

DECLARATION

I hereby declare that all the work presented in this thesis is my own, unless otherwise stated, and that the thesis has been composed by myself.

Alastair A Macdonald

Studies
into Foetal and Neonatal Development
of the Pig (*Sus scrofa* L.)

by

Alastair Anderson Macdonald

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VOLUME I - TEXT

CONVENTIONS

The abbreviations, conventions and symbols used in this thesis are those specified in "Units, Symbols and Abbreviations - A Guide for Biological and Medical Editors and Authors" published in 1972 by the Royal Society of Medicine.

The anatomical nomenclature employed follows the specifications given in *Nomina Anatomica Veterinaria* (Anon, 1968), and the system of enzyme terminology is as recommended by the Commission on Biochemical Nomenclature (1973). References are presented in the text and the bibliography is listed according to the Harvard system. Names of journals are given in full. Results are presented in the form $X \pm Y$ where X represents the estimated mean and Y represents the standard error of the estimated mean unless otherwise stated.

1.

INTRODUCTION

The problem

The statistics available from the United States Mid-West show that early in the first half of the twentieth century, over one-third of piglets born in the spring months died before weaning (Anon, 1924, 1930), and mortality of this magnitude has been reported in Britian for the years before the Second World War (Reid, 1949). Recent surveys and reviews indicate that piglet losses up to weaning still remain in the range of 15 to 30% of those born (Anon, 1959, 1960; Pomeroy, 1960; Kernkamp, 1965; English, 1968; Aumaitre, 1969; Holub, 1971; Edwards, 1972; Leman, Knudson, Rodeffer and Mueller, 1972; Curtis, 1974; Seerley, Pace, Foley and Scarth, 1974), and it has been reported in the United States of America by Tunks (1973) that the average number of pigs weaned per litter has remained constant within the range of 7.10 to 7.35 since 1965 (England, 1974).

Williamson and Payne (1959) reported a piglet mortality of 29.3% in an experimental herd in Fiji and Gonzalez-Chapel and Carlo (1954) in a survey of piglet losses in Puerto Rico observed a mortality rate of 35.7% to weaning. The losses in Jamaica were found to be 21% of live born pigs during the first month of life (Fraser, 1966 a,b), a figure in keeping with that of Williamson and

Table 1.1 World total livestock numbers - 1947/48 and 1970/71.

Country	Livestock Numbers (Millions)	
	1947/48-1951/52	1970/71
Europe	69.2	139.4
North and Central America	75.2	95.2
South America	35.6	83.3
Asia	20.9	49.1
China	68.4	223.0
Africa	4.4	6.9
Oceania	1.9	3.5
U.S.S.R.	19.7	67.4
World	295.3	667.7

Source: Food and Agricultural Organisation (1972).

Table 1.2 Numbers of animals slaughtered and quantity of meat produced in the world in 1971/72.

Animals	Animal numbers slaughtered (Millions)	Edible meat produced (Million tonne)
Cattle	214.2)	41
Buffalo	6.8)	
Pigs	615.0	40
Sheep	377.2)	7
Goats	128.3)	

Source: Food and Agricultural Organisation (1973).

Payne (1959) if a still-born rate of 5 to 7% is assumed to have occurred (Randall, 1968; Milosavljevic, Miljkovic, Sovljanski, Radovic, Trbojevic and Stancov, 1972).

Thus, the evidence available from the Americas, Europe and other parts of the world would indicate that neonatal mortality in pigs constitutes, at between 15% and 35%, a serious loss in potential animal productivity. It has been demonstrated repeatedly, moreover, that the bulk of this loss occurs within the first seven days after birth (Anon, 1959; English, 1968; Edwards, 1972).

World statistics for livestock numbers (table 1.1) show that there has been a substantial growth in animal production in the twenty years to 1972, and a recent review from the Commonwealth Secretariat (Anon, 1973) intimated that there has been, at the same time, an almost continuous growth in world carcass meat production and consumption, with the expansion in supply failing to keep pace with demand in the last few years.

An analysis of the number of animals slaughtered throughout the world in 1971/72 demonstrates that pig meat constitutes a large proportion of the animal flesh consumed (table 1.2).

It may be deduced, from the information in table 1.2 and the estimates of piglet mortality described above, that world pig production lies below the potential total output to the extent of between 110 million and 330 million animals - if no mortality were to occur between birth and weaning. In terms of potential edible meat production, the loss represents between 7 million and 22 million tonne of pig meat, a substantial amount when compared to present world meat supply (table 1.2).

Estimates of losses in other domestic species of livestock indicate that high neonatal mortality may also be found both in sheep (Purser and Young, 1959; Dennis, 1965; Barton, 1974), and cattle (Withers, 1952, 1953; Wiltbank, Warwick, Vernon and Priode, 1961; Vaccaro, 1974). However, more complete statistics with which to make across-species comparisons have been published in the medical literature. In nineteenth century Europe, infant mortality ranged between 15% and 37% (Knodel, 1968; Wickes, 1953) whereas one hundred years earlier as many as 50% of all children born died before two years of age (Still, 1931; Wickes, 1953; Beaver, 1973). The death rate in Scotland for infants less than one year old had fallen to, and was essentially static at, 152 per

Table 1.3 Rates of perinatal mortality, neonatal mortality and infant mortality - 1871-80 to 1972.

Year	Perinatal mortality (1)	Neonatal mortality (2)	Infant mortality (3)
1871-80	-	-	123
1921	-	37	90.3
1931	-	35.8	81.8
1939	67.5	36.6	68.5
1942	60.7	35.1	69.3
1952	43.7	21.7	35.2
1962	34.8	17.9	26.5
1972	23.7	12.4	18.8

(1) Still births and deaths in the first week of life (rate per 1,000 total births including still births).

(2) Death at ages under 28 days (rate per 1,000 live births).

(3) Deaths during first year of life (rate per 1,000 live births).

Source: Scottish Home and Health Department (1974) and Anon (1933).

thousand births for males and 126 per thousand births for females during the forty years 1860 to 1900, with the further decline in the infant death rate being restricted to the present century (Froude, 1933). The Scottish statistics for the past fifty years are given in table 1.3 and demonstrate that now only just over 2% of all births lead to death within one year of life. On a world wide basis, it is clear that a similar trend is in train. Infant mortality data from 37 countries and territories for the past twenty years have been analysed by the World Health Organisation (Anon, 1970); it was found that between 1951 and 1955 the average infant mortality rate was 43.7 per 1,000 live births with a range from 131.7 in Chile to 19.3 in Sweden. By 1967 the average infant mortality rate had fallen to 22.9 with a range from 99.8 in Chile to 12.9 in Sweden; almost 60% of all infant deaths occurred at less than 7 days of age.

Two conclusions may be reached from an analysis of these observations; that high neonatal mortality has been a feature common both to domestic species of animals and to man himself, and that efforts to gain an understanding of the problem have been demonstrably effective in drastically reducing the wastage around the time of birth in the human species.

A series of studies were therefore made of those aspects of the uterine environment which were considered to play an important role in the development of the foetal pig.

The energy reserves of the foetus.

It was suggested over sixty years ago that the ability of the animal to regulate its body temperature after birth was a function of the reserves of fat deposited during gestation (Thomas, 1911). In some species the amount of fat present at birth was large, 10g/1000g of body weight in the guinea-pig and in excess of 15g/100g in the body of the newborn human baby (Widdowson, 1950). The pig, on the other hand, has been shown to contain only about 1.0g/100 g throughout gestation (Gortner, 1945) with estimates of chemical lipid content at birth ranging between 1.0 and 1.4g/100g (Widdowson, 1950; Manners and McCrea, 1963; Elsley, 1964). In this species carbohydrate in the form of glycogen would appear to provide the main source of energy for the newborn piglet (McCance and Widdowson, 1959).

Studies carried out by a number of workers and summarised by Elsley (1969) have indicated that the

very high concentration of glycogen present in the newly born pig, is deposited in a comparatively short period at the end of gestation. The pattern of accumulation within the organs of the foetal piglet and the relative importance at birth of specific anatomical depôts of glycogen nevertheless remained unexplored. A series of experiments were therefore undertaken to investigate the way in which glycogen was accumulated in the skeletal and cardiac musculature, lung, liver and kidneys of the foetal pig, and the manner in which mobilisation of glycogen took place following birth. The findings are presented and discussed in the thesis.

The nutrition of the foetus.

The substrates which serve as metabolites for the synthesis of foetal proteins, carbohydrates and lipids have not been fully determined. In particular, there is controversy concerning the role played by glucose in the energy metabolism of the foetal sheep (Shelley, 1973; Battaglia and Meschia, 1973). Early workers suggested that the foetus derives most of its energy from carbohydrate sources (Windle, 1940; Villee, 1953b) and more recent studies lent support to that

opinion (Alexander, Britton, Cohen and Nixon, 1969; Crenshaw, 1970). Other studies have raised the possibility that lipids may be catabolised by the foetus to provide energy (Robertson and Sprecher, 1968; Roux and Yoshioka, 1970).

The discovery of high concentrations of fructose in the blood of newly born piglets prompted Goodwin (1956a) and Curtis, Heidenreich and Foley (1964) to propose that fructose was the principle sugar metabolised by the pig foetus. The results of work by Aherne, Hays, Ewan and Speer (1969) led them to conclude that on the contrary, fructose plays only a minor role as an energy source for the foetal pig.

The importance of glucose to the piglet in utero, however, remained unclear and therefore the method of Tsoulos, Colwill, Battaglia, Makowski and Meschia (1971) was employed to evaluate the contribution made by this sugar. The thesis describes an investigation in which the fraction of foetal oxygen consumption required to metabolise aerobically the umbilical glucose uptake was studied at various times during the latter stages of gestation. The results are discussed in the light of observations made in other species and changes occurring in the body composition of the near-term foetus.

The anatomy of nutrient transfer.

Some of the principles governing the maternal-foetal transfer across the placenta were discussed by Faber (1969, 1973) and Forster (1973), and the few observations which have been made on the permeability of the pig placenta have recently been reviewed by Sperhake (1971). Although estimates of the distance between maternal and foetal capillaries have been given by Amoroso (1952), Bjorkman (1965) and Crombie (1970, 1972), no detailed study appears to have been made to quantify the changes thought to occur during pregnancy. Disagreement remains concerning the best estimate of maternal-foetal contact surface area (Dempsey, Wislocki and Amoroso, 1955; Bjorkman, 1965; Baur, 1973).

There is a comparable lack of information concerning the pattern of blood flow described by the maternal and foetal placental vasculature. No work has been found which sought to observe at the level of the scanning electron microscope the three dimensional arrangement of placental capillaries belonging to both circulations. Several experiments were therefore formulated to reveal the angio-architecture of the pig's placenta, and to elucidate the direction of blood flow within its micro-vasculature.

'One must learn by doing; for though you think you know it, you have no certainty until you try.'

(Sophocles)

2. THE PLACENTAL VASCULAR ANATOMY

I N T R O D U C T I O N

Historical

One of the earliest references to the use of an injected medium to assist in making observations on the uterine and foetal placental vasculature was that of Aldes (1667); in the course of observations on the uterus of a cow three or four months pregnant, Aldes (sometimes referred to as Slade, 1667), injected a black coloured liquid into the 'foetal arteries running towards the placenta' and discovered that the maternal cotyledons did not also turn black, but on the contrary, remained uninjected and white. Nevertheless, Cole (1921) believed that it was a more recent paper (which also described the use of injection techniques to facilitate observation) which placed beyond question the independence of maternal and foetal blood circulation in the placenta; the author was Monro (1734), the Professor of Anatomy at Edinburgh and a Member of the Honourable Society of Improvers in the Knowledge of Agriculture in Scotland (Inglis, 1911).

Subsequent investigations of the uterine and foetal vasculature relied heavily on the injection of dyes or Indian ink to help visualise the vessels

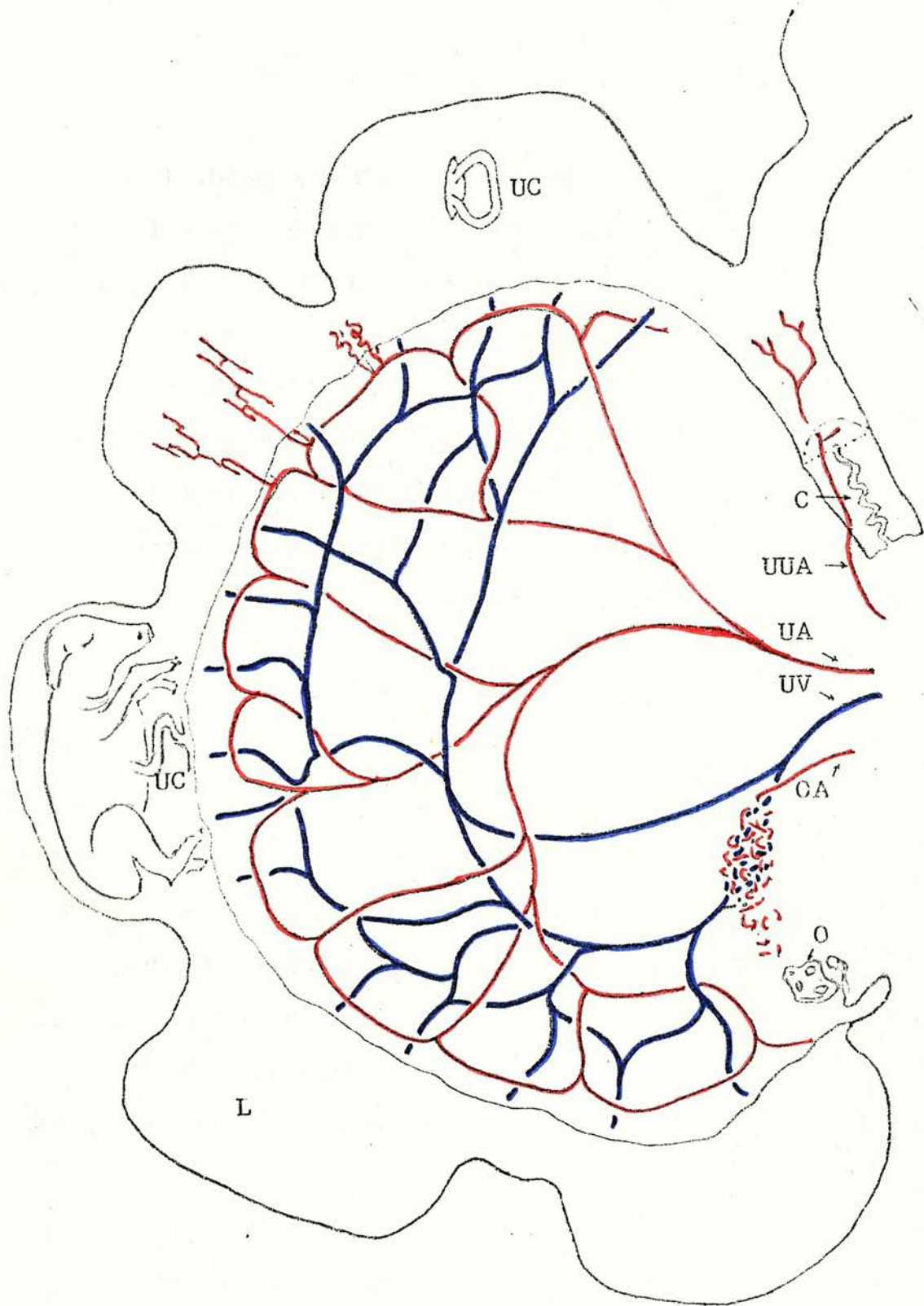


Figure 2.1 Diagram of the uterine blood vessels serving the left uterine horn demonstrating the uterine artery (UA), uterine vein (UV), uterine branch of the urogenital artery, (UUA), ovarian artery (OA) and the foetal umbilical cord (UC). The ovary (O), cervix (C) and a foetal loculus (L) are also depicted.

(von Baer, 1828; Eschricht, 1837; Leopold, 1874; Sieber, 1903; Heuser, 1927), although Tafani (1886) used coloured gelatin. One of the drawbacks with such methods lay in the difficulty in obtaining preparations which would last sufficiently long to make repeated observations. The use of serial histological sections in order to build models of the vascular arrangement was one solution adopted to circumvent this problem (Patten, 1948).

Recent techniques.

Within the last 50 years, methods of making solid casts of the blood vessels have become available (Burt, 1928). The use of rubber was superceded in turn by neoprene latex (Barcroft and Barron, 1946), vinyl acetate (Ramsey, 1949), vinylite resin (Grahame and Morris, 1957) and acrylics (Macdonald, 1971), as deficiencies inherent in each material became apparent.

Numerous authors have examined and described, with the aid of such injection methods, the arterial vasculature of the porcine uterus (Montané and Bourdelle, 1920; Ellenberger and Baum, 1943; Boye, 1956; Grahame and Morris, 1957; Lange, 1959; Barone, Pavaux and

Frapart, 1962; Oxenreider, McClure and Day, 1965; Nunez and Getty, 1969; Macdonald, 1971; Del Campo and Ginther, 1973), although fewer observations have been made on the uterine venous vessels (Montané and Bourdelle, 1920; Ellenberger and Baum, 1943; Lange, 1959; Barone et al., 1962; Oxenreider et al., 1965; Nunez and Getty, 1970; Del Campo and Ginther, 1973). Almost all this work was carried out on the non pregnant animal, and only the studies by Grahame and Morris (1957) and Nunez and Getty (1969) drew attention to architectural differences between the uterine blood vessels of the nulparous gilt and post partum sow.

No studies, other than those of Tafani (1886), Tsutsumi (1962) and Macdonald (1971), were found which described the angio-architecture of the gravid porcine uterus. Furthermore, only the early experiments of Tafani (1886) and Tsutsumi (1962) set out to elucidate the anatomical arrangement between maternal and foetal vessels. Technical difficulties have until very recently hampered studies of the three dimensional appearance of the placental capillaries; photographs published by Lange (1959) and Tsutsumi (1962) illustrate the problems encountered in attempting to obtain a sufficient depth of focus using light microscopy.

The present study was undertaken to explore the vascular anatomy of the pregnant uterus and allanto-chorion, to bring to bear the scanning electron microscope as a suitable instrument with which not only to see clearly in three dimensions the placental micro-vasculature, but also make observations from a large range of viewing points and to obtain thereby an impression of the relationships between the maternal and foetal placental blood vessels at different times during pregnancy.

M A T E R I A L S A N D M E T H O D S

Animals

Ten sows, weighing between 140 kg and 200 kg and of mixed Large White and Landrace breeding were killed at different times during pregnancy. In four cases, the stages of pregnancy were known to be 98, 102, 104 and 109 days from recorded mating dates; in the remaining animals, obtained from the local slaughter house, the relationship between foetal age and mean piglet weight per litter (Warwick, 1928; Pomeroy, 1960; Gjesdal, 1972) was used to estimate prenatal age as 40, 45, 65, 85, 90 and 95 days.

Preparation of the tissues

The reproductive tracts were dissected free in such a way that the origins of the arteries in the aorta and the veins in the vena cava were maintained. Flexible polythene catheters were placed in the left uterine artery, uterine branch of the urogenital artery, ovarian artery and uterine vein (Figure 2.1). The right uterine horn was ligated close to the junction with the body of the uterus. In three cases, the

vessels of the alternate side were cannulated because of damage to the vasculature of the left uterine horn.

Preparation of the cast

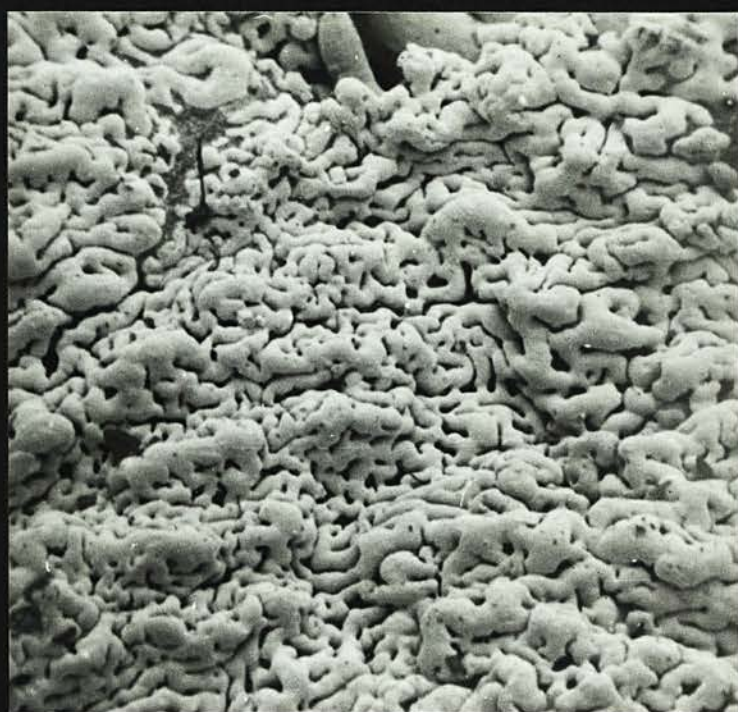
Cold setting all-acrylic cement, "Tensol" cement no. 7 (Imperial Chemical Industries Ltd.) is a thin, clear ready-made solution of methyl methacrylate polymer in the corresponding monomer (Bugge, 1963). The cement was coloured by adding acid resisting pigments such as mercuric sulphide (vermillion), cobalt aluminate (blue) or cadmium sulphide (yellow). Approximately 4 ml/100 ml activator was rapidly stirred into the coloured cement to start polymerisation. The material was then injected into the catheters using disposable hypodermic syringes operated under digital pressures of between 50 and 150 mmHg. Systolic blood pressures lie within the range 100 to 150 mmHg (Engelhardt, 1966) which may explain the low incidence of vessel rupture encountered during injection. When the cement in the maternal blood vessels had hardened, a small incision was made in both the uterine wall and the foetal membranes through which the umbilical cord was exteriorised. The umbilical vein or an umbilical artery was cannulated and the vessels of the allanto-chorion injected with cement in a manner similar to that employed for the maternal

vasculature. Precautions were taken to ensure minimal dislocation of the uterus and its contents.

