and malate were significantly lower in the livers of the small fetuses (table V.13). The most striking feature of the liver of the small fetus was a 16 fold increase in the ammonia concentration (table V.13). The adenine nucleotide concentrations in the livers of both normal and small fetuses were similar (table V.13).

At 60-63 days some of the differences were not so marked (table V.14). Most of the triose phosphate concentrations, α-ketoglutarate, malate and aspartate concentrations were still low in the livers of the small fetuses and ammonia concentrations were much higher than in control livers (table V.14). The adenine nucleotide concentrations in the livers of the small 60-63 days fetuses were more comparable to those of normal younger fetuses with higher ATP and ADP concentrations than in their normal size controls (table V.14).

Table V. 13. Metabolite concentrations in the livers of normal and small fetal guinea pigs at 49 - 51 days of gestation. (nmol./g. wet weight).

	49 - 51 days	
	Normal	Small
Bodyweight (g.)	41.3 ± 7.3	17.1 ± 2.4°
Glucose-6-phosphate	175 ± 37	200 ± 86
Glucose-1-phosphate	6.7 ± 3.9	6.6 ± 3.8
Fructose-6-phosphate	38.9 ± 7.5	35.7 ± 22.1
Fructose-1,6-diphosphate	13.1 ± 4.3	5.9 ± 3.9$^{\triangle}$
6-Phosphogluconate	8.9 ± 8.9	14.2 ± 10.4
Ribulose-5-phosphate	21.5 ± 7.5	6.4 ± 5.5°
Glyceraldehyde-3-phosphate	<0.7	<2.4
Glycerol-3-phosphate	167 ± 57	49 ± 46°
Dihydroxyacetonephosphate	16.4 ± 4.3	7.4 ± 4.3°
Glycerate-3-phosphate	59 ± 21	25 ± 33$^{\triangle}$
Glycerate-2-phosphate	10.5 ± 5.3	8.2 ± 6.5
Phosphoenolpyruvate	46 ± 36	12 ± 8°
Pyruvate	113 ± 31	190 ± 73$^{\triangle}$
Lactate	2210 ± 462	7475 ± 4084$^{\triangle}$
Citrate	303 ± 38	277 ± 115
Isocitrate	4.0 ± 1.9	4.7 ± 2.4
2-Ketoglutarate	105 ± 27	41 ± 50$^{\square}$
Malate	275 ± 81	167 ± 70$^{\triangle}$
Glutamate	2070 ± 265	1707 ± 608
Aspartate	876 ± 167	550 ± 120°
Alanine	864 ± 282	1062 ± 758
NH_4	140 ± 97	2274 ± 1432°
ATP	1700 ± 215	1786 ± 209
ADP	273 ± 70	342 ± 177
AMP	179 ± 74	155 ± 55
Pi	2103 ± 318	2555 ± 360

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are means ± S.D. of 7 - 9 determinations. The normal data are taken from Faulkner and Jones, 1976b.

Table V. 14. Metabolite concentrations in the livers of normal and small fetal guinea pigs at 60 - 63 days of gestation (nmol./g. wet weight).

	60 - 63 days	
	Normal	Small
Bodyweight (g.)	86.3 ± 12.0	41.6 ± 9.4°
Glucose-6-phosphate	256 ± 87	212 ± 131
Glucose-1-phosphate	10.0 ± 7.5	8.7 ± 7.1
Fructose-6-phosphate	44.5 ± 21.9	36.1 ± 21.8
Fructose-1,6-diphosphate	6.3 ± 7.8	4.5 ± 4.0
6-Phosphogluconate	8.9 ± 7.9	4.6 ± 3.6
Ribulose-5-phosphate	14.5 ± 6.6	18.9 ± 4.7
Glyceraldehyde-3-phosphate	<1.0	<2.6
Glycerol-3-phosphate	165 ± 41	25 ± 17°
Dihydroxyacetonephosphate	12.3 ± 5.4	7.8 ± 4.7
Glycerate-3-phosphate	85 ± 33	36 ± 16°
Glycerate-2-phosphate	13.5 ± 8.8	9.7 ± 4.6
Phosphoenolpyruvate	36 ± 20	18 ± 8$^{\triangle}$
Pyruvate	91 ± 41	126 ± 52
Lactate	2211 ± 1162	3978 ± 1844$^{\square}$
Citrate	293 ± 40	317 ± 98
Isocitrate	7.2 ± 4.0	4.8 ± 2.2
2-Ketoglutarate	101 ± 42	75 ± 40
Malate	667 ± 391	375 ± 175$^{\triangle}$
Glutamate	2507 ± 872	2497 ± 444
Aspartate	1263 ± 523	777 ± 286$^{\triangle}$
Alanine	861 ± 299	953 ± 938
NH_4	153 ± 99	2093 ± 1005°
ATP	1422 ± 156	1758 ± 335$^{\triangle}$
ADP	321 ± 35	426 ± 76$^{\square}$
AMP	151 ± 47	130 ± 37
Pi	2294 ± 355	2774 ± 480

$^{\triangle}P<0.05$ $^{\square}P<0.01$ $^{\circ}P<0.001$

The results are means ± S.D. of 7 - 9 determinations. The normal data are taken from Faulkner and Jones, 1976b.

V.6. Discussion

V.6.1. Carbohydrate metabolism

One of the most important functions of carbohydrate metabolism in the fetal liver is the accumulation of glycogen which has to serve as an energy depot during and shortly after birth (Dawes and Shelley, 1968). The effects of intrauterine growth retardation on the quantity of glycogen in the fetal liver have already been mentioned in preceding chapters. Only few data exist in man on this subject while no data at all exist on the metabolic processes involved. In animal experiments over the last 20 years some data have become available on the processes of glycolysis and gluconeogenesis mainly in the fetal rat, guinea pig and sheep.

During normal development of the fetal guinea pig liver most enzymes involved in glycolysis are present at a relatively early stage and remain at a fairly constant activity (Faulkner and Jones, 1975 a, b, 1976a).

An exception to this is the hexokinase activity in the fetal liver that decreases over the latter half of gestation (Faulkner and Jones, 1976a). In the growth retarded fetal liver both at 50 and 60 days the activities of most glycolytic enzymes were less than normal with the exception of hexokinase that showed an increase in activity (table V.1). As will be shown in the next chapter, it is notable that the phosphofructokinase activity was not only substantially lower in the small fetal liver (table V.1), but also in the small fetal heart and skeletal muscle (tables VI.1-VI.4, V.15). The lower glycolytic capacity of the liver from the small fetus was consistent with the substantial reduction in the rate of CO_2 production from glucose and of glucose uptake by incubated liver of these fetuses (table V.3). The reduced glycolytic flux together with the increase of the hexokinase to phosphofructokinase ratio could be one of the factors responsible for the maintenance or even an increase of the glycogen concentration in the liver of the small fetus in spite of the low plasma glucose and insulin concentrations in the small fetus (tables III.10, IV.1, IV.3). The increase of glycogen deposition in the liver of the small fetus (table III.10) is also consistent with the higher rate of glucose incorporation into glycogen in these livers (table V.3). This may coincide with a reduced glycogen turnover because of the late development of the phosphorylase activating system (Jones,1981). In the fetal liver the gluconeogenic enzymes in many species develop early but do not appear to be directly involved in glucose synthesis (Adam and Felig, 1978). Immediately after birth however the gluconeogenic pathway rapidly appears in most species including man (Ballard, 1978).

It has been suggested that the often found hypoglycaemia in small for dates human newborns is in part possibly due to a poor development of the gluconeogenic pathway (Haymond *et al.*, 1974, Mestyan *et al.*, 1975). In the experimentally growth retarded rat there has also been evidence for this (Girard *et al.*, 1975, Kollee, 1980) and similarly the activities of the key enzymes involved in gluconeogenesis were dramatically lower in the liver of the small fetal guinea pig (tables V.2, V.15). Tissue

Table V. 15. Relative changes in tissue composition and enzyme activities of liver, heart and skeletal muscle of small fetal guinea pigs in comparison with normal fetal guinea pigs at 49 - 51 and 60 - 63 days of gestation.