After the cement had set hard, the uterus was rapidly frozen at -20°C and stored at that temperature until tissue digestion was possible. For maceration, the frozen uteri were placed into concentrated hydrochloric acid and left for about one week, at the end of which period, the casts were cleaned of digested material with gently running water.

Microscopy

Pieces of cast, 10 mm^2 in size were dissected from the placental area, coated under vacuum in a mist of gold-palladium and examined using a Cambridge stereo-scanning electron-microscope (II A).



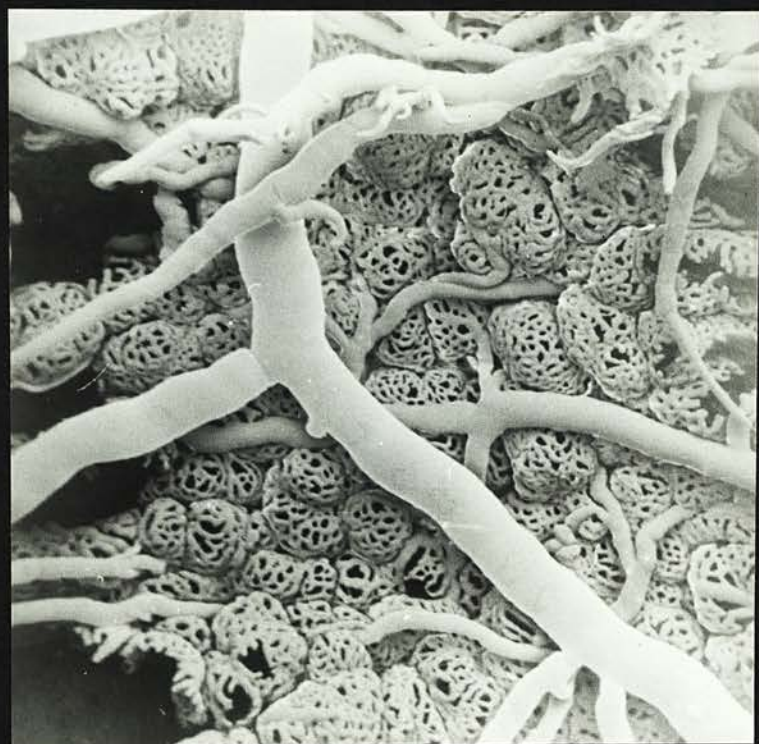
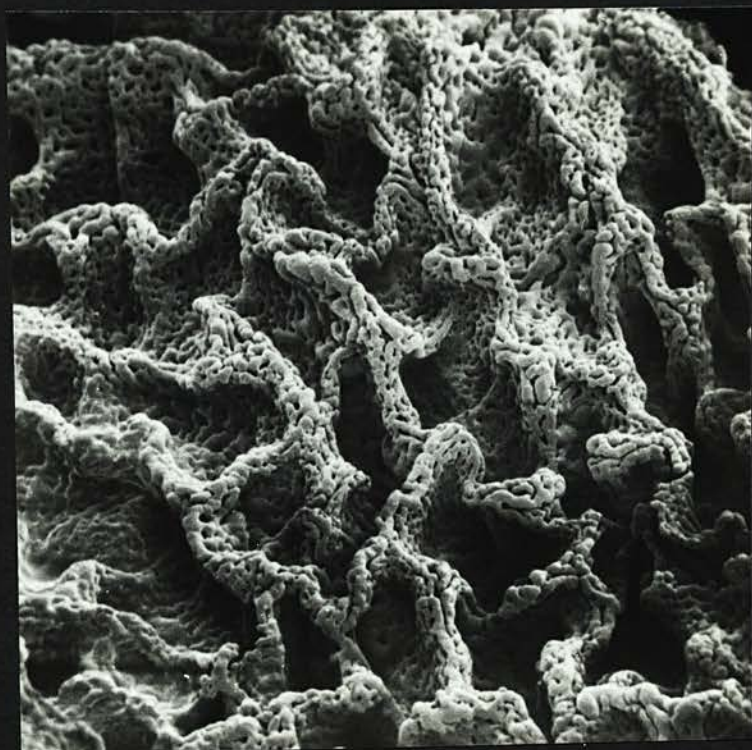
R E S U L T S

Maternal placental blood vessels

In so far as the casts reflected the natural appearance of the placental blood vessels, large changes were to be seen in their arrangement as pregnancy progressed. The much folded appearance of the uterine mucosa was reflected in low power micrographs of the under-lying vasculature (figure 2.2). Closer inspection revealed numerous shallow ripples on the cast, with what seemed to be capillaries of smaller diameter lying in the grooves and rather dilated capillaries constituting the ridge tops (figure 2.3). No clearly defined pattern could be discerned in the arrangement of vessels lying between the arterioles and venules at the early stages of pregnancy.

Figure 2.2 A scanning electron micrograph of part of a vascular cast made of the uterine blood vessels of a pregnant pig forty days post coitum; the capillaries serving the uterine mucosa are depicted (x 20).

Figure 2.3 A close-up of the cast illustrated in figure 2.1 demonstrating the low ridge and trough arrangement of uterine capillaries at this stage of pregnancy (x 100).

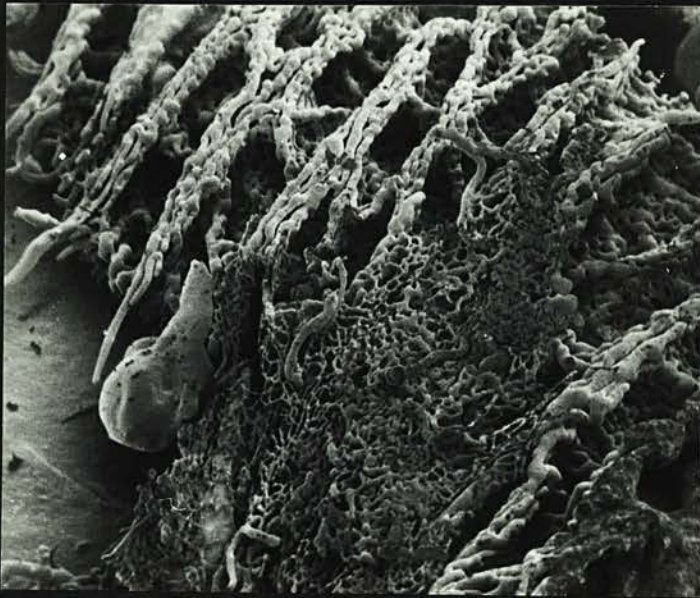


As pregnancy progressed, the topography of the uterine capillary network developed into the more highly structured arrangement illustrated in figure 2.4. The capillary nets descended almost perpendicularly from the ridge tops and ran in a "curtain like" manner with folds and pleats parallel to the circumference of the uterine lumen. Although the ridge tops continued to be demarcated by blood vessels of generally larger diameter, several vessels on the sides and bases of the troughs were demonstrably larger than their immediate neighbours. Towards the end of pregnancy, the low transverse ridges seen in figure 2.4 achieved the dimensions of the earlier formed balk-like vascular networks.

When viewed from the "maternal aspect", the capillary bed of the uterine mucosa had the appearance illustrated in figure 2.5. The paths taken by the arteries suggested that maternal arterial blood was delivered to the ridge tops, subsequently passing down the sides of veins situated beneath the bases of

Figure 2.4 A scanning electron micrograph of the folds and pleats of the uterine mucosal capillaries at ninety-five days of gestation (x 50).

Figure 2.5 The mucosal capillaries of the eighty-five day pregnant uterus viewed from below (x 45).



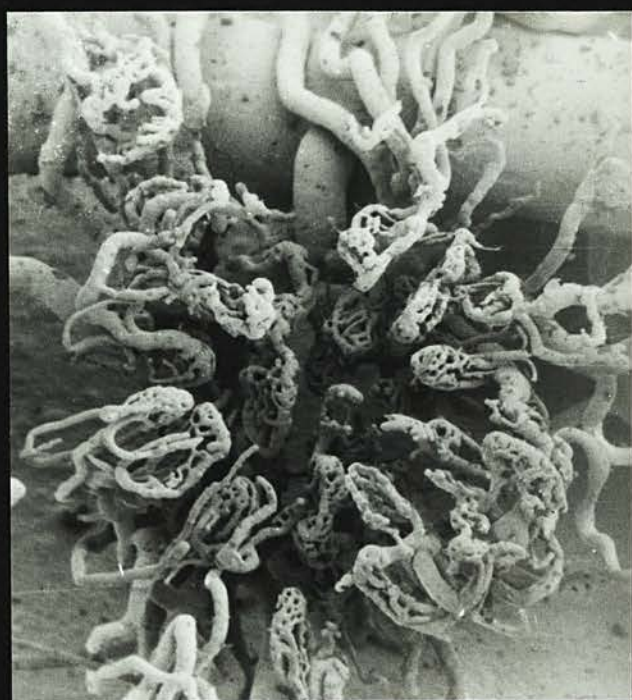
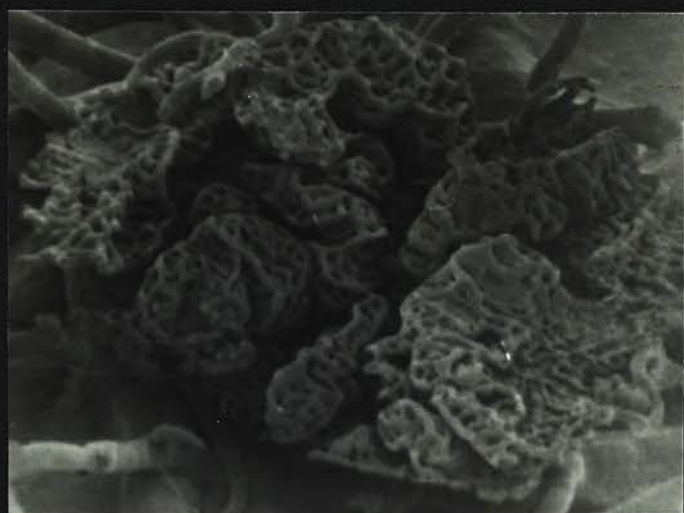
the crypts. Injection of different colours into the arteries and veins of the same uterus lent weight to such a suggestion; when the capillaries were "back-filled" from the venous side, ridgeless "basket" networks of vessels were formed. Material injected into arteries on the other hand passed between the adjacent "basket" structure to the region of the ridge top.

Foetal placental blood vessels

The morphology of the allanto-chorionic vascular net underlying that of the uterine mucosa is depicted in figure 2.6. The trough and ridge pattern of the maternal vessels was readily identified, but the lace like filigree of foetal capillary vessels obscured the type of vascular structure presented to the uterine mucosa by the foetal placental membranes.

Figure 2.6 A view of the foetal allanto-chorionic capillaries overlying the placental vessels of the uterine mucosa of a pig at ninety days of pregnancy (x 25).

Figure 2.7 An allantoic artery and arterioles directed towards the capillary network of the uterine mucosa of a ninety days pregnant pig (x 50).



In another part of the same cast, the capillary net had not been filled and it was possible, therefore, to see how a small foetal artery sent out dendritic arterioles towards the capillary network of the uterine mucosa (figure 2.7). The indentations on casts of the foetal vessels may have been caused by the cells of the vascular smooth muscle.

The cement filled inter-areolar regions of the allanto-chorionic capillary network were largely composed of tightly-spaced raised portions of variable size, arranged in parallel fashion and closely resembling sand ripples left on a beach by the receding tide (figure 2.8). As gestation progressed, the height of these vascular structures increased, and they attained a more uniform club-shaped appearance. The vessels of the areolae were on the other hand, strikingly different in their distribution, being radially distributed about a central depression (figure 2.9). Incomplete

Figure 2.8 A scanning electron micrograph of the veins and capillaries of the allanto-chorion of a foetal pig at eighty-five days of gestation, showing the club and ripple-like projections (x 180).

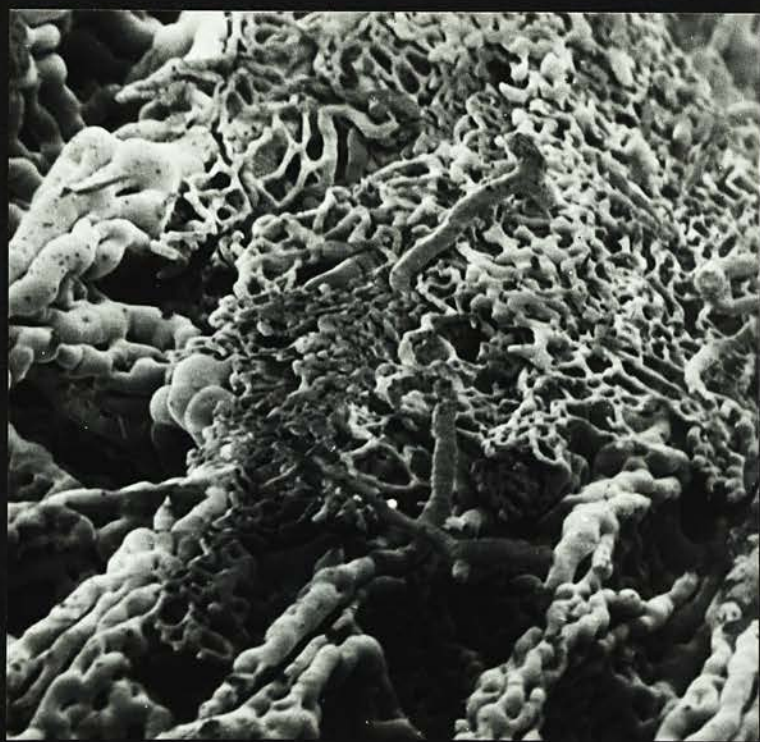
Figure 2.9 Arteries and capillaries of an areolus on the allanto-chorion of a piglet at ninety days of gestation (x 190).



injection of the umbilical arteries produced casts which showed little filling of the inter-areolar capillary bed, but partial exposure of the areolar vascular network. Several arterioles supplied the folds and papillae of the areolae, single arterioles being seen rising to the summits of the papillae, distributing lateral capillaries en route. Venous drainage from each papilla and fold was undertaken by several fine venules.

The distribution of arteries and veins in the inter-areolar region indicated that the capillaries which lay on the surface of each club-like structure arose as laterals from several arterioles at the bases (figure 2.10). The superficial capillary network then appeared to feed towards the interior of the structure, and venous injection casts demonstrated that single venules received tributaries from the cores of the allanto-chorionic capillary folds. There was therefore, anatomical evidence that arterial blood flowed from the base to the tops of the folds via the surface capillaries.

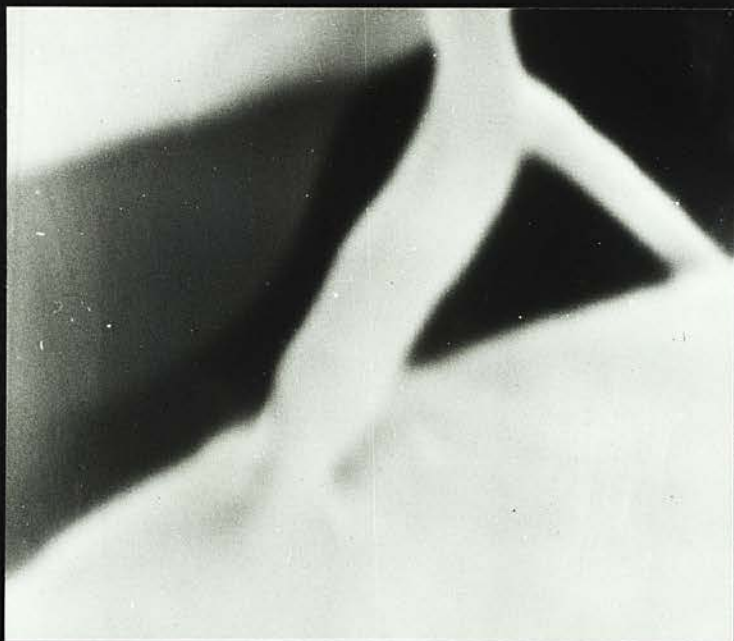
Figure 2.10 The club-like vascular structures of the allanto-chorion of a ninety day old foetus illustrating the way the capillaries turn in towards the core (x 130).



Initially, the interrelationship between foetal and maternal vessels was deduced from the morphology of their capillary nets. The foetal 'club-shaped' structures corresponded to the cross-ridged troughs of the uterine vasculature (figures 2.10 and 2.11). Subsequently, by studying sections of the cast in which the foetal network lay undisturbed over the maternal vessels the inter-locking arrangement of the two circulations was confirmed (figure 2.12).

Figure 2.11 A close-up view into the cross ridged trough of the uterine mucosal capillary network (x 250).

Figure 2.12 A cast of the allanto-chorionic capillaries overlying the uterine placental network. Two foetal "club" structures may be seen on the right fitting into the parallel arrangement of maternal troughs (x 70).



Sub-placental vascular structures

In the course of studying one of the maternal casts, thread like structures were noticed in regions behind the capillary bed of the uterine mucosa. Inspection of other maternal and foetal casts demonstrated similar structures in both. Figure 2.13 depicts a characteristic section of the filament and illustrates the type of branching which was observed. The straightness and slimness were striking. Furthermore, an odd shaped structure was noted (figure 2.14) which appeared to have small discoid appendages distributed on its surface.

The filaments were of smaller diameter than the capillaries, but when they were traced back they were found to originate firmly in both arterial and venous vessels (figure 2.15).

Figure 2.13 A general view of the thread-like cement filled structures (x 40).

Figure 2.14 The unexplained 'body' found attached to the thread-like structure, illustrating the discoid appendages on its surface (x 200).

Figure 2.15 A close-up view of the point of attachment to a uterine vessel of the thread-like structure (x 550).

D I S C U S S I O N

Placental blood flow

The possibility that the foetal and maternal circulations were arranged in such a fashion that blood flowed in a concurrent manner could be inferred from the relative distribution of the placental capillaries. Earlier workers using either injections of coloured gelatin (Tafari, 1886) or neoprene latex (Tsutsumi, 1962) put forward similar proposals. There was, however, a number of differences between the structures noted in the present study and the observations of Tsutsumi. Generally, the uterine arteries were found to send tributaries to the tops of the ridges whereas Tsutsumi (1962) believed that the capillaries passed from the artery, round the 'crypt' to the vein on the opposite side (figure 2.16).

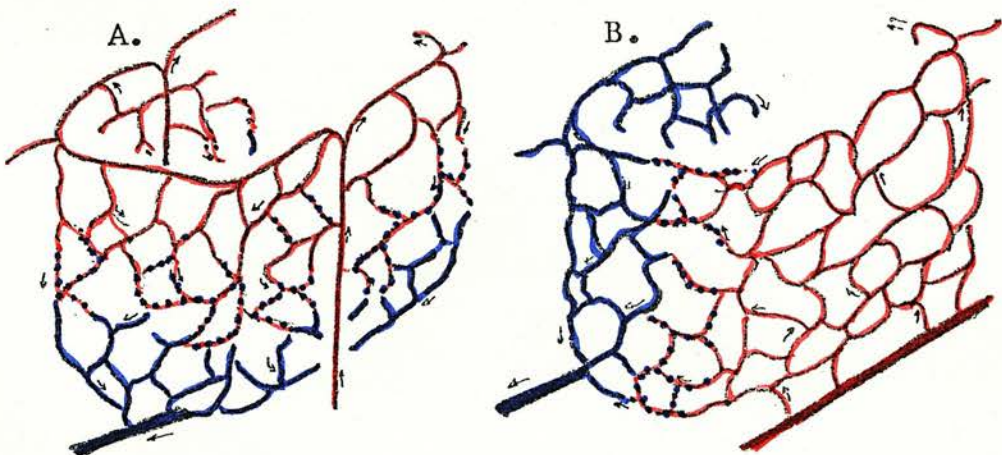


Figure 2.16 Schematic diagrams of the patterns of uterine blood flow according to (A) the present study and (B) that of Tsutsumi (1962).

Only very occasionally did the author observe the vascular arrangement described by Tsutsumi; more often the troughs were seen to lie on a small vein with several short capillary connections made to it; periodically a venule was noted to have become incorporated into the trough for part of its length.

A greater measure of agreement was reached with respect to the allanto-chorionic vasculature. Tsutsumi noted that the distribution of superficial capillaries arose from arterial branches, many of which ran up the full height of the club shaped structures, and that the capillary net connected with the tips of venous tributaries at the base. However, Tsutsumi made no comment concerning the possibility of the venous drainage occurring within the structure, except to state that there was no central vessel.

The difficulty of deducing haemodynamic properties from the examination of structure alone was demonstrated by Silver, Steven and Comline (1973) when they reviewed the published observations of ruminant placental capillaries. Although the pig has a simpler placenta upon which to make observations, the author recognises that caution must be exercised particularly when drawing

conclusions about blood flow in the micro-circulation.

The morphology of the foetal vessels

The three dimensional structure of the foetal allanto-chorionic capillaries was very reminiscent of the plates published by Lieberkuhn (1745) demonstrating the microscopic vascular supply of the intestinal mucous membrane. The present author, like Tafani (1886), thought that the functional similarity between the two capillary beds with regard to nutrient uptake was also quite comparable.

Further support for the analogy was provided by the observation that the capillaries of the uterus and allanto-chorionic areolae (Crombie, 1972) fall under the classification of 'fenestrated' capillaries (Majno, 1965). This suggested that they may play a role in the leaking or absorption of fluids (House, 1974). The inter-areolar capillaries were, however, classified as 'continuous' by Crombie (1972) and in other sites in the body these are generally regarded as a less permeable type of vessel (House, 1974). Pomeroy (1960) found that although uterine tissue continued to increase throughout pregnancy, the foetal placental membranes seemed to stop growing as measured by weight after 65 days. In the

light of the present observations, gross uterine or allanto-chorionic weights would seem to bear only a tenuous relationship to the anatomical and physiological functions of nutrient and waste-product transport across the placenta. Similarly, the expression of uterine or placental blood flow with respect to the gross weights of the corresponding tissues would appear to require caution.

The few observations that have been made on the permeability of the pig placenta have been reviewed recently by Sperhake (1971). The work by Flexner and co-workers on the placental permeability to radioactive sodium ions has remained the most complete investigation in the pig to date (Gellhorn, Flexner and Pohl, 1941; Flexner and Gellhorn, 1942), although points of criticism have been raised. Wislocki (discussion of paper by Amoroso, 1955) considered that the rates of transfer of sodium should have been expressed in terms of "units of absorbing surface" instead of "per unit weight of placenta".

Baur (1973) recently gave a figure of 0.9 m^2 for the villous surface area of a full term pig placenta. However, ultrastructural studies by Dempsey, Wislocki and Amoroso (1955), Bjorkman (1965) and Crombie (1973)

have shown that at the foetal-maternal contact surface, there is a tenfold increase in area because of the microvillous interdigitation. It is conceivable that the figure given by Baur (1973) was an underestimate of true placental exchange surface area since he employed stereological methods at the light microscopic level of magnification. No measurements were made of capillary surface area in the present study.

The question of what constitutes a better estimate of exchange area, therefore, remains unresolved in the case of the pig placenta.

The sub-placental vascular structure

The fine, branching vascular threads found beneath the capillary networks of both maternal and foetal vessels may have been the under-developed remnants of earlier capillary nets. It is known from the work of Evans (1909) and Woolard (1922) that the major vessels arise by growth and differentiation from the embryonic network of capillaries. There was, however, no particular piece of evidence to confirm that the thread-like structures could be explained on this basis. Further studies are, therefore, required to gain a more complete appreciation of the origins and functions of this structure.

C O N C L U S I O N S

The conclusions drawn from the present study were:-

1. The stereo-scanning electron-microscope permitted close observation of the topography of maternal and foetal placental vessels.
2. The anatomy of the capillary networks suggested that maternal blood flows to the ridge tops and drains towards the crypts, the foetal blood apparently being supplied to the superficial capillaries of the club-like structures and flowing from the base towards the top and draining into the core.
3. The relative maternal-foetal blood flow pattern would appear to be concurrent.
4. Previously unreported thread-like vascular structures were observed lying beneath the maternal and foetal placental capillaries.

Suggestions for future studies

The present study exposed to the author a number of areas requiring further study. These may be summarised as follows :-

1. Quantify placental surface area at different stages of gestation.
2. Seek those differences which may exist between the placental vascular areas of large and small foetuses in the same litter.
3. Measure the mean distance between maternal and foetal circulations throughout pregnancy.
4. Ligate one umbilical artery and note the effect on the vascularised area of foetal and maternal placentae.
5. Note the variation in vascularity of placentae resulting from differences in the litter size or the numbers of foetuses in each horn of the uterus.
6. Decrease the maternal flow to individual loculi and note the effect on placental surface area.

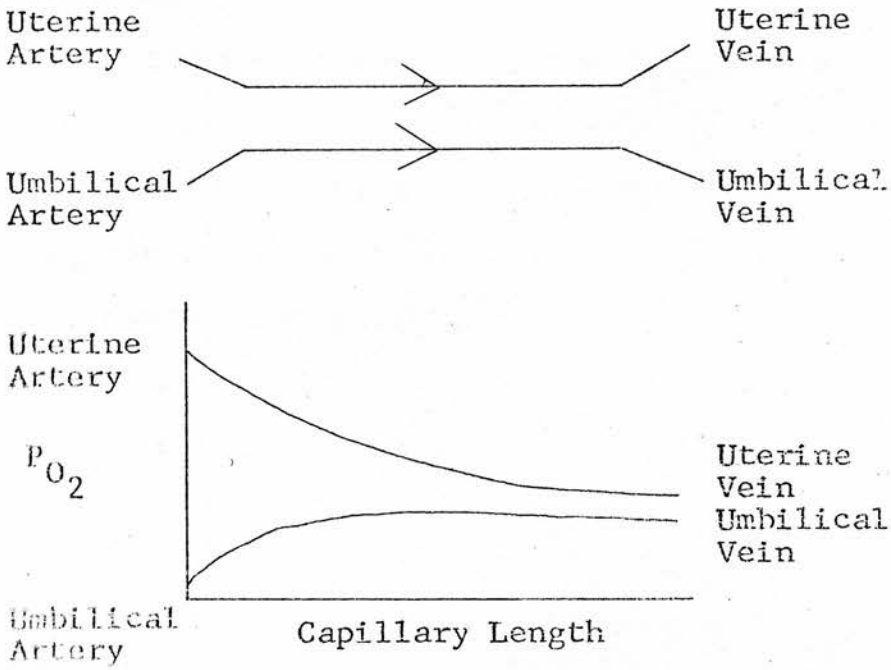
3. MATERNAL - FOETAL RESPIRATORY GAS
RELATIONSHIPS

I N T R O D U C T I O N

The importance of the relative directions of blood flow on the foetal and maternal sides of the placenta as one aspect of an understanding of trans-placental gas exchange has been stressed (Bartels, Moll and Metcalfe, 1962; Dawes, 1968). However, the respiratory physiology of the foetal pig received scant study until very recently, although experiments were described by Vesalius (1543) in the fifth and seventh books of his *De Fabrica*. For example, he relates "If you open the abdomen of the pig (when the animal is shortly about to bear) right to the cavity of the peritonaeum and then lay open the womb in the place where one of the foetuses lies, and freeing the placenta and the membranes from the womb, you place the foetus on the table, you will see through the transparent membranous coat how it tries in vain to breathe, and dies as if suffocated. But if you make a hole in the envelopes of the foetus and free its head from them, you will soon see it revive again and breathe nicely" (Farrington, 1932; Lambert, 1935).

This experiment might have suggested a foetal dependence upon the maternal circulation for air, a

Concurrent Placental Blood Flow.



Countercurrent Placental Blood Flow.

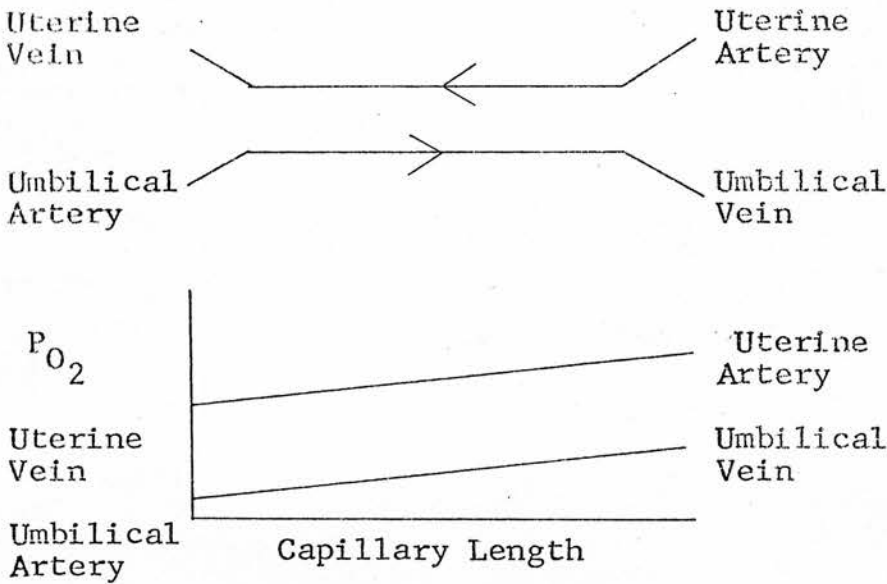


Figure 3.1 Schematic representations of concurrent and countercurrent uterine and umbilical placental circulations, together with diagrams of the time course of change in oxygen tensions during a single transit in placental exchange vessels (adapted from Metcalfe, Moll and Bartels, 1964 and Bartels, 1970) .

postulate of Hippocrates and Aristotle. Such a conclusion was not at that time feasible, however, since it predated the discovery of the circulation of blood, first described in 1628 by Harvey. Nymman in the same year commented that although no continuity existed between maternal and foetal vessels, as Arantius (1564) had earlier asserted, but without proof, the vessels were sufficiently closely apposed to each other that as much air as the foetal required could be obtained with ease from the vessels of the mother.

Diagrams of the possible placental blood flow arrangements which might be found in foetal and maternal vessels are shown in figure 3.1, together with a simplified diagrammatic representation of the corresponding changes in oxygen pressure. In a countercurrent flow system, foetal blood at the venule end of its placental capillary is exposed to oxygen-rich maternal blood at the beginning of the maternal capillary. Such a system is recognised as potentially very efficient for the transfer of diffusible substances.

The discovery in the rabbit of oxygen tensions which were higher in umbilical venous blood than in uterine venous blood (Barron and Battaglia, 1955-56) was the first physiological evidence of a countercurrent

system of placental blood flow. Work by Bartels, El Yassin and Reinhardt (1967) substantiated the finding, and reported that the guinea pig also showed functional evidence of a countercurrent flow system.

Reviews of blood gas studies carried out in the sheep, goat and cow, indicated that those species had concurrent arrangements of placental vessels (Metcalfe, Bartels and Moll, 1967; Bartels, 1970). No comparable work has, however, been carried out on the pig, the work of Harris (1971), Harris and Cummings (1973), Novy, Hoversland, Dhindsa and Metcalfe (1973) and Tweeddale (1973 a,b) being concerned with questions related to respiratory physiology of the foetal pig.

Accordingly, a study was initiated to examine the oxygen and carbon dioxide partial pressures in the foetal and maternal circulations with a view to revealing the nature of the relative foetal and maternal placental blood flows.

M A T E R I A L S A N D M E T H O D S

Animals

Fourteen Large White x Landrace gilts were mated to an A.B.R.O. synthetic sire-line boar (Webb, 1974) and kept under standard commercial conditions until four days before surgery when they were moved to individual penning adjacent to the operating theatre. Food and water were withheld from the animals for the 24h preceding the experiment.

Anaesthesia

Anaesthesia was induced by the intravenous injection of sodium pentobarbitone ('Nembutal', Abbott Laboratories Ltd., 60 mg/ml) to a depth which permitted intubation with a 9.5 mm cuffed McGill tube (Rowson, 1965). The animals were premedicated with atropine sulphate (Evans Medical Ltd. 3000 μ g). General anaesthesia was maintained by closed circuit administration of a variable oxygen/nitrous oxide/Halothane ('Fluothane' I.C.I. Ltd.) mixture.

Sampling Procedures

The uterus was exposed by a midline abdominal incision and the ovarian end of one uterine horn identified

A 2 ml sample of blood was drawn anaerobically from a branch of the uterine artery into a heparinised glass syringe, the dead space of the syringe being filled with heparin saline. Immediately thereafter, a corresponding sample was taken from a vein draining the locular region of the uterus adjacent to the ovary. As rapidly as possible, an incision was made, using thermocautery, through the uterine and allanto-chorionic membranes; the latter, in common with the amnion, often requiring blunt dissection to be penetrated effectively. The umbilical cord was exteriorised, and venous drainage from the placenta sampled before removal of arterial blood from the foetus. In all cases, the syringes were capped immediately and blood gas tensions analysed within five minutes. Each foetus was sampled in turn along the uterine horn, blood being taken as described. It was convenient to continue to take uterine arterial samples from the same site for all the foetuses in one horn.

Analytical Procedures

Measurements of blood pH, P_{O_2} and P_{CO_2} were made at 37°C using Instrument Laboratories 'IL213' digital blood gas analyser. The "direct reading" carbon dioxide electrode (Severinghaus and Bradley, 1958) and polaro-

graphic P_{O_2} electrode were repeatedly calibrated during an experiment with gas mixtures of know composition, and according to daily changes in atmospheric pressure.

C O M M E N T S O N T H E
T E C H N I Q U E S E M P L O Y E D

Surgical techniques

It became clear as the studies progressed that considerably more effort was required to maintain a stable physiological preparation than the author could provide because of deficiencies in both physical resources and personal experience. Lack of knowledge rather than facilities prevented better control of the respiration of the anaesthetised gilt (Cummings, Harris and Agar, 1972), whereas heart rate, blood pressure and blood flow could not be monitored because of the unavailability of suitable apparatus.

Blood gas tensions

That better results could be obtained if blood was sampled from the unstressed animal rather than one lying in dorsal recumbency under general anaesthesia was clear to the author at the outset of the investigation (Macdonald, 1972). However, it was considered that the author lacked sufficient expertise in the special surgical and aseptic techniques vital for successful use of the chronic animal preparation. Every effort

was therefore taken to try to ensure that each animal was treated in a similar manner during the pre-operative period and while the animal was studied under anaesthesia. Variation between gilts nevertheless, did occur. These could be partly related to the particular animal's response to anaesthesia and partly to the depth of breathing and subsequent oxygenation of its blood.

Table 3.1 The summarised results of maternal uterine arterial and venous blood gas analysis.
 Number of samples in parentheses.

Stage of Pregnancy (d)	P _O ₂ (mm Hg)		P _{CO} ₂ (mm Hg)		pH	
	Uterine Artery	Uterine Vein	Uterine Artery	Uterine Vein	Uterine Artery	Uterine Vein
69	181.9 (3)	85.1 (1)	78.2 (3)	84.1 (1)	7.23 (3)	7.16 (1)
70	172.5 (2)	75.0 (1)	107.5 (2)	104.0 (1)	7.19 (2)	7.20 (1)
99	175.4 (4)	68.2 (3)	83.9 (4)	81.3 (3)	7.22 (4)	7.20 (3)
100	137.4 (2)	71.7 (2)	99.9 (2)	105.3 (2)	7.14 (2)	7.14 (2)
101	129.7 (2)	58.5 (3)	63.0 (2)	68.7 (3)	7.26 (2)	7.24 (3)
104	57.3 (4)	43.0 (2)	60.2 (4)	65.1 (2)	7.26 (4)	7.25 (2)
105	145.7 (5)	64.9 (4)	87.8 (5)	95.2 (4)	7.23 (5)	7.20 (4)
108	77.5 (2)	55.0 (2)	56.7 (2)	61.5 (2)	7.22 (2)	7.21 (2)
109	114.0 (1)	33.0 (1)	67.9 (1)	88.1 (1)	7.11 (1)	7.18 (1)
111	241.3 (4)	71.7 (3)	74.8 (4)	72.2 (3)	7.25 (4)	7.22 (3)
113	75.5 (2)	63.0 (2)	109.5 (2)	84.4 (2)	7.20 (2)	7.23 (2)
114	73.1 (5)	71.6 (3)	100.4 (5)	92.2 (3)	7.07 (5)	7.05 (3)
115	229.8 (4)	65.5 (2)	58.4 (4)	63.2 (2)	7.33 (4)	7.35 (2)

R E S U L T S

Measurements made on the gilt

The results of measurements carried out on maternal blood are summarised in table 3.1. The number of observations made on each gilt ranged from one to six according to the duration of surgical intervention.

The high oxygen partial pressures in the uterine artery reflect the high concentration of oxygen in the inspired gases. Hence, at 104 days pregnant mean value of 57.3 mm Hg was found when oxygen represented about 40% of the inspired gas, whereas 175.4 mm Hg was the mean of four determinations at the 99th day of pregnancy when oxygen represented almost 70% of the inspired gas. As may be seen from table 3.1, considerably lower oxygen tension values were found in the uterine vein blood. The difference between the observed uterine venous means of 43.0 mm Hg at 104 days and 68.2 mm Hg at 99 days of pregnancy (25 mm Hg) may be compared with the difference of 118 mm Hg between the two uterine artery blood mean values. The effect of increased concentration of inspired oxygen upon the partial pressure of oxygen in the blood was clearly demonstrated in arterial blood, but substantially moderated by the

tissue, the drainage from which the venous sample was taken. In each case, venous P_{O_2} was lower than arterial P_{O_2} , although considerable ranges of differences existed between arterial and venous samples.

In general, uterine arterial P_{CO_2} values were high, means varying between 56.7 mm Hg at 108 days of pregnancy and 109.5 mm Hg detected on day 113. The mean at 104 days of pregnancy was 60.2 mm Hg compared with 83.9 mm Hg at 99 days. Low P_{CO_2} measurements were not necessarily related to moderately low blood oxygen tensions, since a mean arterial P_{CO_2} value of 58.4 mm Hg was recorded at 115 days when arterial P_{O_2} was 229.8 mm Hg. Eight of the thirteen uterine venous mean P_{CO_2} values were greater than the means of uterine artery observations. Overall, venous P_{CO_2} ranged from 61.5 mm Hg at 108 days to 105.3 mm Hg at 100 days of pregnancy.

Measurement of free H^+ ion concentration yielded nine uterine means greater than their uterine artery counterparts; in the remaining animals the converse was true. Uterine artery pH ranged from 7.07, the mean of five observations at 114 days, to 7.33 the mean of four observations one day later, at 115 days of pregnancy. Observations on blood from the uterine vein revealed a range from 7.05 at 114 days to 7.35 on day 115 of pregnancy.

Table 3.2 The summarised results of foetal artery+vein blood gas analysis: Means \pm standard deviation (number of foetuses sampled in parentheses).

Age (Days)	P _O ₂ (mm Hg)		P _{CO} ₂ (mm Hg)		pH	
	Umbilical Artery	Umbilical Vein	Umbilical Artery	Umbilical Vein	Umbilical Artery	Umbilical Vein
69	-	39.4 \pm 7.0 (11)	-	86.4 \pm 5.9 (11)	-	7.17 \pm 0.05 (11)
70	17.0	76.7 \pm 24.7 (3)	126.4	88.2 \pm 15.9 (3)	7.22	(1) 7.24 \pm 0.08 (4)
99	21.8	52.6 \pm 12.0 (13)	117.6	100.4 \pm 10.7 (13)	7.11	(13) 7.15 \pm 0.05 (13)
100	19.1	58.4 \pm 4.3 (11)	120.0	109.1 \pm 7.9 (11)	7.10	(11) 7.14 \pm 0.03 (11)
101	15.8	47.1 \pm 12.9 (11)	76.5	69.0 \pm 3.1 (11)	7.16	(11) 7.20 \pm 0.03 (11)
104	22.4	35.2 \pm 5.5 (9)	82.9	66.4 \pm 6.1 (9)	7.01	(8) 7.11 \pm 0.09 (9)
105	16.2	52.6 \pm 5.2 (6)	118.5	98.8 \pm 7.1 (6)	7.05	(6) 7.09 \pm 0.03 (6)
108	21.2	40.1 \pm 5.1 (7)	69.7	58.3 \pm 4.7 (7)	7.15	(6) 7.21 \pm 0.03 (7)
109	18.1	59.9 \pm 9.9 (14)	82.1	71.5 \pm 7.2 (13)	7.10	(14) 7.16 \pm 0.05 (14)
111	14.0	62.3 \pm 5.2 (7)	102.6	81.8 \pm 6.0 (7)	7.13	(6) 7.20 \pm 0.03 (7)
113	30.0	50.0 \pm 12.7 (9)	101.7	101.5 \pm 36.5 (9)	7.14	(8) 7.19 \pm 0.05 (10)
114	44.7	75.0 \pm 18.4 (9)	106.5	84.9 \pm 25.9 (9)	6.98	(10) 7.03 \pm 0.02 (14)
115	15.7	59.3 \pm 6.7 (3)	75.3	65.0 \pm 1.3 (3)	7.21	(3) 7.27 \pm 0.03 (3)

Measurements made on the foetus

In order to obtain estimates of transplacental relationships between respiratory gases, the oxygen and carbon dioxide partial pressures in foetal blood were measured.

A summary of the observations on umbilical arterial and venous blood is shown in table 3.2. Means and standard deviations from means are given, with between one and fourteen foetuses being studied at each stage of gestation. Umbilical artery oxygen partial pressures averaged about 18 to 20 mm Hg with umbilical venous drainage results 20 to 40 mm Hg higher. Variation in umbilical oxygen pressure between foetuses was similar in artery and vein but expressed in terms of uterine artery P_{O_2} , the percentage variation was greater in umbilical artery than in umbilical vein.

Foetal P_{CO_2} values were generally greater than 60 mm Hg, but in all cases a lower gas pressure was measured in the venous return from the placenta than in the umbilical arterial supply. Values in the artery ranged between about 70 mm Hg and 130 mm Hg, whereas gas tensions between 58 mm Hg and 110 mm Hg

were found in umbilical vein blood. Both arterial and venous P_{CO_2} values at 99 days were about 30 mm Hg higher than their counterparts at 104 days gestation.

The acidosis indicated partly by the high umbilical P_{CO_2} measurements is illustrated in the pH values found in arterial and venous bloods. In all cases the arterial sample was more acidotic, with values dropping as low as 6.98 at 114 days; this particular litter of piglets appeared to be in the process of natural delivery on the operating table. Despite higher P_{CO_2} values, foetuses at 99 days appeared to have a higher mean arterial pH than those observed at 104 days gestation.