PERCENTAGE OF NORMAL VALUES

Fetal organ	Liver		Heart		Skeletal muscle	
Percentage of gestation	75	90	75	90	75	90
Glycogen	314$^{\square}$	164$^{\square}$	104	164$^{\triangle}$	91	127
Triglycerides	177$^{\triangle}$	56$^{\triangle}$	224$^{\triangle}$	225$^{\triangle}$	-	-
Hexokinase	160$^{\square}$	188°	106	108	100	136
Phosphofructokinase	40$^{\square}$	38°	62$^{\triangle}$	71	73$^{\triangle}$	50$^{\square}$
Phosphoenolpyruvate carboxykinase	31°	17°	-	-	-	-
Aspartate aminotransferase	63$^{\triangle}$	77$^{\square}$	105	96	82	72$^{\triangle}$
Alanine aminotransferase	<10	12°	<10	56$^{\triangle}$	58	38$^{\triangle}$
Glutamate dehydrogenase	23°	27°	101	97	104	100
Carbamyl phosphate synthetase	27$^{\triangle}$	19$^{\square}$	-	-	-	-
Na^{+},K^{+}-ATPase	-	-	107	93	-	-
Ca^{++}-ATPase	-	-	69$^{\triangle}$	81	90	52°

$\triangle P<0.05$ $\square P<0.01$ $\circ P<0.001$

Reproduced from Lafeber *et al.*, 1979.

incubation studies clearly show that the low activities of the gluconeogenic enzymes cause a marked reduction in the capacity of the liver to synthetize glucose, glycogen and glyceride-glycerol from pyruvate, alanine and glycerol (tables V.4, V.5).

V.6.2. Lipid biosynthesis

In the fetal liver in most species usually a high rate of fatty acid synthesis is present in the earlier stage mainly for membrane synthesis and later for triglyceride storage. In the fetal guinea pig most enzymes involved in fatty acid synthesis such as acetyl CoA carboxylase, fatty acid synthetase and ATP citrate lyase show a peak activity at 45-50 days of gestation although the activity of acetyl CoA carboxylase remains low (Jones and Ashton, 1976a). This coincides with a peak activity of enzymes involved in NADPH production such as those of the pentosephosphate pathway while enzymes like malic enzyme and isocitrate dehydrogenase increase towards term (Jones and Ashton, 1976a).

In the growth retarded situation fatty acid synthesis from various precursors except pyruvate is lower in the 50 days fetal liver (tables V.5, V.7). This coincides with

the lower activities of the enzymes associated with fatty acid synthesis but not with a lower fatty acid synthetase activity (table V.6). By contrast there is a higher activity of fatty acid synthesis from various precursors accompanied by higher activities of the associated enzymes in the small fetal liver at 60 days compared to that of the normal fetus (tables V.5, V.6, V.7).

Studies on the cellular structure of the hepatocyte have shown that there is a good correlation between the amount of smooth endoplasmic reticulum and the rate of lipid synthesis (Threadgold, 1976). In the fetal guinea pig hepatocyte smooth endoplasmic reticulum is predominantly present at 45-50 days (fig. III.25).

The livers of the growth retarded fetal guinea pigs however showed little smooth endoplasmic reticulum at 50 days (fig. III.27), but more at 60 days (fig. III.31). This therefore suggests a shift in the maximal fatty acid synthesis. At 50 days of gestational age the effects of growth retardation are more pronounced on those lipids that are incorporated into membranes (table V.8) and this might indicate a lower rate of membrane synthesis at this time. It is likely that the period of membrane synthesis and probably cell proliferation is extended for a longer period i.e. beyond 60 days.

The normal changes in the rate of fatty acid synthesis coincide with an increase in plasma insulin and thyroid hormone concentrations. Both of these hormones have been shown to regulate the rate of the biosynthetic pathway (Lakshmanan *et al.*, 1972, Kumar *et al.*, 1977). The changes found in the concentrations of these hormones in the plasma of growth retarded fetuses (chapter IV) are in agreement with the fact that the period of a high rate of fatty acid synthesis is shifted to a later and/or prolonged period of gestation.

V.6.3. Amino acid metabolism

Many questions still exist concerning the role and functioning of amino acid metabolism in the fetal liver. Villee (1954) showed that labelled alanine and glycine are incorporated in $^{14}CO_2$ to some extent in slices of human fetal liver *in vitro* but whether or not these amino acids play a role *in vivo* cannot be said from these experiments. Battaglia *et al.* have shown that amino acid oxidation takes place in the ovine fetus and so serves as an alternative fuel to glucose (Battaglia and Meschia, 1978). In the fetal rat the available evidence suggests that amino acids are not important metabolic fuels to the liver (Yeung and Oliver, 1967, Snell and Walker, 1973) while in the fetal guinea pig liver the capacity to metabolize a range of amino acids except alanine is comparatively high as is indicated by a relatively high rate of urea production and oxidation to CO_2. As discussed in chapter IV it could be expected that intrauterine growth retardation caused by uterine artery ligation would possibly reduce the supply of amino acids to the fetus. Also the reduction in relative mass of the liver is likely to have a large effect on the amino acid metabolism of this organ. As shown in tables V.9 and V.15 the fetal guinea pig liver shows a marked decrease in the activities of some of the aminotransferases and glutamate dehydrogenase. In addition to these changes some of the key enzymes involved in the

urea cycle show a lower activity in the livers of the small fetus (table V.11). In general the same was found in the heart and skeletal muscle of the small fetus (chapter VI) (table V.15)..

These changes in enzyme activities could well explain the higher amino acid concentration found in the plasma of the small fetuses (chapter IV) particularly of alanine, serine and threonine. The same high concentrations although less pronounced were found in the plasma of the intrauterine growth retarded rat (Manniello *et al.*, 1977) and sheep (Robinson *et al.*, 1979). If it is true that these high concentrations are due to a reduction in peripheral consumption, than the rate of amino acid transport across the placenta of the growth retarded fetus may be impaired.

In the fetal guinea pig liver alanine aminotransferase appears relatively late (i.e. after 45-50 days) (Jones and Ashton, 1976 a, b) and its increase in activity is coincident with the appearance of *de novo* glucose and glycogen synthesis from alanine (Jones and Ashton, 1976a, b) and with an increase in alanine incorporation into fatty acid (Jones, 1973). Thus the low rates of glycogen, glucose, fatty acids and of CO_2 production from alanine in the liver of the growth retarded fetus are very consistent with the low activities of alanine aminotransferase (table V.10). It is also reflected in the reduced ability to produce urea (table V.11). The high ammonia concentrations found in the plasma and the liver of small fetuses (tables IV.1, V.13, V.14) do also fit in this picture. These amounts are much higher than normally found (Faulkner and Jones, 1976; tables V.13, V.14) and are comparable to values normally found in portal vein plasma (Brosnan, 1976, Shih, 1976). Hyperammonaemia is normally only found in conditions of deficiency of urea cycle enzymes (Shih, 1976). Thus the low activities of carbamylphosphate synthetase I and argininosuccinate synthetase in the liver of the growth retarded fetal guinea pig make it comparable with that of infants that have deficiencies in the urea cycle enzymes.

Hyperammonaemia has been reported in prematurely delivered newborns (Rubaltelli *et al.*, 1970, Mestyan *et al.*, 1975). The consequences of this condition are not clear. In children and adults this is associated with conditions of depressed brain acitivity and coma but whether or not this also happens in the fetus or newborn is still unknown.

V.6.4. Liver metabolites

Intrauterine growth retardation also had an effect on liver metabolite concentrations. The normal process of a rise in intracellular $[K^+]$ and a fall in intracellular $[Na^+]$ during development of the liver of the fetal guinea pig seemed to be slowed down. The intracellular ion concentrations found in the livers of the small fetuses were more comparable with those of younger fetuses (table V.12, Faulkner and Jones, 1976). At both ages the $[Na^+]$ was higher and the $[K^+]$ was lower in the livers from the small fetuses (table V.12).