Blood gas relationships between gilt and foetus

In the majority of operations, the pH of the sow's arterial blood declined during surgery. This is illustrated in figure 3.2 which depicts foetal and maternal blood pH values observed at 105 days of pregnancy. The animal (M8) had anaesthesia induced with sodium pentobarbitone at 1010 hours and surgery commenced about one hour later. Oxygen was supplied at a rate of 0.9 litre/min together with 0.4 litre/min of nitrous oxide, the mixture containing 2.5% halothane. The

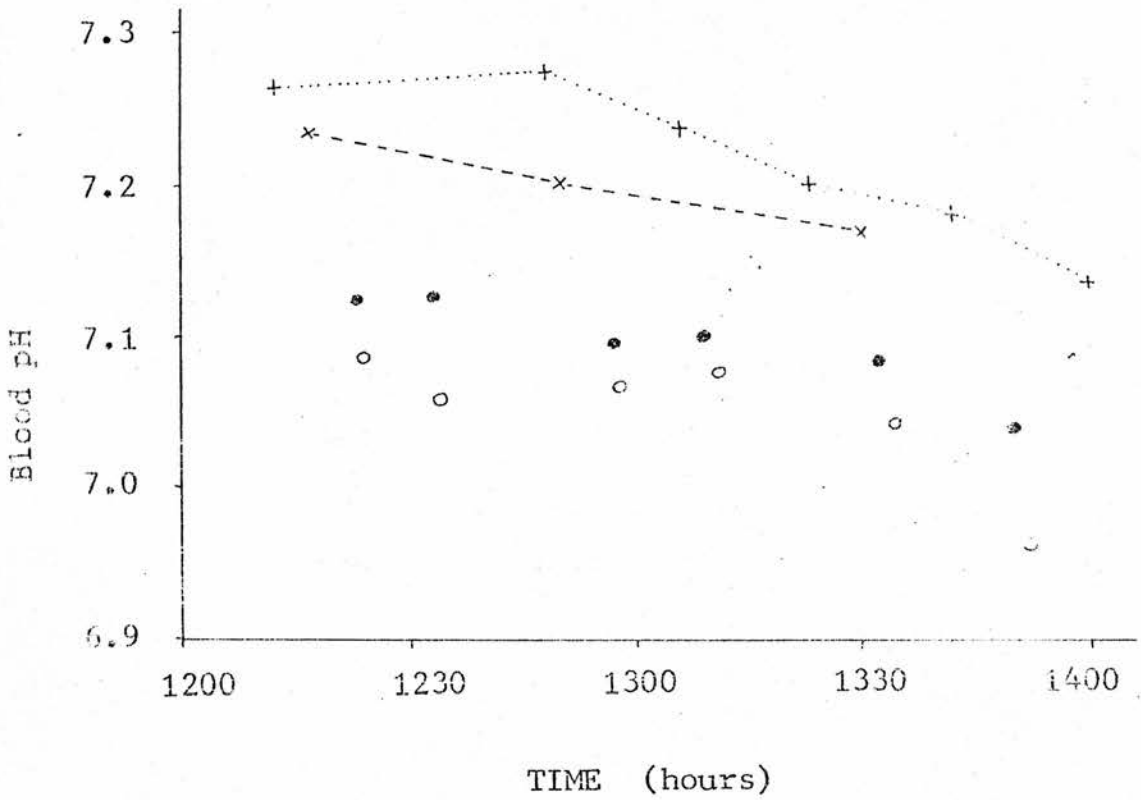


Figure 3.2 Blood pH values from uterine artery (+), uterine vein (x), umbilical artery (o) and umbilical vein (*) for each of six fetuses taken during surgical intervention at 105 days of gestation.

maternal heart rate was 110 to 120 beats/min, and a respiration rate of 12/min was recorded throughout surgery. Foetal heart rates varied between 84 and 108 beats/min according to the foetus being studied.

The uterine artery pH gradually decline from 7.28 to 7.18 one hour later, uterine venous blood apparently declining earlier and at a slower rate. Fewer observations were made on venous blood, however. Both arterial and venous umbilical blood showed similar falls in pH, more exaggerated differences being seen between foetal arterial than venous blood, (as appeared to be the case in the sow). Very little change occurred in umbilical arterial pH between 1234 and 1334 hours despite a progressive fall from 7.27 to 7.18 observed in maternal arterial blood pH.

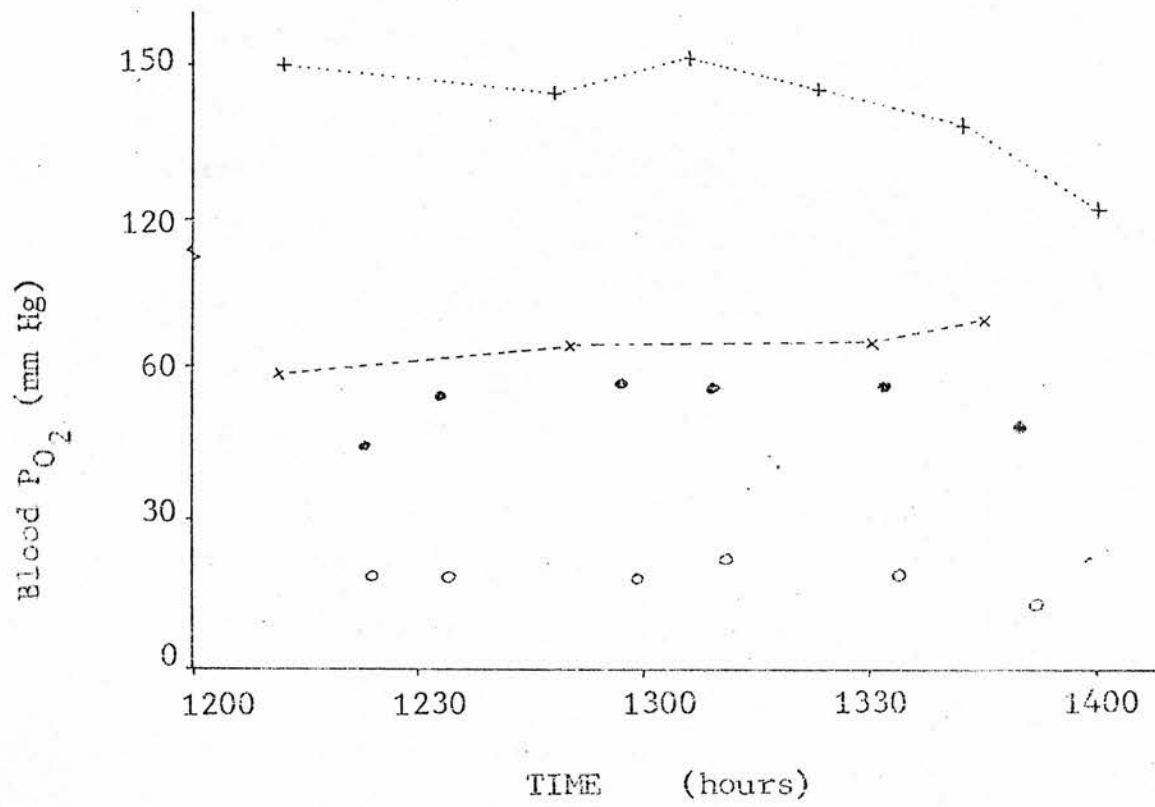


Figure 3.3 Blood oxygen partial pressure values associated with each of six 105 day old fetuses and measured in uterine artery (+), uterine vein (x), umbilical artery (o) and umbilical vein (*).

Throughout this time maternal arterial blood P_{O_2} declined (figure 3.3) having remained fairly stable from 1200 to 1320 hours at 145 to 150 mm Hg; it thereafter fell to about 120 mm Hg by 1400 hours. Uterine venous blood P_{O_2} was observed to rise during this period and only slight changes in foetal blood P_{O_2} were noted other than low umbilical venous blood P_{O_2} in the first and last foetus sampled. Both maternal and foetal arterial P_{O_2} values appeared to change in accordance with one another. But for the divergence observed in the values from the last foetus, the same appeared true for both foetal and maternal venous oxygen partial pressures.

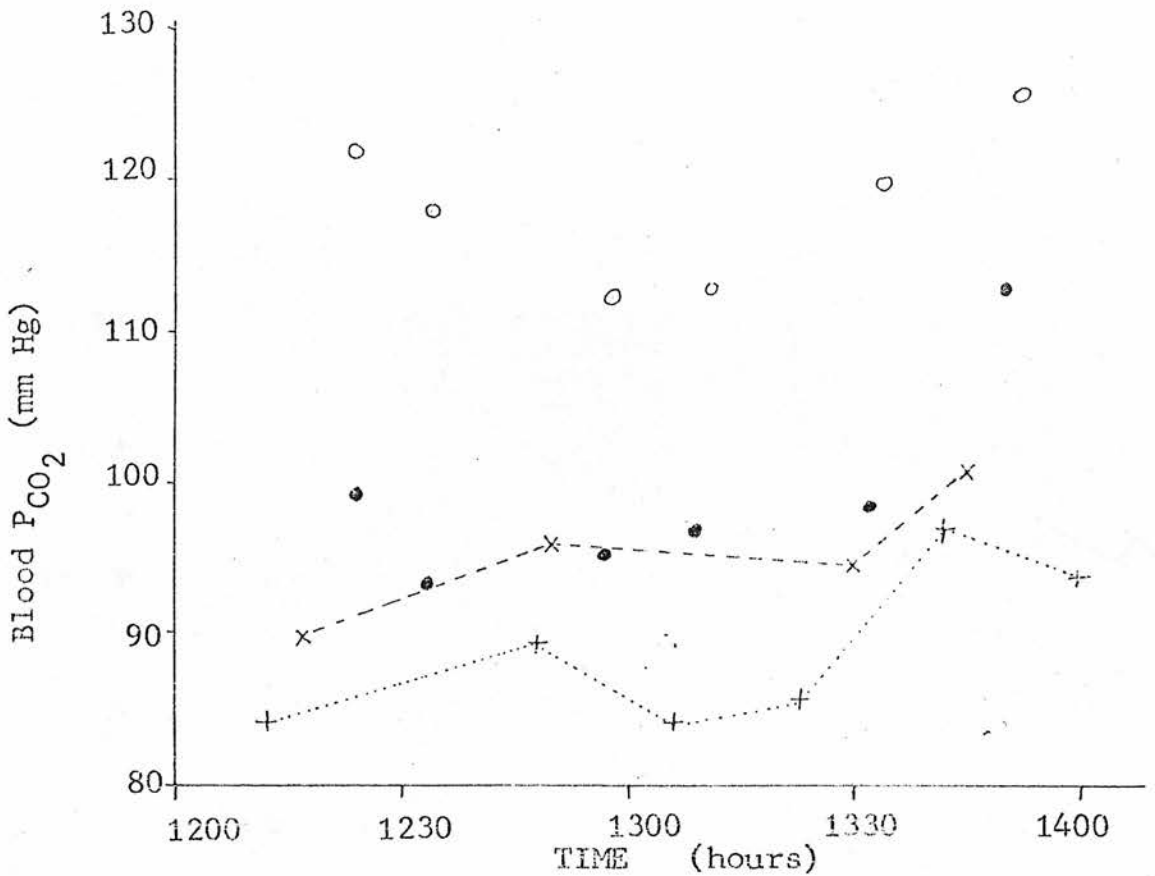


Figure 3.4 Values for the carbon dioxide tensions in blood associated with six, 105 day old foetal pigs and measured during surgery; uterine artery (+), uterine vein (x), umbilical artery (o) and umbilical vein (•).

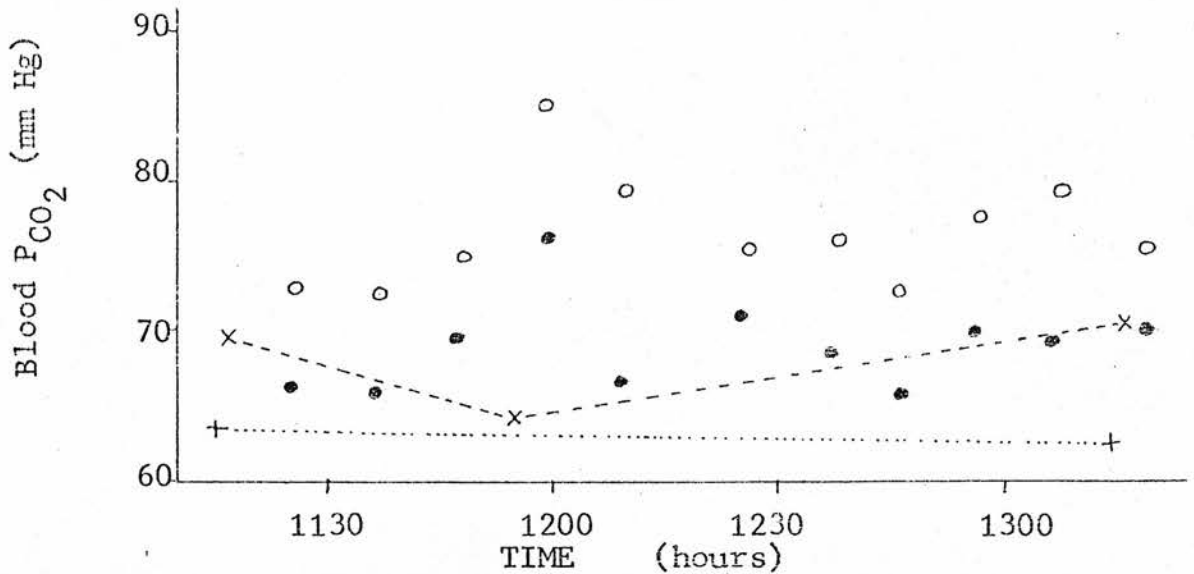


Figure 3.5 Carbon dioxide tensions in blood from uterine artery (+), uterine vein (x), umbilical artery (o) and umbilical vein (•) associated with each of eleven 101 day old foetal piglets.

Figure 3.4 depicts the results of P_{CO_2} determinations and shows clearly a trend towards higher maternal values as surgery proceeded. Initially, there appeared to be a decline in foetal arterial P_{CO_2} , although venous P_{CO_2} increased from about 1230 hours, the time at which uterine vein P_{CO_2} was the same as umbilical vein P_{CO_2} . It was some 30 to 40 minutes later before foetal arterial blood P_{CO_2} was seen to rise.

Fluctuations in uterine arterial P_{CO_2} were apparent, but the low frequency of sampling obscured comparable venous movements, if they existed. A relationship does appear to be present, however, between umbilical arterial and venous P_{CO_2} values as shown in figure 3.5. The observations were made on sow M10 (101 days gestation) and it was apparent that foetal P_{CO_2} values followed one another more closely than they followed maternal values, which would suggest a measure of foetal autonomy with respect to P_{CO_2} .

Table 3.3 Maternal-foetal differences in blood oxygen partial pressures at various times during pregnancy

Oxygen partial pressure difference

Stage of gestation (d)	Uterine vein-umbilical artery (mm Hg)	Uterine artery-umbilical vein (mm Hg)
99	14.6	129.9
100	56.6	91.6
101	30.9	86.6
101	52.0	82.5
104	33.0	57.0
104	19.0	18.0
105	41.0	106.0
105	48.0	87.0
105	47.3	89.7
105	56.6	90.4
108	35.0	45.0
108	32.0	27.0
111	61.0	156.0
111	67.0	163.0
111	51.0	152.0
113	22.0	36.0
114	32.6	17.3
114	0.4	3.8
115	48.0	144.0
115	50.0	168.0

In only two instances out of thirty was umbilical venous P_{O_2} greater than uterine venous P_{O_2} . The calculated difference between uterine venous and umbilical arterial P_{O_2} was, therefore, tabulated together with the difference between uterine arterial and umbilical venous P_{O_2} (table 3.3).

At 104 days gestation, the foetus adjacent to the left ovary had maternal vein minus foetal artery and maternal artery minus foetal vein P_{O_2} differences of 33 mm Hg and 57 mm Hg respectively, whereas the foetus adjacent to the right ovary had P_{O_2} differences of 19 and 18 mm Hg respectively. In the majority of cases the maternal artery minus foetal vein difference was higher than the maternal vein minus foetal artery P_{O_2} difference; in the instance reported above, and for one foetus each at 108 days and 114 days, the converse was true.

Table 3.4 The differences found between foetal and maternal placental arterial oxygen tensions and venous oxygen tensions at various times during pregnancy.

Stage of gestation (d)	Oxygen partial pressure difference	
	Uterine artery - umbilical artery (mm Hg)	Uterine vein - umbilical vein (mm Hg)
99	118.1	13.0
100	135.4	13.0
101	108.7	8.8
101	120.3	14.2
104	74.0	16.0
104	24.0	13.0
105	132.0	15.0
105	127.0	8.0
105	127.2	10.0
105	125.6	21.0
108	63.0	17.0
108	49.0	10.0
111	198.0	19.0
111	214.0	16.0
111	207.0	- 4.0
113	44.0	14.0
114	44.9	5.0
114	8.9	- 4.7
115	178.0	14.0
115	218.0	0

Maternal-foetal arterial and venous differences in oxygen partial pressure were calculated and the results presented in table 3.4. In all cases, the arterial differences were greater than those between maternal and foetal veins. The two instances when umbilical venous P_{O_2} was greater than uterine venous P_{O_2} occurred at 111 and 114 days of pregnancy. Records made during surgery provided no explanation for this occurrence.

D I S C U S S I O N

There was little evidence in the observations of foetal and maternal blood gas tensions throughout gestation to support the notion of countercurrent placental blood flow in the pig. The findings that in the majority of instances, uterine artery - umbilical vein P_{O_2} differences were substantially larger than uterine vein - umbilical artery P_{O_2} , tended to militate against the occurrence of the theoretically more efficient system of relative blood flow (Metcalfe et al., 1967; Bartels, 1970). On the other hand, the results showing the arterio-arterial P_{O_2} difference between mother and foetus to be always significantly larger than the veno-venous difference, lent weight to the suggestion, arising from the anatomical evidence, that the two blood streams largely run a concurrent course (Bartels, 1970; Silver, Steven and Comline, 1973).

The fact that the present series of results were not gained on the unanaesthetised animal raised questions with regard to the influence that inhalation of high concentrations of oxygen would have on uterine and umbilical P_{O_2} levels, and the subsequent conclusions.



The volumes of oxygen supplied to the gilts during anaesthesia were about twice the normal requirement of an unstressed animal, according to the observations of Harris and Cummings (1973).

Lawn, Mills and Prior (1970), and Harris and Cummings (1973) demonstrated that the effect of increasing above normal the availability of oxygen to the maternal animal had a greater effect on the oxygen content of foetal than maternal arterial blood, a result in keeping with the relationship between the partial pressure of oxygen and blood oxygen saturation found in foetal and maternal pig blood (Tweeddale, 1973b; Novy et al., 1973). Moreover, both maternal and foetal blood samples had P_{CO_2} values which were sufficiently high as to suggest that the animals were suffering from moderate degrees of hypercapnia. Lawn et al., (1970) believed that a rise in uterine blood flow would result from maternal hypercapnia, causing a rise in the amount of oxygen going to the foetus without an increase in maternal arterial oxygen content.

In work with the pregnant mare, Comline and Silver (1970) found that there was a steep rise in the foal's umbilical vein blood P_{O_2} when the maternal arterial P_{O_2} was raised, resulting in a reversal of the umbilical

vein - uterine vein gradient. They tentatively suggested that these results confirmed the evidence that there may be a true countercurrent arrangement of the cotyledonary vessels in the mare.

Despite these various factors, of 20 foetuses studied at different stages of gestation, only 3 failed to show umbilical vein P_{O_2} values lower than the corresponding uterine vein results. It may be significant that the 3 foetuses inconsistent with the rest were aged within three days of the expected date of birth.

Other factors, such as the anaesthetic employed, which is known to have effects on the cardiac output and peripheral resistance (Sawyer, Lumb and Stone, 1971), and the trauma and duration of surgery, also raised questions regarding the closeness with which these results approximate to normality. Studies undertaken on the sheep foetus would suggest that complete confidence in the hypothesis of concurrent blood flow could only result as the consequence of a series of investigations on the unstressed animal (Silver et al., 1973). Technical problems remain to be solved before such a situation may be obtained in the pig.

The pattern of relative blood flow is important when the rate of perfusion of the placenta is compared with the placental tissues' diffusing capacity for the molecule under study (Bartels et al., 1962). There would be little or no advantage in a counter-current flow pattern if the mass blood flow was high in proportion to the diffusing capacity of the placenta.

No attempt was made in the present study to quantify uterine blood flow, but Dickson, Bosc, du Mesnil du Buisson and Locatelli (1969) reported studies in which the 4 - amino antipyrine method of Huckabee and Walcott (1960), modified from Kety and Schmidt (1948), was applied to pregnant sows. Mean values of 374 ± 33 ml/min/kg of uterus and tissue contents, and 348 ± 75 ml/min/kg tissue were observed at 75 days and 95 days respectively. However, Dickson et al. (1969) stated that they could not regard these estimates as reliable and that they were likely to be underestimates of true rates of blood flow.

In studies employing artificial placentae, Lawn and McCance (1964) found umbilical blood flows of 100 to 200 ml/min/kg in foetuses of 60 to 85 days gestational

age, although with an earlier device, lower flow rates were found (20 to 50 ml/min/kg).

No other measurements of porcine uterine or foetal blood flow appear to have been reported. Engelhardt (1966) summed up the situation when he noted that knowledge of circulatory physiology of swine was frequently fragmentary with a serious lack of data from conscious undisturbed animals.

Recent studies on conscious sheep produced estimates for uterine blood flow of 200 to 300 ml/min/kg tissue at the end of pregnancy (Huckabee, Crenshaw, Curet and Barron, 1972), and the review of uterine blood flow by Lewis (1969) indicated that blood flow varied between 50 and 300 ml/min/kg according to species. Umbilical blood flow in the sheep had a value of about 220 ml/min/kg of foetal body weight which at term represented 40% of cardiac output (Rudolph and Heyman, 1970, 1974).

Cardiac output estimates have been made on the new born pig by Gruskin, Edelmann and Yuan (1970) who reported a mean value of 885 ml/min/m². In the present study, the surface area of the new born piglet was found to be between 800 and 1200 cm² for body weights ranging between 1.0 and 1.5 kg. These observations, taken together, would suggest that cardiac output in

the piglet at the end of gestation was in the region of 110 ml/min/kg of body weight, low in comparison with the foetal sheep at term; if the relationship between cardiac output and placental flow in the perinatal lamb also holds for the piglet, then only about 50 ml/min/kg would be the estimated umbilical blood flow.

This figure is within the lower range described by Lawn and McCance (1964); however, in view of the number of assumptions made as a basis for carrying out the calculation, little confidence should be put in this estimate. Furthermore, semi-quantitative observations made by the author while sampling foetal umbilical blood, indicated that withdrawal of blood in late pregnancy at a rate of about 60 ml/min was much slower than the delivery of blood to the vessel by the foetus.

There are, therefore, too few observations from which to reach a firm conclusion with respect to blood flow in the placenta of the pig. Such evidence as there is available would suggest that the blood flow to the pregnant uterus of the pig is high when compared with that of the sheep or goat, whereas umbilical blood flows may be similar between the species.

C O N C L U S I O N S

It was entirely appreciated that the conditions under which the present series of results were obtained did not represent the condition of the unstressed maternal or foetal pig. Nevertheless, a number of conclusions may be reached as a consequence of the observations made, namely -

1. The evidence of blood gas tension gradients would lend support to the suggestion that the two circulations flow in concurrent fashion in the placenta.
2. During wide fluctuations in maternal arterial P_{O_2} levels there is relatively little change in foetal P_{O_2} levels.
3. Foetal blood P_{CO_2} levels may differ between foetuses within the same litter.

SUGGESTIONS FOR
FURTHER STUDIES

The pig was highlighted during this study as a species which had been largely unexplored in matters concerned with the transplacental movement of respiratory gases. There were several possibilities whereby slow growth in utero might be explained on the basis of impaired transplacental oxygen supply or carbon dioxide removal. It may, therefore, be suggested that further acute and chronic studies will elucidate the rates of placental transfer of respiratory gases and define the oxygen consumption and carbon dioxide production of the uterine tissue, foetal placenta and foetus itself.

The blood flow in the uterus remains to be measured and the distribution of the uterine blood flow has not been reported in the pig. The quantity of lymph produced in the pregnant uterus may vary at different times during pregnancy and its relationship to uterine arterial blood flow could suggest ways in which blood flow to the uterus can be controlled. The cardiac output and distribution of foetal piglet blood flow remains

to be studied. Variations in placental flow may have detrimental and/or beneficial effects on the foetus. There may occur developmental changes in the control the foetus can exert over the distribution of its cardiac output. There is also the possibility that, through the proper use of drugs given to the mother, man may exert a direct influence over placental blood flow.

4.

THE NUTRIENT - GLUCOSE

Table 4.1 Concentration of glucose in the whole blood of foetal pigs at various ages.

Age of Foetus (d)	Blood Glucose Concentration in Umbilical Artery (mg/100 ml)	Blood Glucose Concentration in Umbilical Vein (mg/100 ml)
82	50.0	49.0
95	40.5	46.0
96	46.5	43.0
110	30.0	42.0
111	41.5	46.5
112	32.0	37.0

Source: Modified after Aherne et al. (1969)

Table 4.2 Concentration of glucose in the blood sera of foetal pigs at various ages.

Age of Foetus (d)	Glucose Concentration in Jugular Venous Blood Serum (mg/100 ml)
85	137 ± 16
92	168 ± 14
99	136 ± 14
106	134 ± 14

Source: Modified after Tumbleson, Hutcheson and Fogg (1970)

I N T R O D U C T I O N

Foetal blood glucose concentration

Early estimations of blood sugar concentration carried out on foetal pigs (Aron 1924) indicated that with a mean value of 139 ± 8 mg/100 ml, they were higher than the maternal value of 100 mg/100 ml recorded. Subsequent work by Goodwin (1956b) and Aherne et al. (1969) has shown that most of the blood sugar estimated by total reducing substances methodology was fructose. Values for blood glucose obtained from foetuses at the end of gestation were given by Goodwin and they are in close agreement with the estimates made near term by Aherne et al. (1969) shown in table 4.1. Neither Goodwin nor Aherne employed the glucose specific oxidase method of sample analysis, and Goodwin (1956b) particularly drew attention to his lack of confidence in the methods then available to him. Tumbleson, Hutcheson and Fogg (1970) did, however, use an enzymatic method to estimate glucose concentrations in serum samples obtained from pig foetuses by jugular puncture at 85, 92, 99 and 106 days' gestation. Their results (table 4.2) are at variance with those reported in earlier work, being three to four times higher.

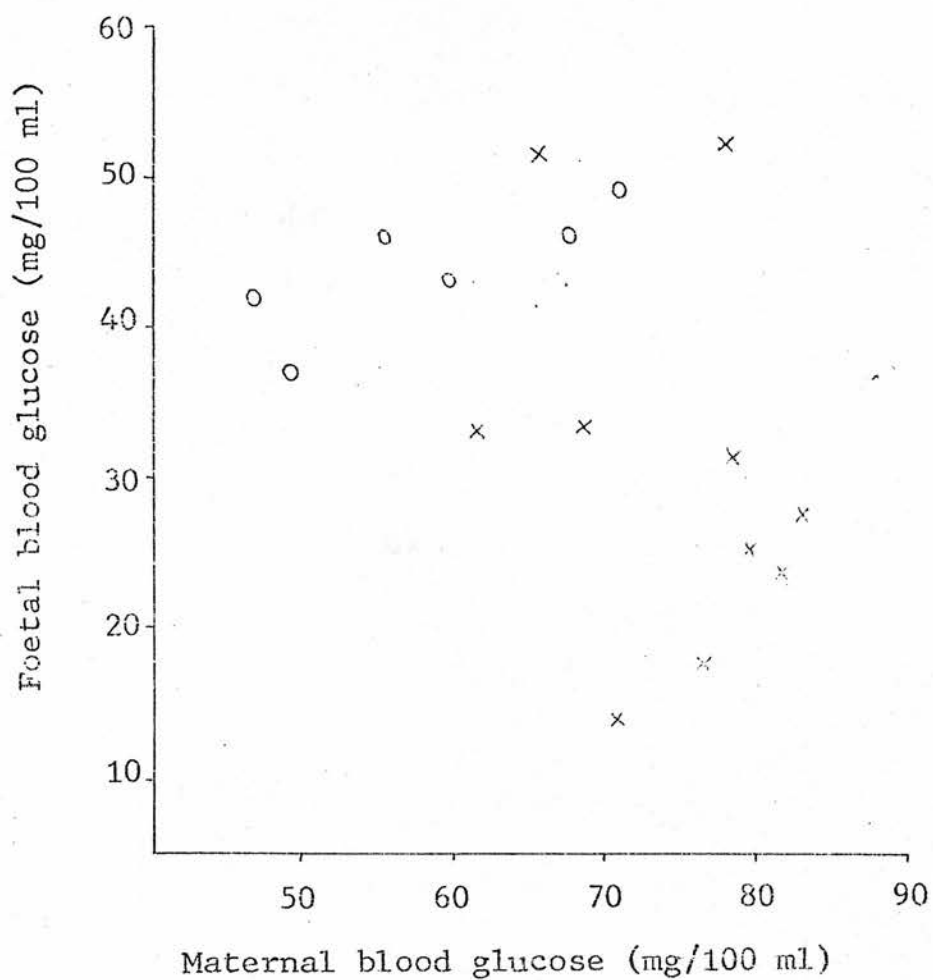


Figure 4.1 Relationships found between foetal and maternal blood glucose according to the results of Aherne et al. (1969) (o), and Wachholz and Wahlstrom (1973) (x).

Maternal blood glucose concentration

Glucose estimations have been carried out on blood from pregnant sows by a large number of workers during studies other than those specifically related to carbohydrate metabolism. Meisters (1938) for example, was reported by von Volker Wege (1967) to have investigated blood glucose levels in 43 sows at the end of pregnancy, and found between 63.0 mg/100 ml and 108 mg/100 ml, with mean glucose concentrations of about 80 mg/100 ml. These levels correspond with the values for serum glucose concentration at 30, 60 and 90 days pregnancy reported by Nachreiner and Ginther(1972).

The few recently published measurements of both maternal and foetal blood glucose concentration were summarised and depicted in figure 4.1. Wachholz and Wahlstrom (1973) obtained blood from 515 fetuses aged either 60 or 95 days, whereas Aherne et al. (1969) studied 12 fetuses of six different ages. The paucity of observations in the latter study and the lack of agreement between the two series of results precluded any firm conclusion concerning the transplacental relationship with respect to glucose concentration.

The author concluded, therefore, that not only have few studies been carried out to examine the blood

glucose concentrations of the foetal pig, but even fewer studies have apparently dealt in depth with the relationship between foetal and maternal blood glucose concentrations.

M A T E R I A L S A N D M E T H O D S

Animals and sampling procedures

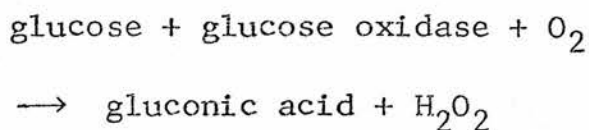
The animals studied and method of blood collection used have been described in the section dealing with blood gases.

Analytical procedures

The 2 ml blood sample drawn for glucose estimation was immediately centrifuged to a maximum acceleration of 14,000 g for 5 minutes (Jobling Microcentrifuge 320). The plasma was separated off, placed in a glass vial on ice until the day's surgery was completed, then stored at -20°C to await biochemical analysis.

The glucose specific glucose oxidase (E.C.1.1.3.4.) method of Huggett and Nixon (1957), automated as described by Trinder (1969), was employed to measure the glucose concentration.

In the reaction



glucose is oxidised to gluconic acid and hydrogen peroxide. The latter product can, in the presence of

peroxidase, oxidise a suitable oxygen acceptor to give colourogenic oxidation products, the colour intensity of which is proportional to the amount of glucose initially present. Phenol in the presence of 4-amino phenazone was used to produce the colour, the optical density of which was measured at 505mm using an 8 mm cell.

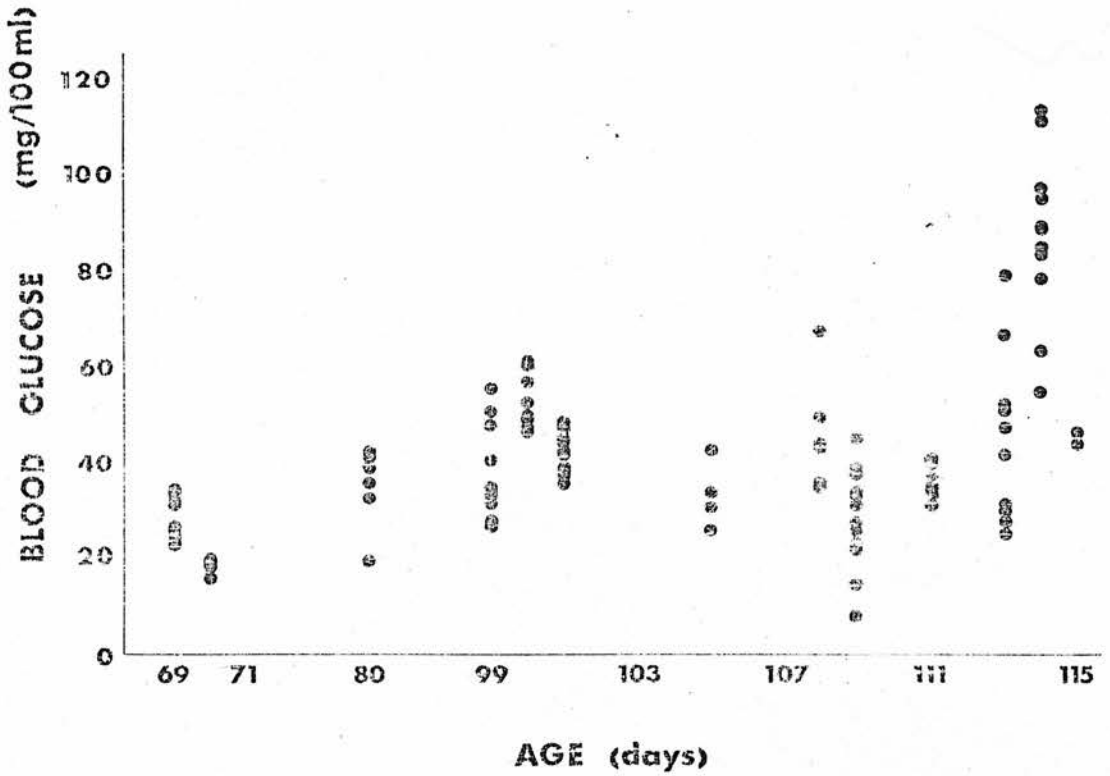
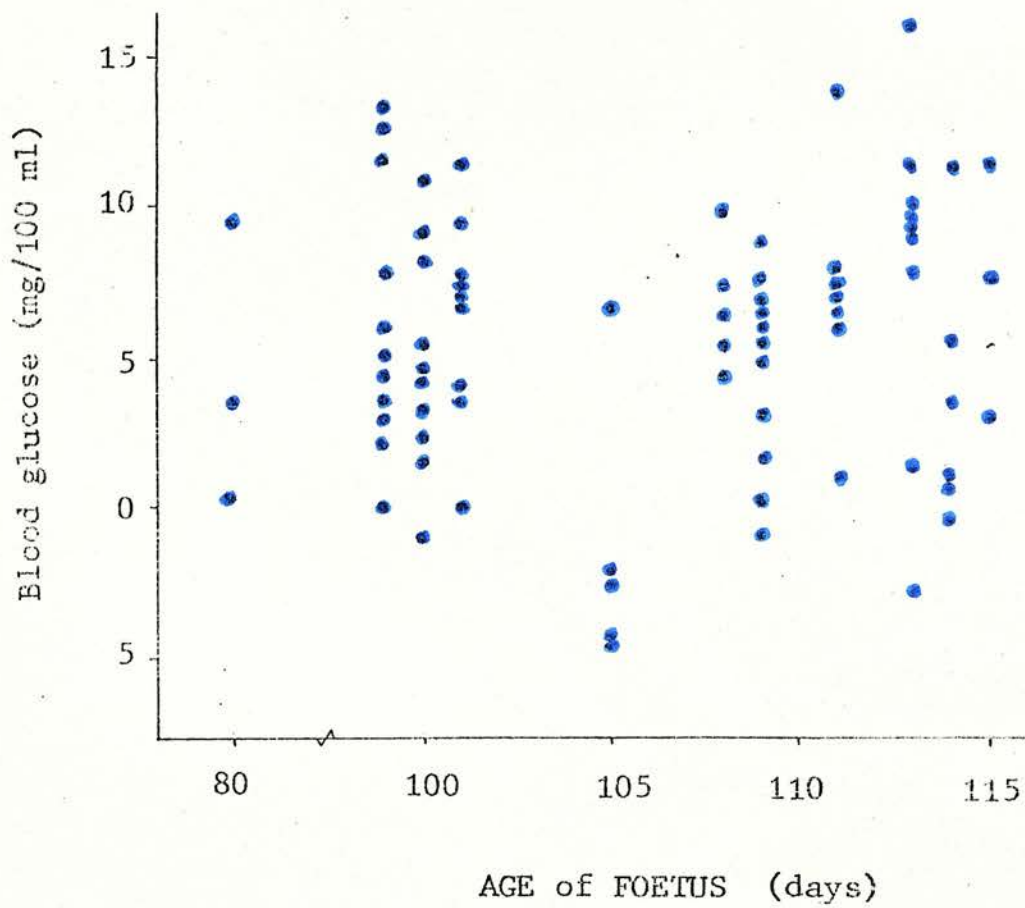


Figure 4.2 Values for glucose concentration measured in blood samples from the umbilical vein of piglets at various stages of gestation.



R E S U L T S

Measurements made on the foetus

Considerable within - and between - litter variation was seen when the results of foetal umbilical vein glucose concentration were plotted against gestational age (figure 4.2). At 69 and 70 days' gestation, less than 35 mg/100 ml of plasma were found. Only at 100, 108, 113 and 114 days were results greater than 60 mg/100 ml found.

When the values for umbilical artery glucose were subtracted from those of umbilical vein and the results plotted against gestational age, there was no clear suggestion of an increasing vein-artery difference as gestation progressed towards parturation (figure 4.3).

The scatter at each age reflected the lack of uniformity found in the values for both umbilical artery and vein. Vein-artery differences close to zero, or negative in value were seen at almost all ages.

Figure 4.3 The glucose concentration differences between umbilical venous and arterial blood at different ages during gestation.

The relationship between foetal size and the arterio-venous difference was investigated because it was expected that a difference in foetal size might influence the potential area of uptake and the rate of utilisation of the glucose molecule (figure 4.4). No clear trend was apparent however.

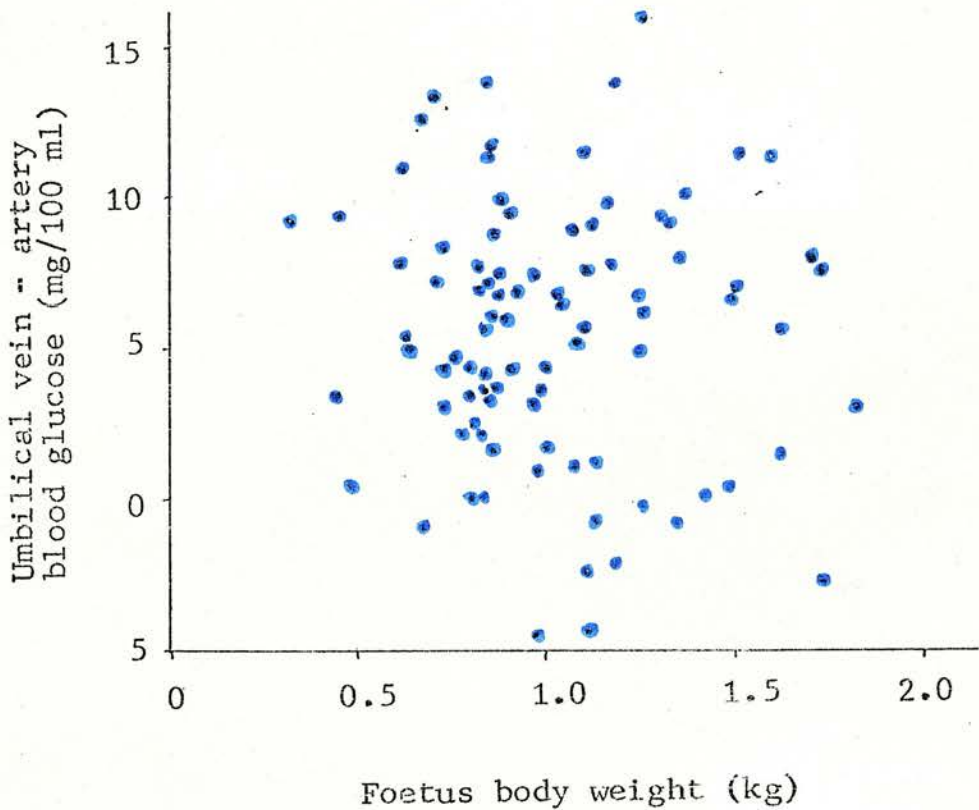


Figure 4.4 The relationship between umbilical veno-arterial difference in glucose concentration and foetal body weight.

Examination of the relationship between umbilical vein glucose and foetal body weight failed to reveal an affinity which was more significant than that due to gestational age.

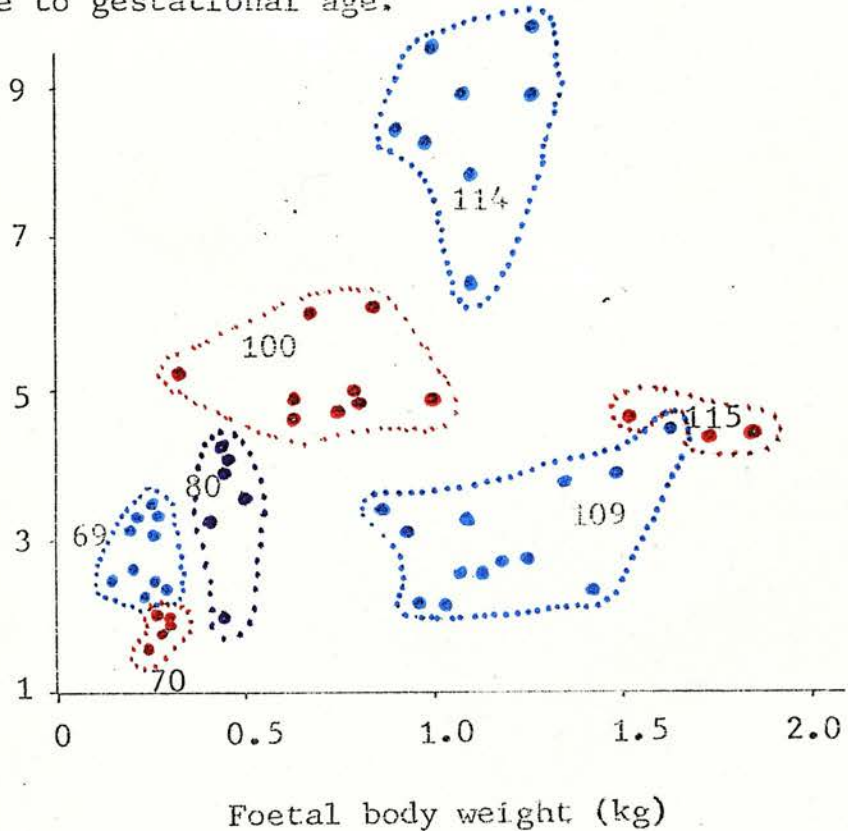


Figure 4.5 Values for umbilical vein glucose in litters of foetal piglets aged 69, 70, 100, 109 and 115 days, related to foetal body weights.