One of the striking observations on the metabolism of the liver of the growth

retarded fetus, as discussed previously, is the fact that glycogen deposition is increased despite hypoglycaemia and hypoinsulinaemia. Partly this was explained by a rise in hexokinase and fall in phosphofructokinase activity. Faulkner and Jones (1978) have demonstrated that in the perfused liver of the fetal guinea pig the glycolytic flux is largely controlled by phosphofructokinase, whose activity is modulated mainly by the fructose 1,6 diphosphate concentration. Thus the lower fructose 1,6 diphosphate concentration in the liver of the small fetus (table V.13, V.14) together with the low phosphofructokinase activity (table V.1) probably operate together to reduce glycolytic flux and divert glucose into glycogen. This is supported by the low triose phosphate concentration (tables V.13, V.14). The low triose phosphate concentration in the livers of the small fetuses also suggest that gluconeogenic flux is low, as is supported by the low gluconeogenic enzyme activities and incorporation experiments as discussed before. The changes in ribulose 5 phosphate and 6-phosphogluconate reflect in a marked fall in the $NADP^+/NADPH$ ratio of the liver of the small fetuses (tables V.13, V.14). The latter is consistent with a lower rate of fatty acid synthesis at 49-51 days of gestational age while both metabolites are returning to normal values at 60-63 days (tables V.13, V.14).

Thus in summary the fetal liver at 49-51 days appears to have a reduced rate of glucose and amino acid consumption, a reduced rate of fatty acid, urea and phospholipid synthesis and an elevated incorporation of glucose into glycogen. By 60-63 days fatty acid synthesis, glycogen synthesis, urea production and amino acid oxidation have increased and are in some respect more comparable to the metabolism of livers 5-10 days younger. One may therefore conclude that intrauterine growth retardation causes a retardation or delayed maturation of the fetal liver metabolism in some respects but a significantly altered pattern in other.

CHAPTER VI THE INFLUENCE OF INTRA-UTERINE GROWTH RETARDATION ON THE DEVELOPMENT OF THE FETAL HEART AND SKELETAL MUSCLE

VI.1. Introduction

Marked structural differences between adult and fetal heart and skeletal muscle have been demonstrated in several species including the fetal guinea pig (chapter III) (Stave, 1964). Part of these differences can be explained by their different functions. The fetus *in utero* is surrounded by amniotic fluid that is kept at the right temperature. It is therefore more than likely that the fetal skeletal muscle has a different workload and function for total body energy homeostasis than the adult skeletal muscle. The adult heart has a high oxygen consumption and a relatively low resistance to anoxia (Opie, 1968). In contrast the fetus at birth must be able to withstand prolonged periods of asphyxia.

Changes in metabolism of the fetal compared to the adult heart and skeletal muscle have been demonstrated that are often closely correlated to structural changes. An example of the latter is the lower activity of mitochondrial enzymes found in the young fetal compared to the adult heart in various species (Rolph *et al.* 1981; Lang, 1965; Edwards *et al.*, 1975) in correlation with a smaller number of less well developed mitochondria as demonstrated in stereological studies (Rolph *et al.*, 1981; Smith and Page, 1977). Another example is the deposition of glycogen in the fetal heart and skeletal muscle towards term (Dawes and Shelley, 1968).

Many of the metabolic changes in fetal heart and skeletal muscle are influenced by changes in substrate and hormone supply (Jones, 1976; Shelley *et al.*, 1975), and changes in hormone receptivity (Wildenthal *et al.*, 1976). For example there is evidence that the fetal heart oxidizes little fatty acid and is largely dependent upon carbohydrates as its source of metabolic fuel (Warshaw, 1972; Beatty *et al.*, 1972; Clark, 1973), then after birth the heart progressively attains the ability to use fatty acids and ketones as its major fuel.

Intrauterine growth retardation causes changes in circulating substrates and hormones in the small fetus (chapter IV) while also marked changes in structure of heart and skeletal muscle were demonstrated (chapter III). In this chapter an attempt is made to connect these changes with alterations in the metabolism of fetal heart and skeletal muscle. To that end the activities of some glycolytic enzymes, some enzymes involved in amino acid metabolism, some mitochondrial enzymes and Ca^{++} ATP-ase activity were measured in the heart and skeletal muscle of normal and small fetuses at 50 and 60 days.

Table VI. 1. Enzyme activities in cardiac muscle of normal and small fetal guinea pigs at 49 - 51 days of gestation (μmol./min. per gram wet weight).

	49 - 51 days	
	Normal	Small
Bodyweight (g.)	41.0 ± 2.3 (9)	18.6 ± 3.4° (9)
Hexokinase		
- cytosolic	1.22 ± 0.18 (4)	1.29 ± 0.14 (4)
- particulate	4.67 ± 1.04 (2)	5.08 ± 0.37 (2)
Phosphofructokinase	4.17 ± 1.11 (6)	2.47 ± 0.76△ (6)
6-Phosphogluconate dehydrogenase	0.33 ± 0.04 (4)	0.30 ± 0.03 (4)
Lactate dehydrogenase	207.58 ± 15.62 (4)	213.35 ± 19.80 (4)
Alanine aminotransferase		
- cytosolic	<0.03 (3)	<0.03 (3)
- particulate	<0.13 (3)	<0.13 (3)
Aspartate aminotransferase		
- cytosolic	27.27 ± 4.39 (4)	28.27 ± 3.87 (4)
- particulate	27.34 ± 6.67 (6)	19.78 ± 4.17△ (6)
Glutamate dehydrogenase	1.37 ± 0.13 (4)	1.38 ± 0.31 (4)
Citrate synthetase	18.80 ± 2.47 (4)	15.47 ± 4.26 (4)
Isocitrate dehydrogenase		
- cytosolic	5.61 ± 1.92 (6)	8.32 ± 1.61△ (6)
Ca^{++}-ATPase	14.31 ± 2.56 (4)	9.68 ± 3.33 (4)
Na^{+}, K^{+}-ATPase	8.80 ± 2.11 (5)	6.09 ± 2.07 (5)

△P<0.05 °P<0.001

The results are means ± S.D. with the number of observations in parentheses.

VI.2. Enzyme activities in the hearts of normal and small fetal guinea pigs

VI.2.1. Enzymes of glucose metabolism

Previous studies have demonstrated that despite wide variations between individuals the mean activities for hexokinase and pyruvate kinase in the fetal guinea pig heart show little change during the latter half of gestation (Faulkner and Jones, 1975, 1976). However while the glycolytic activity remains relatively constant the phosphofructokinase activity increases (Faulkner and Jones, 1976). The present data

Table VI. 2. Enzyme activities in cardiac muscle of normal and small fetal guinea pigs at 60 - 63 days of gestation (μmol./min. per gram wet weight).

60 - 63 days

	Normal	35-50 g.	< 35 g.
Bodyweight (g.)	79.4 ± 11.5 (13)	42.7 ± 6.0° (8)	25.6 ± 8.0° (5)
Hexokinase			
- cytosolic	1.35 ± 0.17 (10)	1.21 ± 0.26 (6)	1.54 ± 0.25 (4)
- particulate	5.88 ± 0.87 (7)	5.64 ± 0.91 (4)	5.91 ± 0.30 (3)
Phosphofructokinase	5.51 ± 2.34 (12)	4.00 ± 2.63 (8)	2.58 ± 0.90△ (4)
6-Phosphogluconate-dehydrogenase	0.32 ± 0.03 (7)	0.29 ± 0.01 (4)	0.29 ± 0.01 (3)
Lactate dehydrogenase	271.95 ± 57.05 (8)	289.40 ± 62.40 (5)	320.50 ± 86.60 (3)
Alanine aminotransferase			
- cytosolic	0.24 ± 0.18 (11)	0.16 ± 0.08 (7)	0.07 ± 0.01△ (4)
- particulate	<0.13 (8)	<0.13 (5)	<0.13 (3)
Aspartate aminotransferase			
- cytosolic	55.32 ± 14.34 (10)	54.01 ± 7.55 (6)	49.62 ± 23.03 (4)
- particulate	43.91 ± 10.14 (13)	36.13 ± 8.59 (8)	29.38 ± 6.54△ (5)
Glutamate dehydrogenase	2.29 ± 0.58 (9)	2.27 ± 0.89 (5)	2.19 ± 0.59 (4)
Citrate synthetase	32.41 ± 9.63 (5)	30.11 ± 5.08 (3)	25.30 ± 7.66 (2)
Isocitrate dehydrogenase			
- cytosolic	11.12 ± 2.06 (8)	14.60 ± 2.72 (5)	9.91 ± 1.93 (3)
Ca^{++}-ATPase	22.25 ± 5.70 (9)	18.80 ± 1.63 (5)	17.62 ± 6.62 (4)
Na^{+}, K^{+}-ATPase	13.82 ± 1.65 (8)	12.82 ± 3.87 (9)	

△P<0.05 °P<0.001

The results are means ± S.D. with the number of observations in parentheses. Small fetuses are subdivided into those moderately growth retarded (35 - 50 g.)and those severely growth retarded (< 35 g.). Na+, K+-ATPase activity in the hearts of small fetal guinea pigs was determined in fetuses weighing < 50 g.

on the normal fetal guinea pig heart confirm this (tables VI.1, VI.2). Phosphofructokinase was the only enzyme assayed that had a lower activity in the heart of the growth retarded fetuses (tables VI.1, VI.2).

Table VI. 3. Enzyme activities in skeletal muscle of normal and small fetal guinea pigs at 49 - 51 days of gestation (μmol./min. per gram wet weight).

	49 - 51 days	
	Normal	Small
Bodyweight (g.)	41.0 ± 2.3 (9)	18.6 ± 3.4^{o} (9)
Hexokinase - particulate	0.35 ± 0.05 (5)	0.34 ± 0.08 (5)
Phosphofructokinase	1.11 ± 0.25 (5)	0.56 ± 0.36$^{\Delta}$ (5)
6-Phosphogluconate dehydrogenase	0.35 ± 0.08 (4)	0.35 ± 0.04 (4)
Lactate dehydrogenase	62.8 ± 13.3 (4)	63.7 ± 14.3 (4)
Alanine aminotransferase		
- cytosolic	0.12 ± 0.04 (4)	0.10 ± 0.06 (4)
- particulate	0.19 ± 0.08 (4)	0.16 ± 0.05 (4)
Aspartate aminotransferase		
- cytosolic	6.92 ± 0.80 (5)	5.92 ± 1.20 (5)
- particulate	13.05 ± 1.19 (6)	9.77 ± 2.22$^{\Delta}$ (6)
Glutamate dehydrogenase	1.38 ± 0.15 (4)	1.44 ± 0.28 (4)
Citrate synthetase	4.62 ± 0.56 (4)	3.37 ± 1.04 (4)
Isocitrate dehydrogenase		
- cytosolic	0.89 ± 0.19 (4)	0.93 ± 0.24 (4)
Ca^{++}-ATPase	15.50 ± 2.00 (8)	14.81 ± 1.07 (8)

$^{\Delta}P<0.05$ $^{o}P<0.001$

The results are means ± S.D. with the number of observations in parentheses.

VI.2.2. Aminotransferase activities

Normally the cardiac alanine aminotransferase activity can only be consistently detected in the fetal guinea pig in the cytosolic fraction around 60 days (Rolph *et al.*, 1981). Although alanine aminotransferase was also detected in the hearts of small 60 days fetal guinea pigs, its activity remained significantly lower in the hearts of the severely retarded fetuses compared to the normal hearts at this age (table VI.2). The aspartate aminotransferase activity showed a twofold increase in both normal and small fetal hearts between 50 and 60 days of gestation (tables VI.1, VI.2). There were no changes in the cytosolic fraction but the particulate fraction remained significantly lower in the small fetal heart at 50 days and the severely retarded heart at 60 days (table VI.1, VI.2).

Table VI. 4. Enzyme activities in skeletal muscle of normal and small fetal guinea pigs at 60 - 63 days of gestation (μmol./min. per gram wet weight).

	60 - 63 days		
	Normal	35-50 g.	<35 g.
Bodyweight (g.)	79.4 ± 11.5 (13)	42.7 ± 6.0° (7)	25.6 ± 8.0° (5)
Hexokinase - particulate	0.27 ± 0.09 (12)	0.34 ± 0.07 (7)	0.47 ± 0.09□ (5)
Phosphofructokinase	4.38 ± 1.64 (13)	2.70 ± 1.57△ (8)	1.21 ± 0.44□ (5)
6-Phosphogluconate dehydrogenase	0.22 ± 0.04 (8)	0.26 ± 0.09 (5)	0.27 ± 0.04 (3)
Lactate dehydrogenase	215.0 ± 65.3 (8)	184.8 ± 68.2 (5)	153.3 ± 42.1 (3)
Alanine aminotransferase			
- cytosolic	2.14 ± 0.93 (11)	0.87 ± 0.66□ (7)	0.32 ± 0.19□ (4)
- particulate	0.61 ± 0.18 (11)	0.37 ± 0.20 (7)	0.21 ± 0.18□ (4)
Aspartate aminotransferase			
- cytosolic	25.11 ± 6.86 (13)	15.86 ± 5.46△ (8)	12.72 ± 3.75△ (5)
- particulate	16.99 ± 3.34 (13)	12.99 ± 3.73△ (8)	9.36 ± 1.78△ (5)
Glutamate dehydrogenase	2.09 ± 0.47 (9)	2.11 ± 0.84 (5)	1.97 ± 0.92 (4)
Citrate synthetase	9.74 ± 1.44 (9)	6.26 ± 1.62□ (5)	4.73 ± 1.16□ (4)
Isocitrate dehydrogenase			
- cytosolic	2.96 ± 0.86 (8)	3.04 ± 0.76 (5)	1.73 ± 0.19 (3)
Ca^{++}-ATPase	44.14 ± 13.40 (10)	34.71 ± 6.89 (5)	21.81 ± 7.42△ (6)

△P<0.05 □P<0.01 °P<0.001

The results are means ± S.D. with the number of observations in parentheses. Small fetuses are subdivided into those moderately growth retarded (35 - 50 g.) and those severely growth retarded (< 35 g.)

VI.2.3. The activities of enzymes associated with the tricarboxylic acid cycle

The normal developmental increases were found in the glutamate dehydrogenase and citrate synthetase activities between 50 and 60 days with no changes between normal and small fetal hearts (tables VI.1, VI.2). At 50 days the cytosolic isocitrate dehydrogenase activity was slightly higher in the small fetal heart (table VI.1).

VI.2.4. ATP-ase activities

No significant differences were found in Ca^{++} ATP-ase and in Na^{+},K^{+}-ATP-ase activities of the normal and small fetal hearts at 50 and 60 days of gestation (tables VI.1, VI.2).

VI.3. Enzyme activities in the skeletal muscle of normal and small fetal guinea pigs

VI.3.1. Enzymes of glucose metabolism

Most of the glycolytic enzymes in the fetal guinea pig skeletal muscle show substantial changes during development (Faulkner and Jones, 1976; Rolph *et al.*, 1981) (tables VI.3, VI.4). The skeletal muscle hexokinase activity however remains stable (tables VI.3, VI.4) (Faulkner and Jones, 1976). As in the fetal heart, growth retardation was associated with lower phosphofructokinase activities in the skeletal muscle at both 50 and 60 days of gestation (tables VI.3, VI.4). The hexokinase activity was significantly higher in the severely retarded fetal skeletal muscle at 60 days (table VI.4). No changes between normal and growth retarded skeletal muscle were found for the 6-phosphogluconate dehydrogenase and lactate dehydrogenase activities (tables VI.3, VI.4).