Figure 4.5 illustrates the way in which the individual litter has a greater influence on foetal blood glucose than age. Litters with a wide spread of foetal weights, such as that at 100 days, failed to show anything other than normal distribution about a mean, whereas the samples taken from piglets at 109 days suggested a slight increase in blood glucose level with increasing body weight.

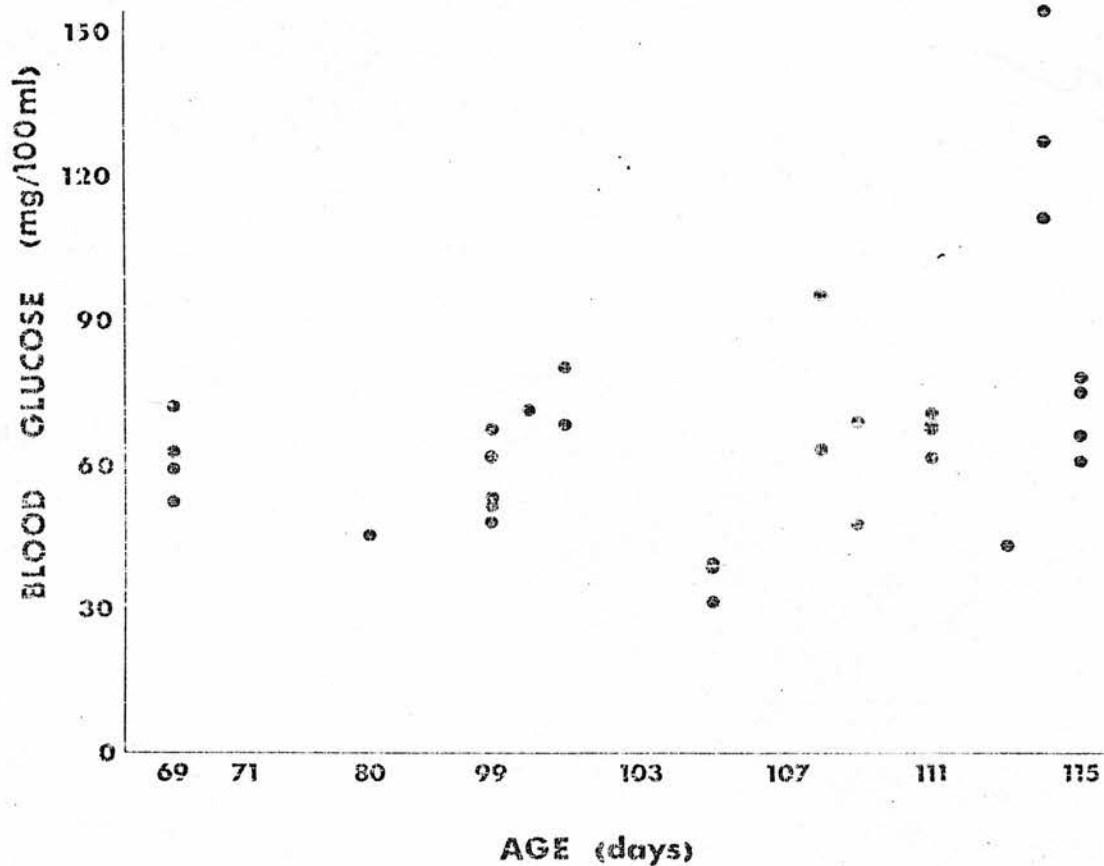


Figure 4.6 Values for glucose concentration measured in blood samples from the uterine artery of different gilts at various stages of pregnancy.

Measurements made on the gilt

When uterine artery blood glucose values were plotted against stage of pregnancy, the pattern illustrated in figure 4.6 demonstrated that there was no apparent trend of increasing or decreasing blood glucose level as the animals approached parturition. Most values lay between 40 and 80 mg/100 ml, the conspicuously different animal being the one sampled on the 114th day of pregnancy. Its blood glucose levels ranged between 110 and 160 mg/100 ml; this particular animal appeared to be about to deliver two foetuses while it lay on the operating table.

Blood glucose relationships between gilt and foetus

The fact that some variation in maternal arterial glucose concentration was present at each stage of pregnancy prompted analysis by plotting the values for each gilt against the time of sampling. No consistent pattern was found. However, foetal results when plotted on the same graphs suggested that during surgery, when maternal levels rose (or fell), foetal values showed a similar trend.

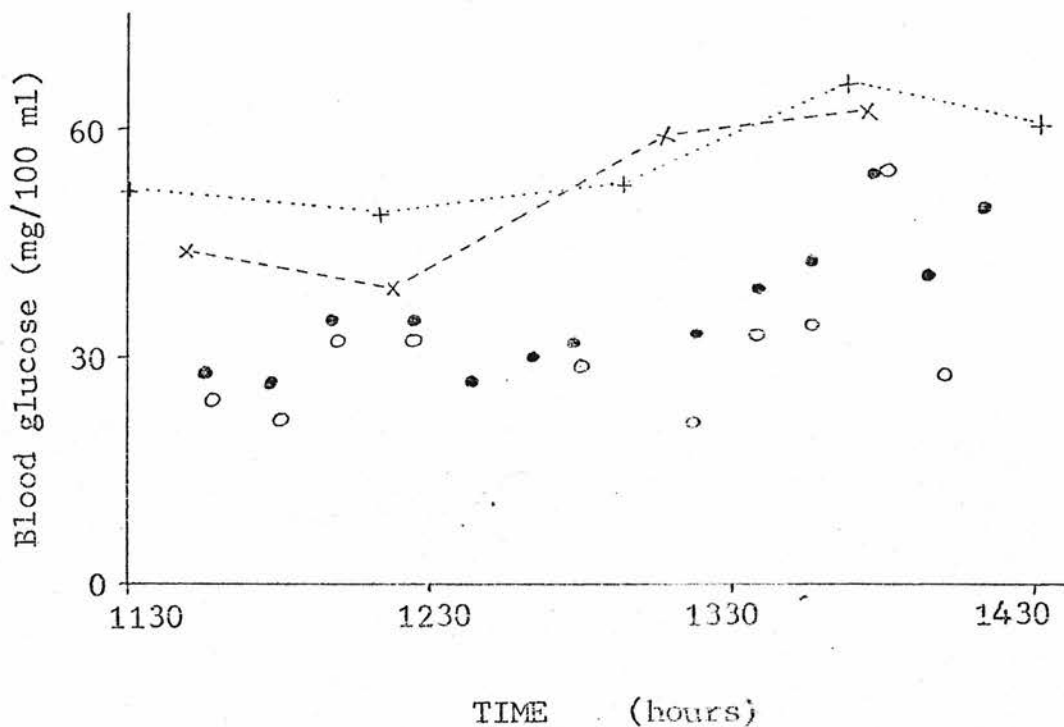


Figure 4.7 Observations on a litter of piglets (99 days). Blood sampled serially from the umbilical artery (o) and umbilical vein (•) together with values for uterine maternal artery (+) and vein (x).

The litters of foetuses studied at 99 (figure 4.7) and 101 days' gestation for example, showed increased blood glucose levels with increasing maternal concentration, and at 108 days' gestation the foetal values declined in parallel with the fall in maternal glucose level.

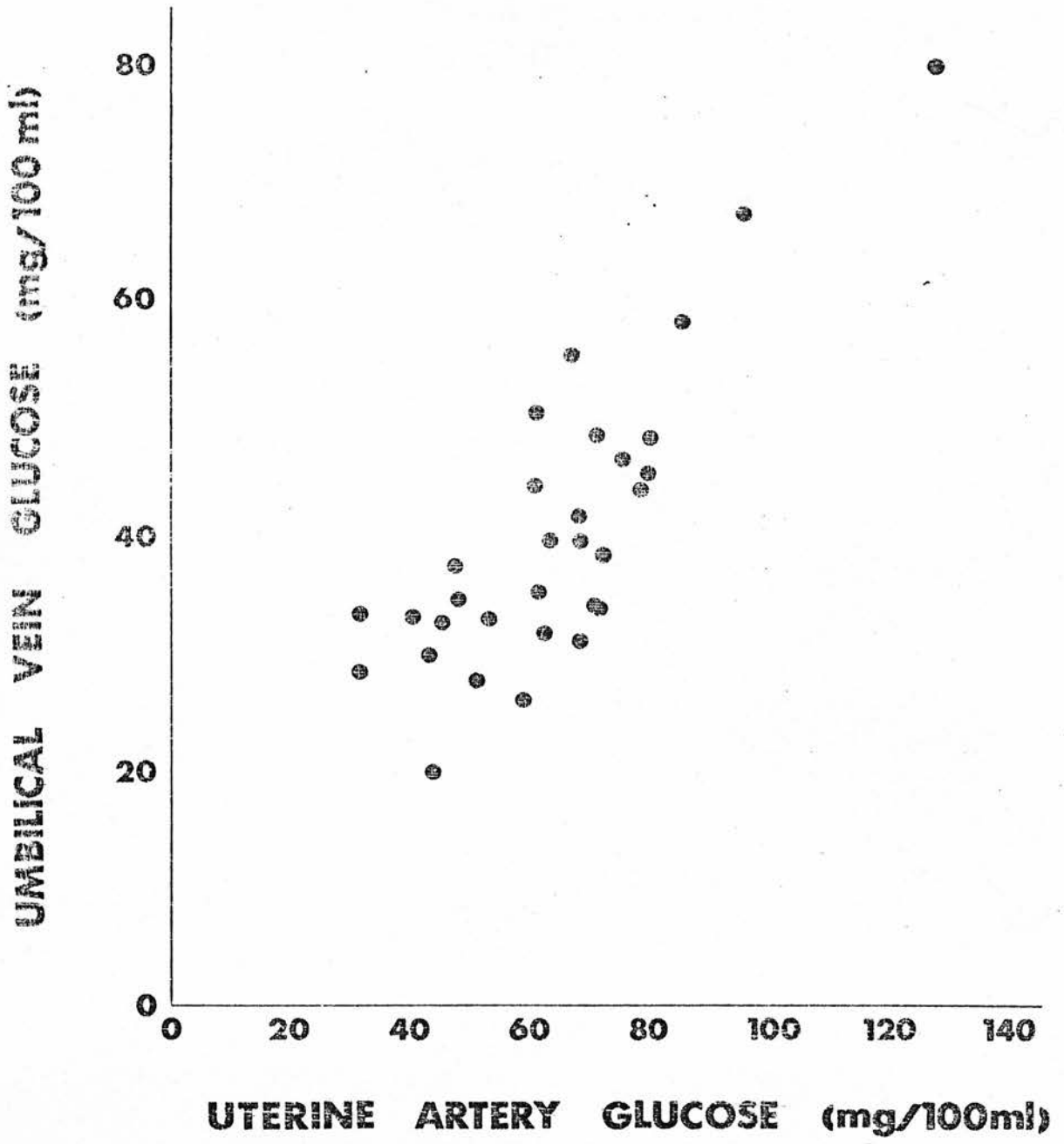


Figure 4.8 The relationship found between pairs of uterine artery and umbilical vein samples with respect to blood glucose concentration.

When the concentrations of glucose in the umbilical vein were plotted against maternal arterial levels (figure 4.8), a regression was calculated which could be described by the linear equation -

$$Y = 13.21 (\pm 6.57) + 0.428 (\pm 0.012) X$$

where Y = umbilical vein glucose concentration

X = uterine artery glucose concentration

(For an analysis of variance see appendix 1)

The difference in plasma glucose concentration between uterine and umbilical arterial blood ranged between 10 and 40 mg/100 ml in the majority of instances (figure 4.9). At each stage of gestation sufficiently wide variation between foetuses was encountered such that no continuous change in the inter-arterial concentration gradient could be detected.

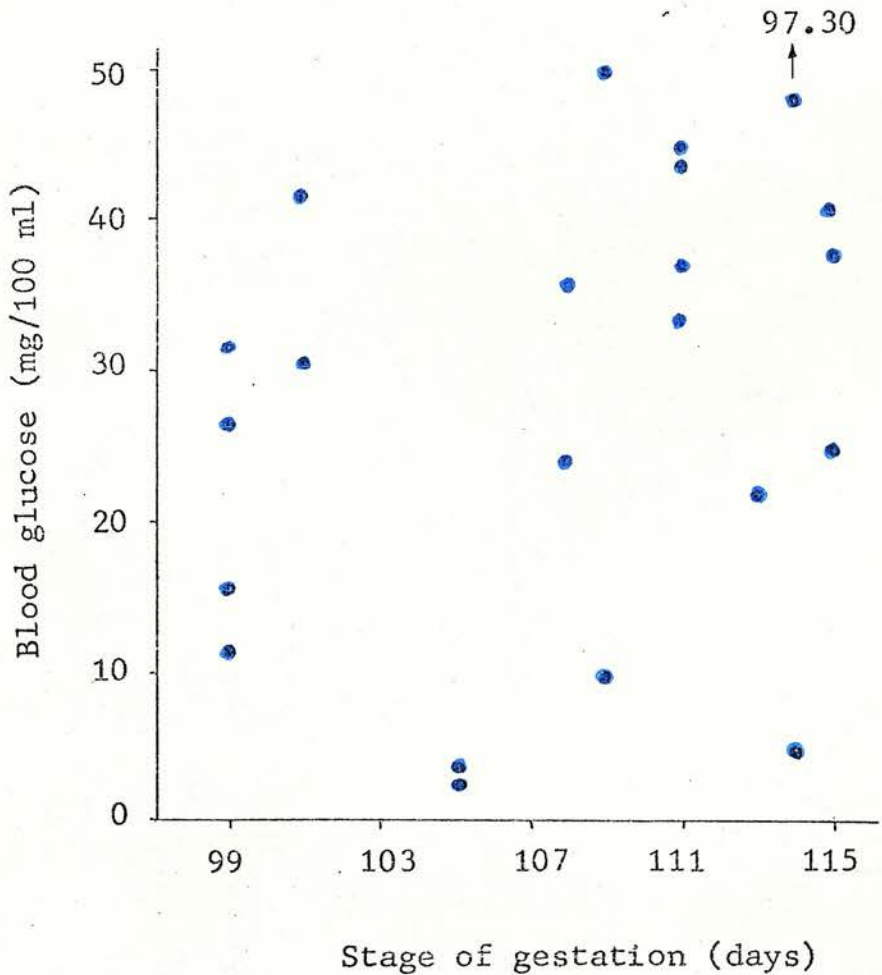


Figure 4.9 The glucose concentration difference between uterine and umbilical arterial blood plasma at different stages of gestation.

Comparable variability was present when the difference between uterine and umbilical venous glucose concentrations were calculated. However, over 80% of the values found were below 30 mg/100 ml, lower than the measured uterine minus umbilical arterial differences. The uterine artery-vein difference in general lay between 1 and 9 mg/100 ml, with several close to zero or negative values found (figure 4.10). No trend towards a higher or lower series of results with stage of gestation was present.

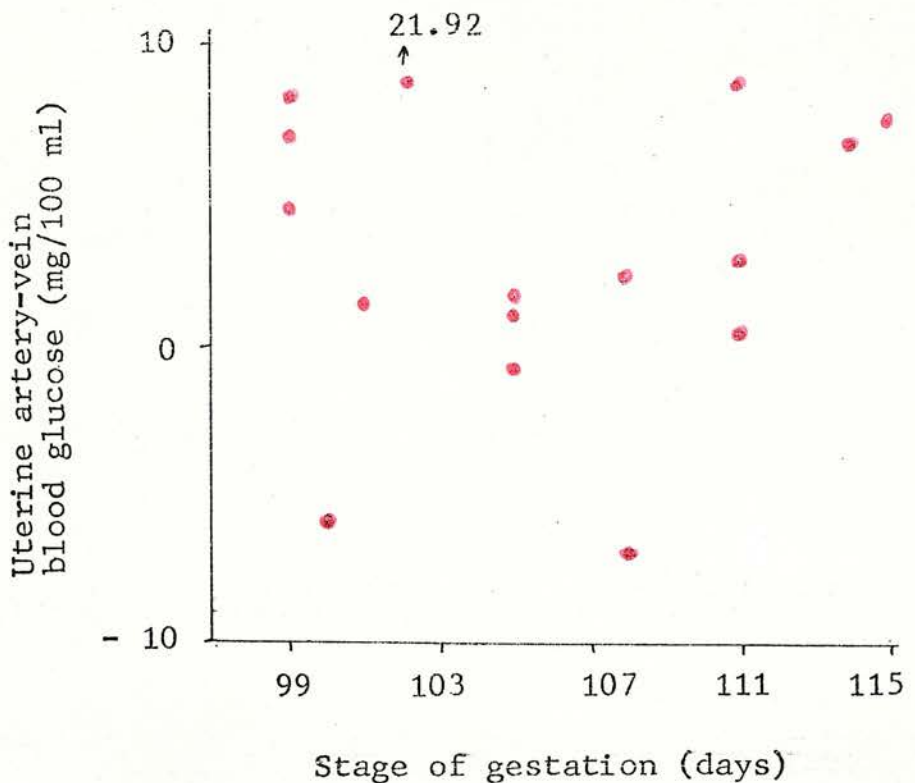


Figure 4.10 The difference in glucose concentration between uterine arterial and venous blood plasma at different stages of gestation.

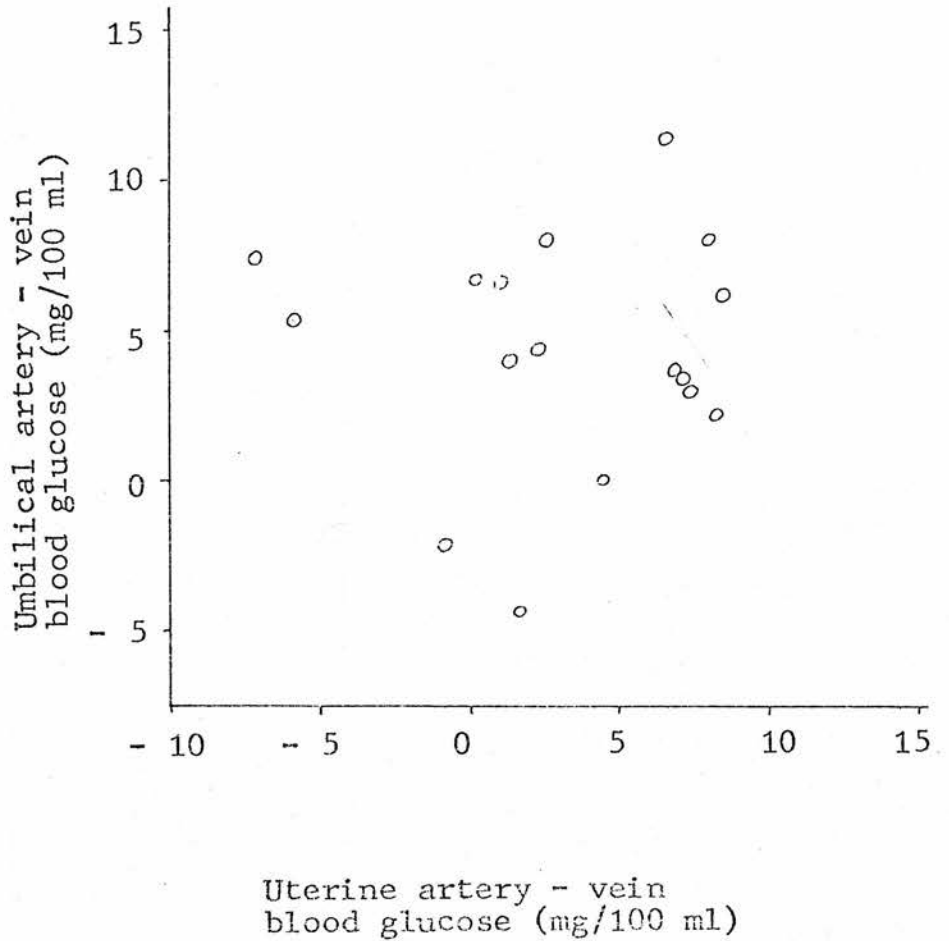


Figure 4.11 The relationship between uterine and umbilical arterio-venous differences in plasma glucose concentration.

When the uterine and umbilical artery-vein glucose results were compared, no correlation was apparent (figure 4.11). Furthermore, there was no evidence to support the view that the foetal difference was consistently greater than the corresponding maternal glucose concentration difference.

D I S C U S S I O N

In steady state conditions, when blood flow and glucose concentration remain stable, the difference in concentration between the umbilical vein and artery would represent not only the amount of glucose transferred across from the maternal tissue, but also the quantity of the carbohydrate monomer being utilised by the foetus. Similarly, the uterine artery - vein difference would reflect the glucose metabolism of the uterine tissues and contents.

Thus, between 1 and 9 mg of glucose were removed from the maternal circulation at the rate of 1% of the uterine blood flow per minute, and between 0 and 12.5 mg were being abstracted by the foetal circulation at the rate of 1% of umbilical blood flow per minute. In some instances the umbilical vein - artery difference was negative and glucose appeared to be being transferred to the uterine tissues.

The fraction of foetal oxygen consumption required to metabolise aerobically the glucose acquired by the foetus may be estimated from the dimensionless glucose/oxygen quotient, defined as :

$$\frac{6 \times \Delta \text{glucose}}{\Delta \text{oxygen}}$$

Δoxygen

where $\Delta \text{glucose}$ = umbilical venous - arterial difference of blood glucose concentration (mmol)

Δoxygen = umbilical venous - arterial difference of blood oxygen content (mmol)

Tsoulos et al. (1971) was the first to employ the ratio to provide quantitative evidence of the role of glucose in lamb fetuses. The formulae of Edwards and Martin(1966) and Novy et al. (1973) were used to estimate the oxygen content of foetal pig blood from the oxygen partial pressures recorded. Details of the calculations are given in appendix 2.