VI.3.2. Aminotransferase activities

The activities of alanine aminotransferase and aspartate aminotransferase show normally a large increase between 50 and 60 days of fetal development of the guinea pig (tables VI.3, VI.4). This increase was significantly less in the small fetuses at 60 days (table VI.4).

VI.3.3. The activities of enzymes associated with the tricarboxylic acid cycle

The activities of the enzymes that are associated with the tricarboxylic acid cycle in the fetal skeletal muscle of the guinea pig increase between 50 and 60 days of gestation (tables VI.3, VI.4) (Rolph *et al.*, 1981). Of these enzymes only the activity of citrate synthetase was significantly lower in the growth retarded fetal skeletal muscle at 60 days (table VI.4).

VI.3.4. ATP-ase activity

Ca^{++}ATP-ase activity also increases between 50 and 60 days in the fetal skeletal muscle of the guinea pig (tables VI.3, VI.4) (Rolph *et al.*, 1981). The activity of this enzyme was about half the normal at 60 days gestation in the severely retarded fetuses (table VI.4), however no significant change in activity was found at 50 days of gestation (table VI.3).

VI.4. Discussion

The most striking difference between the influence of intrauterine growth retardation on the fetal heart and that on the fetal skeletal muscle, is their reduction in weight (chapter III). The weight of the heart is reduced in proportion to body weight in the fetal guinea pig, rat, rhesus monkey, sheep and pig (Oh *et al.*, 1970; Myers *et*

al., 1971; Creasy *et al.*, 1972; Widdowson, 1971; chapter III) while skeletal muscle mass, although not specifically measured in the growth retarded fetal guinea pigs, is reduced to a greater extent than body weight in the pig and monkey (Widdowson, 1971; Hill, 1974).

An important factor to consider, when the fetal heart is compared with the fetal skeletal muscle, is the time of onset of their development during fetal life. In the fetal guinea pig most development of the heart takes place before 50-55 days while the skeletal muscle still develops after this period. This is illustrated by the changes in structural development and enzyme activities that are minimal in the fetal guinea pig heart between 50 and 60 days while marked changes are still found during this period in the fetal guinea pig skeletal muscle (tables VI.1-VI.4; Rolph, 1980).

Since it seems reasonable to assume a greater effect of growth retardation during phases of rapid changes in development (discussion Dahlem workshop, 1978), it is reasonable to expect changes in metabolic activity at an earlier stage in the heart than in the skeletal muscle. Thus at term the delay in the development of the heart may already have been overcome while it is still present in the skeletal muscle.

In the fetal guinea pig heart few changes in glycolytic enzyme activities (that develop relatively early) are found between normal and small fetuses both at 50 and 60 days. The aminotransferase activities however develop relatively late (tables VI.1, VI.2) and particularly in the 60 days severely retarded fetuses significantly lower activities were found for cytosolic alanine aminotransferase and particulate aspartate aminotransferase (table VI.2). Similar but even more pronounced differences in the activities of these enzymes were found in the skeletal muscle of the growth retarded fetal guinea pig (tables VI.3, VI.4).

However, the increase in hexokinase activity of the severely growth retarded skeletal muscle at 60 days and the lower activity of phosphofructokinase in heart and skeletal muscle of growth retarded fetuses at 50 and 60 days (tables VI.1, VI.4.) illustrates that not all changes can be explained as a delay of normal development. It could well be that the fetal heart and skeletal muscle adjust to the situation of growth retardation in the same way as is seen in the growth retarded liver (chapter V) (table V.15). The maintenance of glycogen content in these organs (chapter III) may be attributed to the higher ratio of hexokinase to phosphofructokinase activity in the growth retarded compared to the normal fetus. Such a change might channel extracellular glucose into glycogen rather than glycolysis. The maintenance of glycogen content in cardiac and skeletal muscle of the growth retarded fetus has also been observed in the rat (Wigglesworth, 1968) and pig (Widdowson, 1971).

In the growth retarded fetal guinea pig (chapter IV) as well as in the growth retarded fetal rat (Girard *et al.*, 1976) hypoglycaemia and hypoinsulinaemia were found. Because glucose and amino acids are major substrates for fetal growth (Battaglia and Meschia, 1978) a low insulin concentration will reduce their consumption by insulin sensitive organs like for instance the skeletal muscle (Fricke and Clarke, 1973). This will reduce their growth in the long term. The opposite situation exists in maternal diabetes (Cheek and Hill, 1975). The proposed reduction

in uptake of amino acids in insulin sensitive organs of growth retarded fetuses is consistent with their high plasma amino acid concentration (chapter IV) (Manniello *et al.*, 1977; Robinson *et al.*, 1979; Haymond *et al.*, 1974). Furthermore a low rate of amino acid metabolism and interconversion is confirmed by the low alanine and aspartate aminotransferase activities found in growth retarded heart and skeletal muscle (tables VI.1-VI.4).

The apparent sparing of the heart compared to the skeletal muscle in intrauterine growth retardation is confirmed by the measurement of Ca^{++} ATP-ase activity in these organs. The significantly lower activity in the severely growth retarded skeletal muscle at 60 days indicates that the myosin and sarcoplasmic reticulum is affected. This suggests that possibly the functional capacity of this organ is affected under these circumstances.

In summary growth retardation in the fetal guinea pig results in different effects on heart and skeletal muscle development. Growth retardation has a minimal effect on the metabolism of the fetal heart which is vital for survival. The metabolic development of the skeletal muscle is more affected as demonstrated in a reduction in weight and changes in enzyme activities. These changes can not only be explained as a delay of normal development at the periods of rapid development but also as a result of a change in hormone and substrate supply. The functional capacity of the skeletal muscle in the severely growth retarded fetal guinea pig appears to be affected which may have consequences for perinatal survival.

SUMMARY AND CONCLUSIONS

The aim of this thesis was to investigate some of the consequences of intrauterine growth retardation. Many of the causes and clinical manifestations of human intrauterine growth retardation are known and are described in *chapter one.*
Early recognition of this situation in human pregnancy has now become possible because of ultrasonic techniques. However a clear pathophysiological explanation of many of the consequences in the neonate is not available. For various but mainly ethical reasons animal studies are required. In several species intrauterine growth retardation has been investigated by studying naturally occurring "runts", diet restriction to the pregnant mother or surgical manipulation of the pregnant animal. The various experimental approaches that previously have been used, are described in chapter one.

In *chapter two* details of the surgical technique to create intrauterine growth retardation in the pregnant guinea pig are described. At 30 days of gestation (term is approximately 68 days) the uterine artery leading to one uterine horn was ligated. Fetuses were delivered by caesarean section around 50 and around 60 days of gestation. Only fetuses that were significantly reduced in weight were studied, in each case in comparison with a normal littermate from the other uterine horn. In the plasma of these fetuses several metabolites and hormones were determined; of several fetal organs and the placenta weight, composition and structure were studied. Some aspects of the metabolism of the fetal liver, heart and skeletal muscle were studied in more detail.

In *chapter three* the results are given of the changes in growth, weight, composition and structure of several fetal organs because of intrauterine growth retardation. Fetal bodyweight appeared to be affected to a more substantial degree than fetal bodylength. One of the most striking results was the asymmetric nature of the changes in organ weights. It became clear that while the brain was relatively spared in weight, the liver and spleen were reduced more than proportional to the reduction in bodyweight. Nevertheless the brainweight was significantly lower in the intrauterine growth retarded fetuses although few changes were found in its DNA, RNA and protein content except for the severely growth retarded fetuses at 60 days of gestation. Most of these asymmetric changes have also been described in the growth retarded fetuses of other species including man.
Measurement of liver DNA, RNA and protein content, cell counting by means of the light microscope as well as evaluation of the ultrastructure by means of the electronmicroscope, indicate a delay in the normal development of the hepatocyte.