The mean quotient of the 66 fetuses sampled was calculated to be 0.71 ± 0.014 , which would suggest that glucose provided less than three-quarters of the metabolic requirements of foetal pigs under the conditions pertaining to the experiment. Tsoulos et al. (1971) found that at most one-half of the foetal lamb's metabolic requirement was supplied by glucose, although Alexander, Britton and Nixon (1966) concluded from work done on the isolated sheep foetus that glucose was

virtually the only energy source. Battaglia and Meschia (1973) argued, however, on the basis of observations made on unstressed foetuses in utero, that the weight of evidence did not support the view that glucose was the predominant and obligatory metabolic fuel of the foetal lamb. Studies in other species have demonstrated that both injury and anaesthetics can have an influence on energy metabolism (Stoner, 1970; Yoshimura, Kodama and Yoshitake, 1971; Oyama, Takiguchi and Kudo, 1971; Ngai, 1972), which would suggest that caution should be exercised when drawing definite conclusions about the unstressed pig foetus on the basis of the present series of results.

Crenshaw, Cefalo, Schomberg, Curet and Barron (1973) demonstrated on the unstressed sheep foetus that the rate of glucose uptake changed considerably in a single foetus from day to day (from 1.1 to 8.8 mg/kg/min), and daily glucose concentration fluctuations have been reported to range from 5 to 30 mg/100 ml of blood plasma in similar physiological preparations (Comline and Silver, 1970; Silver et al., 1973). It might reasonably be expected therefore, that similar within-foetus variability may occur in the pig.

The present series of results did not include repeated sampling of single foetuses; however, considerable between-piglet within-litter variation was encountered. At 99, 111, 113 and 114 days of gestation the quotient ranged from less than 0.30 to 1.30 or more; less variation occurred at other times. Moreover, an analysis of variance (appendix 3) indicated that the mean value of 0.71 for the glucose/oxygen quotient was obscuring real variation due to a litter or gestational age effect.

These observations suggested that in some foetuses larger quantities of glucose were being removed from the umbilical circulation than could be explained by aerobic metabolism alone. It may be that glucose was being deposited as glycogen, the storage polysaccharide known from work in a number of other species to increase in tissue concentration towards the end of gestation (Shelley, 1961). If this were so, it is conceivable that glycogen was being stored by all the foetuses and that therefore, the glucose/oxygen quotient gave too high an estimate of glucose metabolism. A possible consequence would be that a proportion larger than 30% of the energy metabolism of the pig foetus was being supplied by molecules other than glucose.

C O N C L U S I O N S

On the basis of the present series of results it is concluded that -

1. Foetal umbilical vein glucose concentration is about 43% of the maternal arterial concentration.
2. Foetal blood glucose concentrations rise and fall in accord with maternal levels.
3. Gestational age appeared to have no effect on foetal blood glucose levels.
4. There was no apparent trend of increasing or decreasing maternal blood glucose level as the gilts approached term.
5. Between 1 and 9 mg of glucose was removed from the maternal circulation at the rate of 1% of uterine blood flow per minute.
6. Between 0 and 12.5 mg of glucose was being abstracted by the foetal circulation at the rate of 1% of umbilical blood flow per minute.

7. A proportion larger than 30% of the energy metabolism of the pig foetus was being supplied by molecules other than glucose.

SUGGESTIONS FOR
FURTHER STUDIES

Very little is known about the energy metabolism of the foetal piglet. Studies should, therefore be directed to investigate the role of glucose in the unstressed foetus and the contribution made by amino acids (see appendix 4), free fatty acids and other lipids to energy metabolism. The transfer of these substrates across the placenta of the pig remains to be studied and developmental changes resulting from growth of foetal endocrine autonomy have yet to be defined. The influence of maternal hormones on foetal energy metabolism is poorly understood, and the transfer of hormones across the pig placenta has been little investigated.

5. FOETAL ENERGY STORAGE

I N T R O D U C T I O N

Glycogen

Glycogen has been described by Smith, Taylor and Whelan (1968) as "the most abundant and widespread store of glucose and the most important store of energy in animal systems". Bernard (1855-56, 1859b) was the first to describe glycogen's presence and ubiquitous distribution throughout the tissues and organs of the foetal and postnatal animal. Since that time, although a substantial body of work has been published concerning various aspects of the subject (Cremer, 1902; Pfluger, 1903b; Manners, 1957; Dickens, Randle and Whelan, 1968; Nigam and Cantero, 1972), the tissues of the prenatal animal have been studied relatively infrequently (Creighton, 1896; Shelley, 1961; Dawes and Shelley, 1968). Recently, however, there has been a renewal of interest in the physiology of prenatal life and this has led to the publication of a number of studies on glycogen in foetal tissues.

Glycogen deposition is a suitable model with which to study several aspects of foetal development; it is also an important molecule to species such as the pig where it constitutes the main source of stored

energy for the new born animal (McCance and Widdowson, 1959). The fact that individual foetal tissues and organs accumulate glycogen at different times of gestation (Bernard, 1859b) raises a number of questions with regard to the variation between organs in enzyme development and responsiveness to nervous or endocrine stimuli. There still remains a comparative lack of information describing the patterns of change in organ glycogen content of the pig foetus. It was anticipated, therefore, that more knowledge of glycogen deposition within the foetal organs and tissues might suggest particular times during gestation which were of developmental importance to the growing piglet.

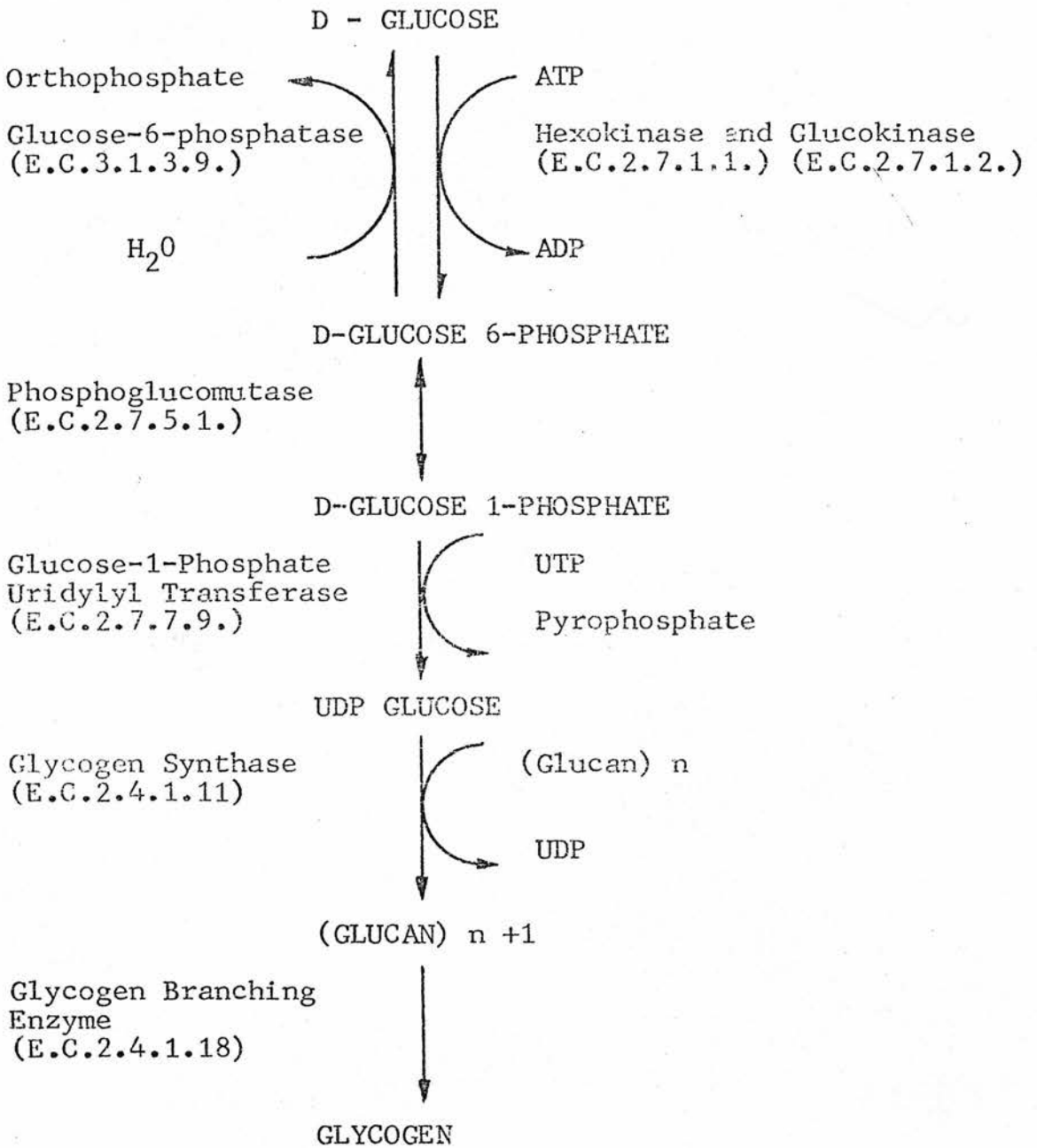


Figure 5.2 A summary map of glycogen metabolism
(modified after Ryman and Whelan, 1971).

Glycogen synthesis

Glycogen is a multi-branched polysaccharide made up of D-glucose residues linked by α - 1, 4 - glycosidic bonds to form long chains which are branched by the formation of α - 1, 6 - linkages (figure 5.1).

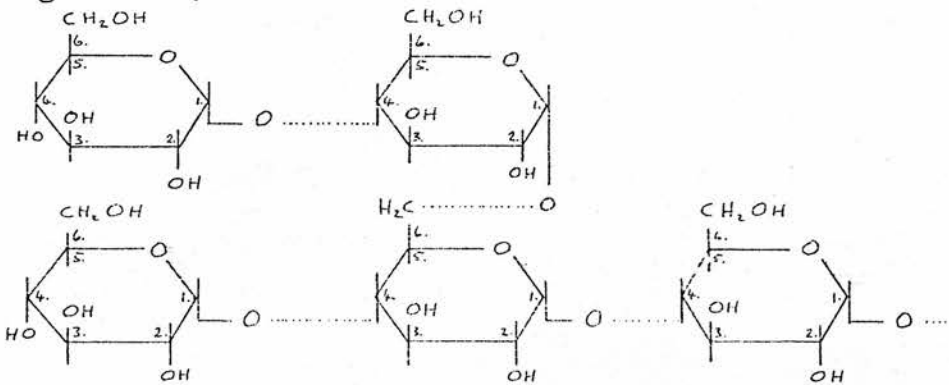


Figure 5.1 The secondary structure of glycogen.

The "Summary map" of glycogen synthesis, published by Ryman and Whelan (1971) is shown in modified form in figure 5.2. Glucose is phosphorylated by hexokinase (E.C.2.7.1.1.) and/or glucokinase (E.C.2.7.1.2.) to glucose-6-phosphate, the phosphate group then being transferred from carbon-6 to carbon-1 by the action of phosphoglucomutase (E.C.2.7.5.1.) to form glucose-1-phosphate. The reaction in which uridine diphosphate-glucose is formed from uridine triphosphate and glucose-1-

phosphate is catalysed by Glucose-1-phosphate Uridyl Transferase (E.C.2.7.7.9.). The enzyme glycogen synthase (E.C.2.4.1.11) catalyses the transfer of the glucose residue to the end of an outer chain in the glycogen molecule. The dendritic structure of the molecule (figure 5.3) is brought about by a second enzyme, Glycogen Branching Enzyme (E.C.2.4.1.18) which transfers small fragments from (1--4) linkage to (1--6) linkages in the same or different polyglucan chain.

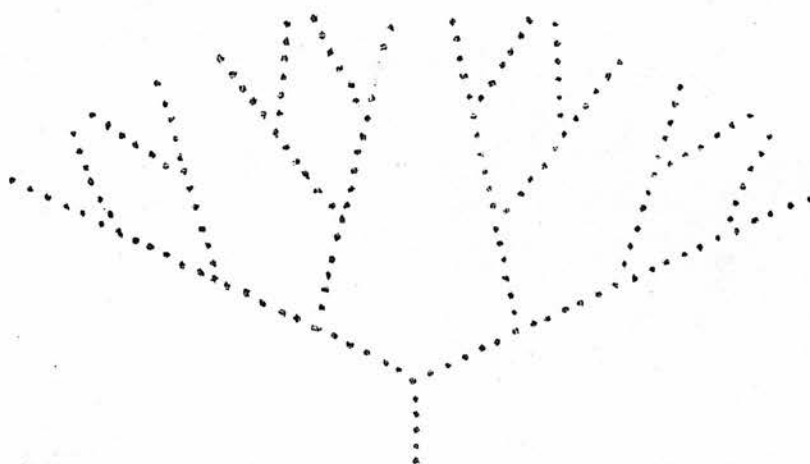


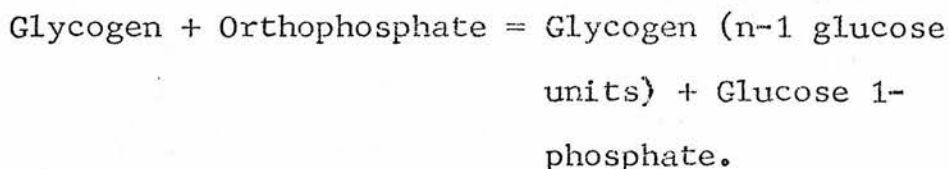
Figure 5.3 A schematic representation of a small segment of the glycogen molecule. (After Smith et al., 1968).

Meyer and Fuld (1941) originally proposed this configuration (later confirmed by the work of Lerner, Illingworth, Cori and Cori, 1952), the structure of which enables the molecule to grow into a spherical shape with an upper molecular weight limit of several

millions. In such a form it possesses several advantages as a storage molecule; not the least of these is the significant contribution made to intracellular osmotic pressure, and as a highly branched, soluble polysaccharide, its accessibility to rapid enzymatic degradation (Smith et al., 1968).

Glycogen degradation

Glycogen can be rapidly mobilised by a combination of enzymes, the key step in this process being the production of α - glucose 1 - phosphate from glycogen by the action of Glycogen Phosphorylase (E.C.2.4.1.1.):



The enzyme is specific for α 1 \rightarrow 4 linked glucose units and its action ceases as it approaches the outer tier of α 1 \rightarrow 6 branch points (Smith et al., 1968). Manners (1968) has shown that debranching is carried out by an indirect method in which the outer chains are rearranged with the subsequent release of free glucose (Ryman and Whelan, 1971).

Liver Glycogen

Glycogen depots form in liver tissue at different stages of gestation in different types of animal. Species with a relatively long gestation period, such as sheep (147 days), rhesus monkey (168 days) and man (280 days) all begin to accumulate liver glycogen at or about mid-gestation (Shelley, 1961); work on animals with short gestations - Corey (1935) and Jacquot (1959) on the rat (22 days), Burton, Greenall and Turnell (1970) on the mouse (24 days), Lochhead and Cramer (1908) and Jost and Jacquot (1955) on the rabbit (31 to 32 days) and Nemeth, Insull and Flexner (1953) and Kornfeld and Brown (1963) on the guinea pig (68 days) - produced results which showed an almost coincident pattern of hepatic glycogen accumulation beginning late in gestation (Shelley, 1961). The few observations made a number of years ago on the pig (114 days) suggested that glycogen might be deposited in a pattern similar to animals of "short" gestation interval. (Pfluger, 1903a; Gierke, 1905; Lubarsch, 1906; Mendel and Leavenworth, 1907; Aron, 1922a).

More recently published information, pertaining to pig foetuses in the last 24 days of gestation, is

Table 5.1 Published estimates of the proportion of glycogen in the liver of the pig foetus during the last 24 days of gestation.

Age of Foetus	90	94	95	104	109	110	114	Reference
Liver glycogen (g/100g)	1.5						9.2	Nocart (1972)
		2.8		8.1			10.9	Padalikova et al. (1972)
				2.0	9.5			Swiatek et al. (1970)
						6.6		Itoh and Hansard (1966)
			0.6			6.7		Zhivkova (1968)
				11.0		25.0		Mersmann et al. (1972)

shown in table 5.1. There is considerable lack of agreement between groups of investigators concerning the proportion of glycogen present at any one time; at 104 days estimates vary between 2 and 11g/100g, whereas between 6.6 and 25g/100g were reportedly present at 110 days.

A similar state of disagreement may be found among the results of work on postnatal piglets. For example, liver glycogen content at birth has been variously reported to be -

- | | |
|---------------------|----------------------------------------------------|
| 26.2 and 24.2g/100g | (Mersmann, Phinney, Mueller and Stanton, 1972) |
| 22.5g/100g | (Mersmann and Houk, 1971). |
| 18.7 to 21.7g/100g | (Seerley, Pace, Foley and Scarth, 1974). |
| 15.0g/100g | (Swiatek, Chao, Chao, Cornblath and Tildon, 1970). |
| 10.9g/100g | (Padalikova, Holub and Jezkova, 1972). |
| 8.5g/100g | (McCance and Widdowson, 1959). |
| 5.2g/100g | (Morrill, 1952a) and |
| 3.4g/100g | (Elneil and McCance, 1965). |

Despite the lack of quantitative agreement between the estimates made by the different groups of workers,

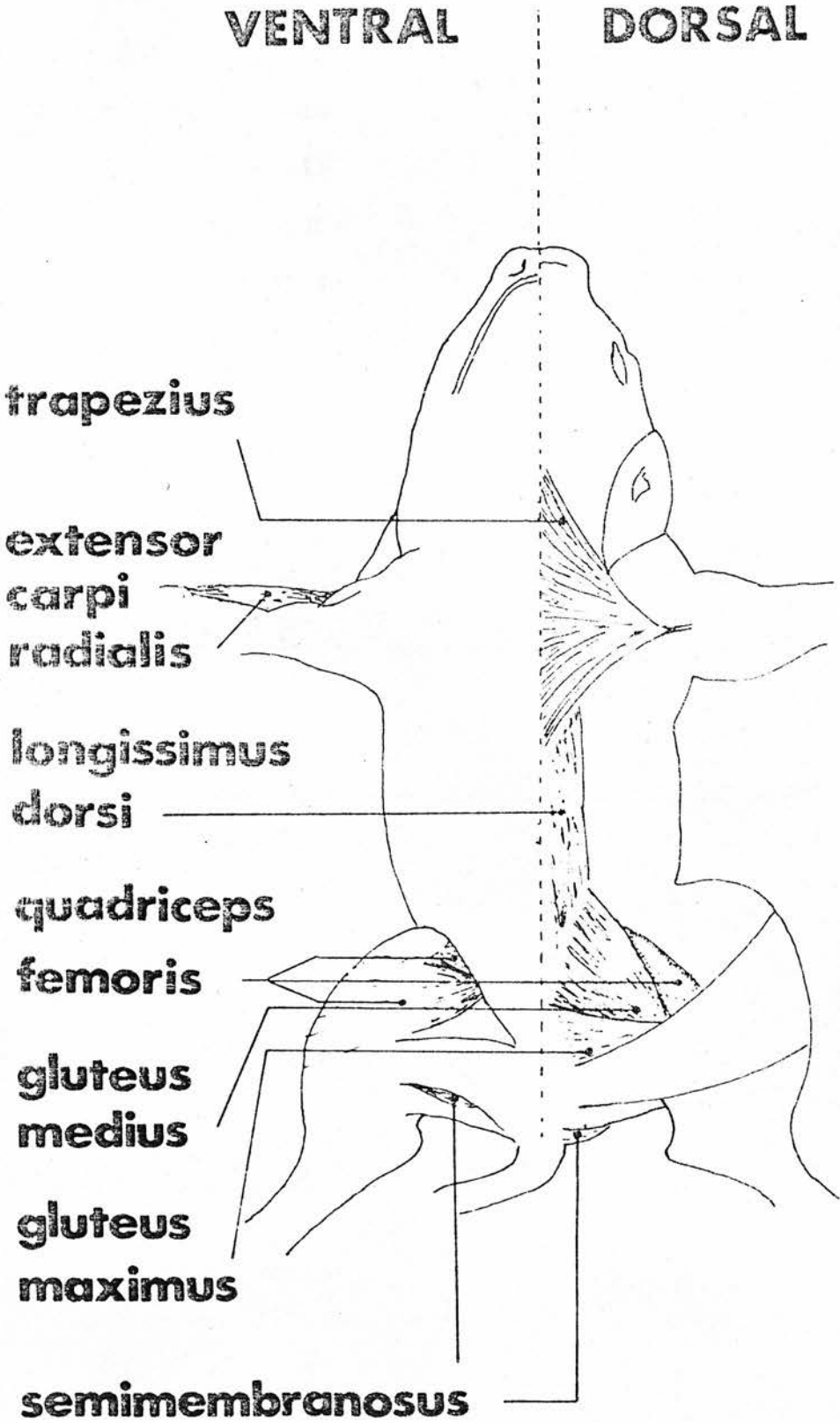


Figure 5.4 A diagrammatic representation of the anatomical location of a number of muscles in the pig foetus. (Adapted from Stuart and Pinder, 1948 and Odlaug, 1969).

they supported early suggestive evidence that liver glycogen accumulated late in gestation. Nevertheless, the inconsistencies in the published data thwarted any attempt to estimate with confidence the amount of energy stored in the liver during gestation or mobilised following birth.

Skeletal Muscle glycogen

Estimates of skeletal muscle glycogen made on foetal rat, rabbit, guinea pig, sheep, monkey and man would suggest that there is no inter species - group difference in glycogen accumulation patterns, in contrast to the situation found in liver tissues (Dawes and Shelley, 1968). Until recently, very few observations had been made on prenatal glycogen deposition in the muscle of the piglet.

The presence of glycogen had been detected by Gierke (1905) in the musculature of pig embryos 1.5 cm (~ 25 days) and 5.0 cm (~ 40 days) in length. However, Swatland and Cassens (1973) made no mention of glycogen with respect to muscle from fetuses 7 to 18 cm in size (~ 50 to 75 days) despite reporting that the polymer could be seen in histochemical sections of longissimus dorsi muscle (figure 5.4) of piglets larger than 21 cm (from ~ 80 days to term).