At 60 days of gestation the hepatocyte has less cytoplasma while the mitochondria and the endoplasmic reticulum show a delay in development. At this time many haematopoietic cells are still visible in the liver of the small fetus while the packed cell volume of the blood is increased. The hepatic glycogen content of the small fetal guinea pigs was remarkably increased in comparison with that of normal littermates. In contrast low hepatic glycogen concentrations have been described in the intrauterine growth retarded rat, while no changes were found in the rhesus monkey. Low glycogen concentrations were measured in livers obtained from human "small for dates" neonates that had recently died, but it seems likely that these newborns suffered from a considerable amount of stress prior to death. Comparable to the situation in several species including man lower fat deposits were found in the small fetal guinea pigs. The relative reduction however was less than that found in other species. Only in the severely growth retarded fetuses at 60 days of gestation a lower hepatic triglyceride content could be demonstrated.
Intrauterine growth retarded fetal guinea pigs showed a delay in skeletal development. This is comparable to the human condition but many differences between individual fetuses were noticed while at present no neonatal data are available in the guinea pig.
In all cases intrauterine growth retarded fetuses had a small placenta that often showed large infarctions. The correlation between fetal bodyweight and placental weight was rather poor in the small fetuses.
In a small group of newborn guinea pigs that were born naturally after approximately 40 days of experimental intrauterine growth retardation no weight loss and an increased growth velocity during the first week after birth were observed.

In *chapter four* the changes in plasma metabolites and hormones because of intrauterine growth retardation are described. In the fetal guinea pig plasma glucose, free fatty acids as well as cortisol are problably to a large extent of maternal origin. The lower plasma level of glucose, free fatty acids and cortisol may therefore be explained by an impaired placental transport. In addition low plasma insulin and high plasma glucagon levels were found in the small fetuses. The levels of some amino acids (i.e. alanine) and of ammonia on the other hand were significantly higher in the plasma of the small fetuses. These high levels could be due to a reduced peripheral consumption of the amino acids and a reduced urea synthesis in the fetal liver as discussed in chapters five and six.
At present many investigators argue that insulin acts as a growth promoting hormone to the fetus while growth hormone on the other hand seems to have no obvious function to the fetus. In our experimental situation we found lower insulin but unaltered growth hormone levels in the plasma of the small fetuses. Plasma sulphation promoting activity in the small fetuses was lower than that found in normal fetuses but at present it is not clear whether this is due to a lower somatomedin activity or to inhibiting factors in the plasma. The lower sulphation promoting activity would be consistent with the poor skeletal development of the small fetal guinea pigs.

The plasma level of ACTH was lower in small fetuses both at 50 and at 60 days of gestation. At 60 days the fetal adrenal gland shows a good response to ACTH and this could therefore also explain the lower level of cortisol at this age. The plasma androstenedione level on the other hand was significantly higher in the small fetuses at 60 days. This fact makes a simple interpretation of the influence of intrauterine growth retardation on the fetal adrenal gland rather difficult.
Although the levels of thyroid hormones were lower than normal at 50 days of gestation, the small fetuses managed to compensate this difference at 60 days to levels that were comparable with those in normal fetal guinea pigs.

Results of enzyme activity measurements, tissue metabolite determinations and tissue-slice incorporation experiments of the liver of normal and small fetal guinea pigs are described and discussed in *chapter five*. In the liver of the small fetuses a reduced glycolytic capacity was found while the hexokinase activity in these livers was increased. In view of the increased liver glycogen content it is suggested that glucose in channeled into glycogen rather than in glycolysis, while glycogenolysis is also possibly affected. In the liver of small fetuses enzyme activity measurement of some key enzymes as well as liverslice incorporation experiments indicate a very low rate of gluconeogenesis. In the normal fetal guinea pig liver at 60 days of gestation gluconeogenic enzyme activity can be detected but gluconeogenesis is only present at a high rate at and after birth. This is comparable to the situation in man. The findings in the intrauterine growth retarded fetal guinea pigs are consistent with the suggestion of a reduced gluconeogenic capacity of the liver of human "small for dates" neonates.
Normally fatty acid synthesis in the fetal guinea pig liver is at a high rate at 50 days of gestation while this rate shows a decrease towards 60 days. The opposite was found in the livers of the small fetuses. This may be explained as a delay of the normal development. The high rate of fatty acid synthesis at 60 days is problably related with the observed high rate of acetate incorporation into lipid fatty acids.
The changes found in the amino acid metabolism of the small fetal guinea pigs are consistent with the higher plasma levels of some amino acids and of ammonia. The aminotransferase activities in the liver of the small fetuses were lower while *de novo* synthesis of glucose, glycogen and fatty acids from alanine was low in the incorporation experiments. High levels of ammonia were also found in the livers of the small fetuses while the ureasynthesis in liverslices and the activity of some key enzymes of the urea cycle were low.

In *chapter six* the investigations of changes in some metabolic pathways of the fetal guinea pig heart and skeletal muscle because of intrauterine growth retardation are described. These changes were determined by means of enzyme activity measurements. In general few changes were seen in the fetal heart while some changes were found in the skeletal muscle that were comparable to those in the liver. In the skeletal muscle of the growth retarded fetal guinea pigs lower glycolytic enzyme activities were found. The tissue glycogen content of both fetal heart and

skeletal muscle of the small fetuses was maintained. The hexokinase activity in the skeletal muscle of the small fetuses at 60 days was increased, similar to the situation in the liver. In these fetuses low aminotransferase activities were found in the skeletal muscle similar to the situation in the liver. This could also indicate a lower aminoacid turn over by the skeletal muscle of the small fetuses. Virtually no differences because of intrauterine growth retardation were found in the activities of some important enzymes involved in the citric acid cycle. Finally in the severely growth retarded fetal guinea pigs at 60 days of gestation significantly lower Ca^{++}-ATPase activities were demonstrated in the skeletal muscle. Since this enzyme is essential for the functional capacity of the muscle, this finding could have major consequences.

In general this investigation on the consequences of intrauterine growth retardation in the fetal guinea pig has shown many changes that indicate a delay of the normal development of various fetal organs. Some of these changes may be caused by a reduced placental transfer of nutrients followed by altered hormone levels. Other changes however indicate an adaptation of the small fetus to its specific deprived situation. The small fetus is sometimes able to achieve a full compensation in a later stage, however some of the changes may be permanent. The investigations in the small fetal guinea pigs have also shown that in severely affected fetuses more dramatic changes can be observed than in moderately affected fetuses.

As the causes of intrauterine growth retardation are very heterogeneous, it must be made clear that the type of experimental intrauterine growth retardation studied in the fetal guinea pig is only comparable to that in man in those instances in which there are clear indications of a poor placental function. Many developmental changes are highly specific to each species and some findings in experimental animal studies are therefore not comparable to the human situation. Nevertheless some general mechanisms can be observed that are also relevant to the human condition. First of all there seems to be a reduced placental transfer of nutrients to the fetus. As a consequence some fetal organs are relatively serious affected (i.e. the liver). At present it is impossible to say whether the small newborn will be able to compensate for these changes or will suffer from permanent damage to its organs. Therefore more specific follow-up studies in "small for dates" infants are necessary both in man and in experimental animals.

Obstetricians are now able to follow human intrauterine growth retardation by means of ultrasonic techniques and it could be suggested to them to performe a preterm caesarean section in those cases in which severe intrauterine growth retardation is caused by a poor placental function.

Finally it is obvious that "small for dates" neonates need frequent and high caloric feedings. In cases of hypoglycaemia extra glucose must be added because of the possible reduced gluconeogenic capacity of the liver of these newborns. At present there is not yet enough evidence to support suppletion of other nutrients or hormones to "small for dates" newborns.

SAMENVATTING

Dit proefschrift heeft als doelstelling de gevolgen van intrauteriene groeivertraging voor de foetus en de pasgeborene te onderzoeken. Vooral na de invoering van de echoscopie kan het achterblijven in de foetale groei bij de mens eerder en duidelijker herkend worden. Veel oorzaken en klinische symptomen van intrauteriene groeivertraging, zoals beschreven in *hoofdstuk één*, zijn inmiddels bekend maar omtrent de pathofysiologie ervan tast men toch nog grotendeels in het duister. Omdat experimenteel onderzoek hiernaar om velerlei maar vooral ethische redenen bij de mens niet mogelijk is, heeft men naar antwoorden gezocht door middel van bestudering van een soortgelijke situatie bij diverse diersoorten. Hierbij kan men gebruik maken van in de natuur ook vrij vaak voorkomende "small for dates" pasgeborenen bij diverse diersoorten; men kan de moeder ondervoeden en vervolgens de foetus en pasgeborene bestuderen of men kan chirurgisch trachten de foetale groei te beïnvloeden. De belangrijkste tot nu toe in de literatuur beschreven dierexperimentele methoden staan vermeld in hoofdstuk één. Om diverse redenen maar vooral ook vanwege de langere zwangerschapsduur kozen wij de cavia als proefdier.

In *hoofdstuk twee* wordt de operatietechniek beschreven. Bij 30 dagen zwangerschapsduur (deze bedraagt bij de cavia ongeveer 68 dagen) werd de arteria uterina naar één baarmoederhoorn afgebonden; deze is dan volledig afhankelijk van de bloedvoorziening via de arteria ovarica. Rond de 50e en 60e zwangerschapsdag werd een sectio caesarea verricht waarna uitsluitend die foetale cavia's werden bestudeerd die duidelijk in groei waren achtergebleven, telkens in vergelijking met een foetus van normale grootte uit de andere baarmoederhoorn. In het plasma van deze foetale cavia's werden diverse metabole stoffen en hormonen bepaald, terwijl vele foetale organen en de placenta werden onderzocht op gewicht, samenstelling en structuur. Bovendien werden enkele metabole processen bestudeerd in de foetale lever, en in het foetale hart- en skeletspierweefsel.

In *hoofdstuk drie* worden de veranderingen in groei, gewicht, samenstelling en structuur van diverse foetale organen en de placenta als gevolg van de intrauteriene groeivertraging beschreven. Het totale foetale lichaamsgewicht blijkt relatief meer achter te blijven dan de foetale lengtegroei. Zeer opvallend was ook de asymmetrie in de groeiachterstand van de diverse foetale organen. Vooral bleek dat het hersenweefsel relatief weinig in gewicht was achtergebleven terwijl de lever en milt daarentegen in verhouding veel ernstiger waren aangedaan. Desondanks was in absolute zin vooral bij de 60e zwangerschapsdag het hersengewicht lager dan

normaal, hoewel zeer weinig veranderingen werden aangetoond in DNA, RNA en eiwitgehalte van de hersenen (met uitzondering van de ernstig groeivertraagde foetus). Deze bij de cavia aangetoonde veranderingen vertonen overeenkomsten met die welke na intrauteriene groeivertraging bij andere diersoorten en ook bij de mens zijn vastgesteld. Hierbij past ook de ernstige mate van ontwikkelingsachterstand zoals die wordt waargenomen in de foetale lever. Zowel het onderzoek van DNA, RNA en eiwitgehalte, de bepaling van celaantallen onder de lichtmicroscoop als de bestudering van de structuur van de hepatocyt met behulp van de elektronen microscoop, tonen aan dat de levercel achterblijft qua celgrootte en ontwikkeling van celbestanddelen als mitochondria en endoplasmatisch reticulum. Ook de op een relatief laat tijdstip nog verhoogde aanwezigheid van haematopoietische cellen in de lever (gepaard gaande met een hoge haematocriet van het bloed) zou kunnen wijzen op een vertraagde ontwikkeling.

Interessant is dat er in de lever van de groeivertraagde cavia foetus zowel kwantitatief als kwalitatief een verhoogd glycogeen gehalte kon worden aangetoond. Tot dusverre werden in de lever van de groeivertraagde foetale rat lagere glycogeen gehalten beschreven terwijl bij de foetale rhesus aap geen verschillen werden gezien. Bij de mens neemt men aan dat het glycogeengehalte van de lever bij "small for dates" pasgeborenen verlaagd is maar men moet wel bedenken dat deze opvatting stoelt op waarnemingen bij overleden pasgeborenen welke zeer waarschijnlijk een periode van uiterste stress hebben doorgemaakt.

De bij de mens, alsook bij andere proefdieren beschreven reductie in vet depots na intrauteriene groeivertraging werd ook teruggevonden bij de kleine cavia foetus (o.a. reductie in perirenaal vet en vetgehalte van de lever). Deze reductie was bij de cavia foetus echter minder uitgesproken, met uitzondering van de ernstig groeivertraagde foetus.

Naast lever en milt is ook het skeletspierweefsel vrij ernstig in groei beperkt gebleven in de kleine foetale cavia's terwijl het hart daarentegen tot de organen behoort waarvan het gewicht in verhouding tot het lichaamsgewicht onveranderd is gebleven. In zowel hart- als skeletspierweefsel werden géén verschillen in glycogeen gehaltes vastgesteld tussen normale en kleine foetale cavia's. Bij de intrauterien groeivertraagde foetale cavia treft men in het merendeel der gevallen een achterstand in skeletrijping aan. Dit is een bevinding die overeenstemt met die bij de menselijke "small for fates" pasgeborene. Aangezien er echter grote individuele verschillen zijn tussen de foetale cavia's en er op dit moment nog geen wezenlijke vervolgstudies bij "small for dates" pasgeboren cavia's zijn verricht, zijn verdere uitspraken of speculaties ten aanzien van een mogelijke inhaalgroei in een later stadium onmogelijk.

Bij het vervolgen van een weliswaar klein aantal op natuurlijke wijze geboren cavia's welke een experimentele intrauteriene groeivertraging hadden ondergaan bleek dat onmiddellijk na de geboorte geen gewichtsverlies optrad met zelfs een grotere groeisnelheid gedurende de eerste week na de geboorte.

In alle gevallen van intrauteriene groeivertraging was de placenta kleiner dan

normaal; de correlatie tussen het foetale lichaamsgewicht en het placentagewicht is echter slecht. Vaak werden grote placentainfarcten gezien.

In *hoofdstuk vier* worden de veranderingen in metabole stoffen en hormonen van het foetale plasma tengevolge van intrauteriene groeivertraging besproken. Glucose, vrije vetzuren alsook cortisol in het bloed van de cavia foetus zijn voor een groot deel afkomstig van de moederlijke circulatie. Aangezien voor glucose, vrije vetzuren en cortisol lagere spiegels werden aangetroffen in het plasma van de kleine foetale cavia's zou men kunnen denken aan een verminderd transport via de placenta. De eveneens lagere insuline spiegels naast een verhoogde glucagon spiegel sluiten hierbij aan. De spiegels van bepaalde aminozuren (zoals bijvoorbeeld alanine) alsook de ammoniak spiegel zijn daarentegen sterk verhoogd. Wellicht zijn deze laatste waarnemingen te verklaren, op grond van de bevindingen beschreven in hoofdstuk vijf en zes, door een verminderde omzetting van aminozuren door de weefsels en door het niet goed functioneren van de ureumcyclus.

Tal van onderzoekingen geven aanwijzingen dat insuline bij de foetus groeistimulerend werkt. Groeihormoon lijkt daarentegen niet functioneel te zijn in de foetus. In onze experimentele situatie werd in het plasma van de kleine foetale cavia's geen veranderde groeihormoon spiegel aangetoond. Momenteel is het nog niet duidelijk of de in het plasma van de kleine foetale cavia aangetoonde verminderde activiteit van sulfaat incorporatie in kraakbeen stimulerende stoffen (hetgeen goed past bij de achterstand in skeletontwikkeling), te wijten is aan een lagere somatomedine activiteit of mogelijk aan remmende factoren in het plasma. In het plasma van de intrauterien groeivertraagde foetale cavia's werd een lagere ACTH spiegel aangetroffen dan normaal. Rond de 60e zwangerschapsdag is de foetale bijnier van de cavia al goed gevoelig voor ACTH. De lagere ACTH concentraties op dat moment zouden eveneens de oorzaak kunnen zijn van de lagere cortisol spiegels. Tegelijkertijd is de androsteendion spiegel in het plasma van de kleine foetale cavia echter significant hoger, zodat een eenvoudige verklaring voor het effect van intrauteriene groeivertraging op de foetale bijnier niet voor handen is.

In *hoofdstuk vijf* worden de resultaten besproken van bepalingen van enzym activiteit, weefsel metaboliet gehaltes en incorporatie experimenten in de levers van normale en intrauterien groeivertraagde foetale cavia's. Hierbij werd een verminderde capaciteit van de glycolyse vastgesteld terwijl de hexokinase activiteit hoog was in de lever van de kleine foetale cavia's. Op grond hiervan en mede op grond van experimenten waarbij de incorporatie in glycogeen werd gemeten kan men veronderstellen dat ondanks de relatieve hypoglycaemie glucose verhoogd wordt omgezet in glycogeen en minder gebruikt wordt voor de glycose; ook de glycogenolyse zou mogelijk gestoord kunnen zijn. De bevindingen omtrent de samenstelling en structuur van de lever, zoals beschreven in hoofdstuk drie, sluiten hier goed bij aan.

Voorts werd in de lever van de kleine foetus een verlaagde activiteit van sleutelenzymen van de gluconeogenese gevonden. Bij de normale foetale cavia worden rond de 60e dag van de zwangerschap reeds actieve enzymen van de

gluconeogenese aangetroffen hoewel waarschijnlijk pas tijdens of kort na de geboorte sprake is van een normale activiteit van de gluconeogenese. Waarschijnlijk geldt dit ook zo voor de mens. De bevindingen in de lever van de intrauterien groeivertraagde foetale cavia's stemmen overeen met die in de lever van de intrauterien groeivertraagde pasgeboren rat en sluiten mogelijk aan bij de waargenomen verminderde activiteit van enkele sleutelenzymen van de gluconeogenese in de menselijke "small for dates" pasgeborene.
In de lever van de kleine foetale cavia wordt een verminderde vetzuursynthese rond de 50e zwangerschapsdag en verhoogde vetzuursynthese rond de 60e dag aangetroffen. Normaal wordt het omgekeerde waargenomen. Getracht is deze verandering te verklaren als een vertraging in de normale ontwikkeling. Terwijl in de normale lever vooral rond de 50e zwangerschapsdag veel membraansynthese plaats heeft, lijkt dit in de kleine foetale cavia rond de 60e dag nog in verhoogde mate het geval.
De veranderingen waargenomen in het aminozuur metabolisme van de lever van de intrauterien groeivertraagde cavia foetus sluiten geheel aan bij de in hoofdstuk vier beschreven hogere plasma aminozuur en ammoniak waarden. Niet alleen is de activiteit van sommige aminotransferases verlaagd maar ook is op basis van incorporatie experimenten komen vast te staan dat er minder alanine voor *de novo* glucose, glycogeen en vetzuur synthese wordt gebruikt dan normaal het geval is. Naast hoge ammoniak waarden in het leverweefsel wordt een verminderde ureumsynthese bij incorporatie experimenten in de lever van de kleine cavia foetus aangetroffen, terwijl de activiteit van enkele enzymen van de ureumcyclus verlaagd is.

In *hoofdstuk zes* worden de resultaten van een mogelijke beïnvloeding van intrauteriene groeivertraging op enkele metabole processen in het foetale hart- en skeletspierweefsel besproken aan de hand van veranderingen in enzym activiteiten. In de eerste plaats valt hierbij op dat er zeer weinig veranderingen aanwezig zijn in het hartspierweefsel. De veranderingen in het skeletspierweefsel zijn in veel opzichten vergelijkbaar met de veranderingen die zijn waargenomen in de lever. Vooral in de ernstig groeivertraagde foetus rond de 60e zwangerschapsdag worden eveneens lagere activiteiten van glycolyse enzymen gevonden; daarnaast blijft het glycogeengehalte onveranderd.
Voorts zijn ook hier verlaagde aminotransferase activiteiten waar te nemen mogelijk passend bij een verminderde omzetting van aminozuren door het spierweefsel. Vrijwel géén verschillen werden aangetoond in de activiteiten van enkele belangrijke enzymen van de citroenzuurcyclus.
In het skeletspierweefsel van de ernstig groeivertraagde foetale cavia's rond de 60e zwangerschapsdag werd een significant lagere activiteit van Ca^{++}-ATPase aangetoond, een enzym dat van groot belang is voor de contractiefunctie van de spier.

In het algemeen zijn bij dit onderzoek van de intrauterien groeivertraagde foetale cavia veranderingen aangetoond die vaak wijzen op een verschuiving van het normale ontwikkelingsprogramma naar een later tijdstip. Deels zou men deze

"vertraging" kunnen verklaren als zijnde het gevolg van een verminderde toevoer van bepaalde voedingsstoffen via de placenta, waardoor weer veranderingen ontstaan in hormoonspiegels in de kleine foetus. Anderzijds zijn er ook metabole veranderingen aangetoond die duiden op een specifieke aanpassing van het metabolisme aan de omstandigheden waarin de groeivertraagde foetus zich bevindt. Soms is de foetus in staat tekorten te compenseren maar veelal is dit ook niet het geval en is het de vraag of bepaalde achterstanden wel ingehaald kunnen worden. Verder blijkt dat de meeste veranderingen in ernst toenemen naarmate de groeivertraging heviger is.

In hoeverre kunnen de bevindingen die in dit onderzoek bij de cavia naar voren zijn gekomen behulpzaam zijn bij het bestuderen van de problemen zoals die zich bij intrauteriene groeivertraging bij de mens tijdens en na de partus kunnen voordoen? Ter beantwoording van deze vraag moet allereerst duidelijk gesteld worden dat de oorzaken van intrauteriene groeivertraging zeer heterogeen van aard zijn. Zeer waarschijnlijk is het experimentele model dat hier beschreven wordt in de cavia uitsluitend vergelijkbaar met die vormen van intrauteriene groeivertraging bij de mens waarbij sprake is van een samenhang met een slechte placentafunctie. Daarnaast zijn er natuurlijk soort specifieke mechanismen, zodat niet alle situaties zoals die zich voordoen bij het proefdier vertaald kunnen worden in vergelijkbare omstandigheden bij de mens. Desondanks zijn er op grond van wat momenteel bekend is bij mens en dier enkele algemeen geldende mechanismen te herkennen. In de eerste plaats zijn ér de gevolgen van onvoldoende toevoer van voedingsstoffen naar de foetus. De gevolgen hiervan voor de foetus zijn dat bepaalde organen of orgaansystemen onevenredig zwaar getroffen kunnen worden (een voorbeeld hiervan is de lever). In hoeverre hier sprake is van een "vertraging" in de ontwikkeling dan wel van een onherstelbare achterstand, zal moeten worden afgewacht. Het is daarom van het grootste belang vervolgonderzoek te verrichten niet alleen bij de mens maar ook bij het proefdier.

Nu ultrasound follow-up onderzoek van de zwangerschap bij de mens technisch goed mogelijk is, kan men zich afvragen of het niet verstandig zou zijn om bij die vormen van ernstige groeivertraging welke het gevolg zijn van een slechte placenta-functie, een sectio caesarea te verrichten, waarbij de geboorte preterm plaatsvindt. Verder is het duidelijk dat men "small for dates" pasgeborenen frequent en ruim calorisch moet voeden met snel opklimmende (borst)voeding. Er moet een frequente controle plaatsvinden van de bloedsuikerspiegels en zonodig moet extra glucose worden toegediend, vooral omdat zeer waarschijnlijk kort na de geboorte maar mogelijk ook nog op latere leeftijd onvoldoende gluconeogenese plaats vindt in de lever. Uitgebreider (proefdier)onderzoek, vooral naar de mogelijk blijvende gevolgen van intrauteriene groeivertraging is noodzakelijk, voordat men kan stellen dat substitutie van bepaalde stoffen of hormonen aan deze groep pasgeborenen ook werkelijk zinvol is.

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