An early attempt to quantify the glycogen content of foetal muscles during gestation was made by Mendel and Leavenworth (1907). They analysed the decapitated, eviscerated carcasses of foetuses ranging in size from 6.2 to 21.5 cm (from ~ 45 to 85 days) and obtained results which suggested that there was a fairly constant composition of 0.5g/100g glycogen, although 1.1g/100g was found in the two largest foetuses. Three values of about 1.1g/100g, representing piglets aged between 50 and 65 days of gestation, were published by Dawes and Shelley (1968) and Widdowson (1971) estimated that 3g/100g was the glycogen content of quadriceps femoris muscles (figure 5.4) from a group of pigs aged 91 to 96 days. Not until the recently published work of Padalikova et al. (1972) has there been any published study which sought to describe the accumulation of glycogen in the skeletal musculature of the foetal pig at specified times during gestation. Their results revealed that there was a six-fold increase in muscle glycogen content from 1.4g/100g at 74 days to 8.5g/100g on day 114; they also suggested that there might be a change in the rate at which glycogen was being laid down, at around 94 to 104 days. A number of workers have reported differing values for skeletal muscle glycogen content at birth;

Curtis, Heidenreich and Foley (1966) found 8.0g/100g in gluteus medius muscle and 7.2g/100g in longissimus dorsi muscle, whereas Dalrymple, Kastenschmidt and Cassens (1973) recorded lower quantities in semi-membranosus (6.2g/100g) and trapezius (5.2g/100g) muscles (figure 5.4). McCance and Widdowson (1959) did not specify the muscle or group of muscles analysed when they stated that 7.2g/100g was the concentration of glycogen found in the new born piglet; a report from the same group later gave a value of 6.06g/100g, without any reference to anatomical location (Elneil and McCance, 1965).

The variance among the results published could conceivably be explained on the count that they represent the glycogen content of different areas of the musculature. The values given by Padalikova et al. (1972) were high in comparison with the concentrations of glycogen found by other workers. If the figure of 6.8g/100g for the carbohydrate content of minced "boneless skeletal tissue" from whole carcasses of new born piglets (Brooks, Fontenot, Vipperman, Thomas and Graham, 1964) truly represents the mean percentage content of muscle glycogen, then it is unlikely that

the pattern of glycogen deposition in the quadriceps femoris muscle will adequately represent the pattern in the musculature as a whole. It would, as a consequence, fail to provide a good estimate of the quantity of energy being stored in muscle during gestation.

No convincing estimates appear to have been published which seek to describe the accumulation of glycogen in foetal pig musculature and quantify its role 'as a local supply of reserve material for the furnishing of muscular energy' (Starling, 1900).

Lung glycogen

Glycogen in foetal lung tissue was first described by Bernard in the course of a series of lectures given in 1855 - 56 (Creighton, 1896); the entire lung of a small lamb embryo had been placed in 'an acidified tincture of iodine' with the result that the bronchial tree had become wine red in colour, an indication of the presence of 'a sort of animal starch' (glycogen), whereas the surrounding gelatinous mass remained colourless. Some time later Creighton (1896) recognised in calf foetuses that it was the columnar epithelial cells of

the bronchi and terminal bronchioles which contained glycogen; subsequently, the carbohydrate polymer was also found in the epithelial cells of the lung, trachea and nose of the pig embryo (Gierke, 1905; Gage, 1917).

In a review of a number of quantitative studies, Shelley (1961) was able to show that although only relatively small concentrations of glycogen were present in the lungs of various species early in gestation, these amounts increased to peak values after mid-gestation, and subsequently fell to near adult levels before birth; the fall in glycogen concentration was associated with the loss of the glycogen-rich epithelial tissue from the foetal lung (Faure-Fremiet and Dragoiu, 1923). The cellular development of the pig lung would seem to be closely analogous to that found in other mammalian species (Flint, 1906; Clements, 1938; Krastev and Vitanov, 1967), and Parhon and Milcou (1938) identified a similar glycogen pattern in foetal pig lung. Nevertheless, recent observations made on the glycogen concentration of foetal pig lung during the second half of gestation revealed non-significant variation about a mean value of 2.4g/100g (Padalikova et al. 1972).

This discrepancy between the pig and other species deserved reinvestigation, particularly since changes are believed to occur in the endocrine status of the foetal pig towards the end of gestation (Aron, 1922b; Marchut, 1971; Lockwood and Misbin, 1972; Dvorak, 1972, 1973) which would be consistent with the hormone induced variation in lung glycogen concentration found in other species (Kikkawa, Kaibara, Motoyama, Orzalesi and Cook, 1971; Alescio and Dani, 1972).

Kidney glycogen

The parallelism between the development of bronchial and renal tubular systems was remarked on by von Kolliker (1879) and found to be particularly well demonstrated when following the course of glycogen deposition in each (Creighton, 1896). Bernard's (1859b) observation that glycogen was confined to the collecting elements of the kidney (the collecting ducts, renal pelvis and ureter) received support from the work of Creighton (1896) and Rothenberg and Swartz (1957). Very few quantitative measurements have been made of prenatal renal glycogen; Shelley (1960) reported 0.28 to 0.58g/100g in foetal lambs, which was slightly higher than the values found by Vिलlee (1953a) in human

foetal kidney cortex but within the range for guinea pig foetuses (Shelley, 1961). Hinde (1949) reported 0.57g/100g in new born rabbits.

No studies of a similar nature appear to have been carried out on the pig foetus, although Kunska (1971) has reported that during the first half of gestation large amounts of glycogen could be detected histochemically in the epithelium of the renal collecting ducts of pig foetuses over 5 cm in length.

Heart Glycogen

Glycogen in the cardiac muscle of pig embryos 0.8 to 1.6 cm in length was reported by Gage (1917) to be so abundant that it made the histological sections almost opaque. In older foetuses 7 cm in size, however, less glycogen was found, an observation consistent with the inverse relationship between glycogen and maturity which Shelley (1961) reported in other species. Support for such a notion came when quantitative studies on the pig foetus produced results which suggested a decline in cardiac muscle of glycogen concentration from 2.6g/100g on day 74 of gestation to 2.0g/100g by day 94. (Padalikova et al., 1972). In contrast with other species however, the fall in glycogen content did not continue

to term; almost twice as much glycogen (0.17g) was present on day 104 as could be detected (0.09g) on day 94, which represented a highly significant increase ($P < 0.001$) from 2.0 to 3.0g/100g (Padalikova et al., 1972). The concentration of glycogen in the heart at term varies between species, with around 4g/100g in man, 2.5 to 3.0g/100g in rat and 1.0 to 2.0g/100g in rabbit, lamb and monkey (Dawes and Shelley, 1968). McCance and Widdowson (1959) found 1.5g/100g in the heart of the new born piglet, but a subsequent publication from the same laboratory (Elneil and McCance, 1965) reported finding only 0.34g/100g, a glycogen concentration similar to the composition of the new born guinea-pig heart (Dawes and Shelley, 1968). The paucity of information on cardiac muscle glycogen in the foetal and neonatal pig is surprising in view of the close linear relationship found between the cardiac carbohydrate concentration at birth and the ability of the new born animal of other species to survive anoxia (Dawes, Mott and Shelley, 1959; Mott, 1961).

Table 5.2 The number of foetal piglets studied and the stage of gestation at which observations were made.

Litter identification	Number of foetuses in litter	Days after mating
1	12	69
2	7	70
3	12	80
4	13	99
5	11	100
6	11	101
7	13	~ 101
8	10	104
9	6	105
10	6	106
11	15	108
12	14	109
13	7	111
14	11	113
15	15	114
16	4	115

Table 5.3 The number of neonatal piglets studied and the ages at which observations were made.

Litter identification	Number of piglets in litter	Hours after birth
17	11	0
18	12	0
19	11	0
20	13	6
21	8	24
22	12	36
23	10	120

M A T E R I A L S A N D M E T H O D S

Animals

Observations were made on 167 foetal piglets from seventeen litters and 77 neonatal piglets from seven litters, described with respect to age in tables 5.2 and 5.3. The animals were from a population of pregnant gilts similar to those employed in earlier studies. Each gilt was checked daily for signs of oestrus, by parading the boar round the pens; mating took place on the day following the first signs of behavioural oestrus. The stage of gestation and foetal age were estimated by designating the date of mating as 'day 0' and calculating from then to the date on which the observations were made. Eight to twelve weeks after mating, the ultrasonic 'Doppler Foetometer' (Centaur, Edinburgh) was used to detect foetal flow, thereby confirming less objective visual diagnoses of pregnancy.

Housing

Six to ten gilts were loose housed in groups on straw bedding in pens providing around 2.5 m^2 of floor area per animal. Each group of pigs had access to a

Table 5.4 The composition of the diet fed to experimental gilts during pregnancy and for the first seven days following parturition.

<u>Component</u>	<u>kg/100 kg</u>
Ground barley meal	64.5
Ground wheat	13.7
Wheatings	9.1
White-fish meal	2.2
Extracted soya bean meal	2.2
Molasses	4.6
Limestone	1.69
Dicalcium phosphate	1.69
Mineral-vitamin mixture *	0.47

* Constituent quantities per kilogram of mineral-vitamin mixture.

Vitamin A	(g)	133.20
Vitamin D ₃	(mg)	22.25
Vitamin B ₂	(g)	1.78
Vitamin B ₁₂	(mg)	4.44
Vitamin E	(g)	3.11
Vitamin K	(g)	0.89
Nicotinic acid	(g)	5.33
Pantothenic acid	(g)	4.44
Iron	(g)	44.44
Cobalt	(g)	0.44
Manganese	(g)	17.78
Copper	(g)	4.44
Zinc	(g)	31.11
Iodine	(g)	1.33

larger area of open ground twice daily, to facilitate oestrous detection and permit feeding. Animals were moved from the holding pens between three and five days before surgery, or no less than seven days before expected parturition, weighed, and transferred to the indoor pre-operative penning facility adjacent to the operating theatre. Individual pens, 2 m² in size accommodated the animals, with former 'group pen mates' being placed in pens adjacent to one another, where possible, to minimise stress. After surgery, the hysterectomised animals recovered in pens with 4 m² of floor area.

Feeding régime

The gilts were reared to puberty according to normal commercial practice and then gradually changed onto a cubed diet formulated to contain 17.8 MJ/kg gross energy with 13% crude protein in the dry matter. The composition of the diet is given in table 5.4. Each gilt was individually fed 2.1 kg of the diet throughout pregnancy and for the first week post partum. Water was given ad libitum at the time of feeding.

Surgical procedures

Food and water were withheld for twenty-four hours before the operation. The animal was snubbed, and anaesthesia induced by intravenous injection, through an ear vein catheter, of sodium pentobarbitone (Nembutal, Abbot Laboratories Ltd., 60 mg/ml) to a depth which permitted intubation; a 9.5 mm cuffed McGill tube was used, placement in the trachea being facilitated by a Rowson (1965) pattern laryngoscope. All animals were premedicated with atropine sulphate (Evans Medical Ltd., 3000 μ g) to control salivation. General narcosis was maintained by the administration of an oxygen/nitrous oxide / halothane (Fluothane, I.C.I. Ltd.) anaesthetic regime via a partial rebreathing circuit with carbon dioxide absorption. Halothane was supplied at 1 to 2.5% concentration in a gas flow of 1500 ml/min in which the ratio of oxygen to nitrous oxide was 2:1.

Respiration rates of 10 to 12 breaths/min were recorded, with between 4.5 and 8.0 litres/min of mixed gases being inspired. Blood pressures were not recorded, but heart rates lay between 90 and 120 beats per minute. The animal was placed on its back on the



operating table and its legs restrained with ties. The abdomen was washed first with soap and water and then with either 70% alcohol or a solution of chlorhexidine gluconate (Hibitane, I.C.I. Ltd.) in alcohol. The site of the operation was covered with tincture of iodine and 0.1% w/v tincture methiolate (Thiomersal, Eli Lilly & Co. Ltd.). The animal was draped with sterile cloths as shown in figure 5.5.

The uterus was exposed by a midline incision and the ovarian end of one uterine horn identified. The loculus in this position was eased out of the abdominal cavity and the foetus removed through an incision made in the uterus, allanto-chorion and amnion. The foetus was sexed and weighed; the liver, heart, lungs, left and right kidney, and right extensor carpi radialis muscle rapidly removed, weighed and plunged into liquid nitrogen. The time at which each organ or tissue was obtained was recorded. The foetus adjacent to the one sampled was next treated similarly and the remainder in the uterine horn in like manner. The same procedure was followed for the contralateral uterine horn. Foetal organs and tissues were stored at -20°C until tissue digestion and analysis for glycogen content. Details of anaesthetic and surgical procedures are contained in appendix 5.

Figure 5.5 A general view of the surgical preparation to remove pig foetuses under general anaesthesia.

New born animals

Each gilt was observed from day 113 of gestation, and the precise time at which each piglet was delivered was recorded. Piglets were removed from the gilt at birth, and if the litter had been assigned to a time of sampling other than at delivery, they were collected in an enclosed warm (20°C) environment until the last piglet had been delivered; they were returned to the gilt together. Piglets were killed as close to the assigned time of sampling as possible. Thus new born pigs were studied immediately each individual was born, and similarly 6, 24, 36 and 120 hour old piglets were killed as individuals exactly 6, 24, 36 or 120 hours following delivery. Tissue collection procedures were as described for foetal piglets.

Tissue digestion

The method used for the digestion of the tissues and subsequent separation of glycogen was based upon the techniques of Bernard (1857) and Pfluger (1902) modified by Good, Kramer and Somogyi (1933).

The whole organ or tissue sampled was placed into a boiling tube containing hot 30% potassium hydroxide

solution (2 ml/g of tissue), and heating was continued in a boiling water bath for 25 minutes. The tubes were removed from the bath, and after the contents had cooled to room temperature, the volume of solution was recorded; two 3 ml aliquots were pipetted into separate centrifuge tubes, or, where only small volumes of alkaline digesta present, the whole amount was placed in one tube.

Approximately 3.5 ml of 95% ethanol was added and the solution brought to the boil (just) and allowed to cool to room temperature overnight. A white flocculate was observed forming in the tubes. The following morning the tubes were centrifuged for 15 minutes at 800 g (1600 rev/min in an M.S.E. Mistral 4L). The supernatant was decanted and the precipitate washed with 3 ml of 95% ethanol and the tube and contents centrifuged at 800 g for a further 15 minutes. The supernatant was again decanted and traces of alcohol remaining were driven off by gentle heating in the water bath. Into each tube was dispensed 2 ml of 2N hydrochloric acid after which the tubes and contents were heated in the boiling water bath for a further 120 minutes. When the tube and contents had returned to room temperature, 2N sodium hydroxide was added dropwise until the pH of the solution was in the range pH 5

to 7. The volume of the solution was made up to 5 ml with distilled water, then mixed well and decanted into glass vials for storage at - 20°C.

Glycogen estimation

The glucose produced by the acid digestion of the white flocculus of glycogen was measured using the glucose specific enzymic assay system described above. The total quantity of glycogen present in the organ or tissue was estimated by means of the equation -

$$\text{Total glycogen (mg)} = \frac{X_1 + X_2}{2} \times \frac{5}{100} \times \frac{V}{3}$$

where X_1 and X_2 = the glucose concentrations found in the duplicate samples (mg/100 ml).

V = the recorded volume of primary digesta (ml).

The glycogen composition of the organ or tissue was calculated as the quotient of total glycogen and organ or tissue wet weight, and was expressed in terms of 'grams of glucose per hundred grams of fresh tissue'.

Histological techniques

Portions were removed from the median lobe of the liver, the lower lobe of the left lung, the left kidney, the right extensor carpi radialis muscle, and a transverse section made of the ventricular muscle of the heart. All the tissues were fixed immediately in Gendré's acetic alcohol picroformalin (Gendré, 1937; Culling, 1957). After dehydration and embedding in paraffin wax, 5 μ m sections were cut and stained. Glycogen was identified by Best's carmine method (Best, 1906; Drury and Wallington, 1967), and by a periodic acid Schiff (PAS) technique, modified from Hotchkiss (1948) but using de Tomasi's Schiff reagent at half strength (Culling, 1957). The unconventionally high concentration of diastase was used as it was found that 0.1% diastase incompletely digested the high concentrations of glycogen present in most of the foetal tissue.

C O M M E N T S O N T H E
T E C H N I Q U E E M P L O Y E D

Glycogen estimation

It has been recognised since the late nineteenth century that less glycogen was obtained by using either aqueous or dilute acid extraction of tissue rather than hot alkaline digestion (Stetten and Stetten, 1960). Nevertheless, Meyer (1943) commented that strong alkali can degrade the glycogen molecule and recently it was shown that enzymatic digestion of heart tissue liberated about 27% more glycogen than was released by hot potassium hydroxide solution (Bartley and Dean, 1968). Steps were therefore taken to rigorously standardise the technique used. All the prenatal tissues were digested and the glycogen extracted during a single short period of time. Tissues from neonatal animals were similarly but separately treated.

The decision to subject the complete organ to biochemical analysis was taken from several reasons, the most important of which were the speed and precision which could be attained by removing and weighing a single, easily identifiable entity; the choice of skeletal muscle was also made on this basis. Although the

technique of clamping the liver between the jaws of tongs precooled in liquid nitrogen can enable hepatic tissue to become frozen within less than 0.1 of a second (Hohorst, Kreutz and Bucher, 1959), it was considered that the technique was in appropriate because of the numbers of tissues and foetuses to be sampled; questions were also raised with regard to the possible variation of glycogen deposition within the organ and the accuracy with which comparable samples could be obtained from each foetus.

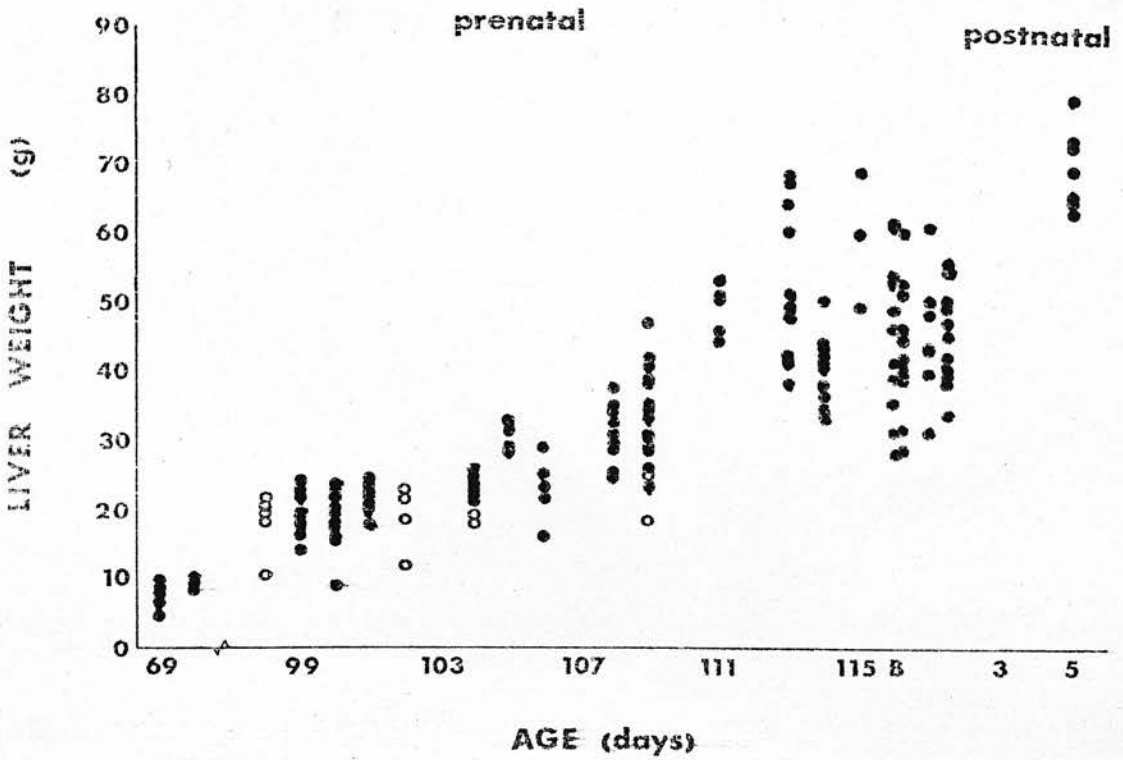


Figure 5.6 Individual foetal and neonatal liver weights of piglets aged between 69 days post coitum and 5 days post partum (open circles represent data from similar piglets in a related study unpublished).

RESULTS

Liver weight

Foetal liver weight increased from a litter mean of 9.4 g on day 70 of gestation to around 20 g by 99, 36 g by day 109 and over 50 g at the end of gestation (figure 5.6). Variation between litters with respect to liver weight was influenced by litter size; for example mean liver weight at 114 days (15 foetuses) was 39.4 g whereas on day 115 (4 foetuses) mean liver weight was 59.6 g. Of the several models tested, the equation -

$$Y = 41.7 (\pm 13.1) - 0.461 (\pm 0.155)A \\ + 1.003 (\pm 0.275)AW - 69.5 (\pm 34.8)W$$

where Y = litter mean foetal liver weight (g)

A = age of foetus (d)

W = litter mean foetal body weight (kg)

(An analysis of variance is shown in appendix 6)

was found to give the best fit to the data. The independent variate AA was tested and found to make an insignificant ($P > 0.05$) contribution to the overall model. Liver weights recorded at birth and during the first 36 hours thereafter were lower than those found on days 111, 113 and 115 of gestation (figure 5.6). Five days after birth, liver weights were in the region of 70 g.

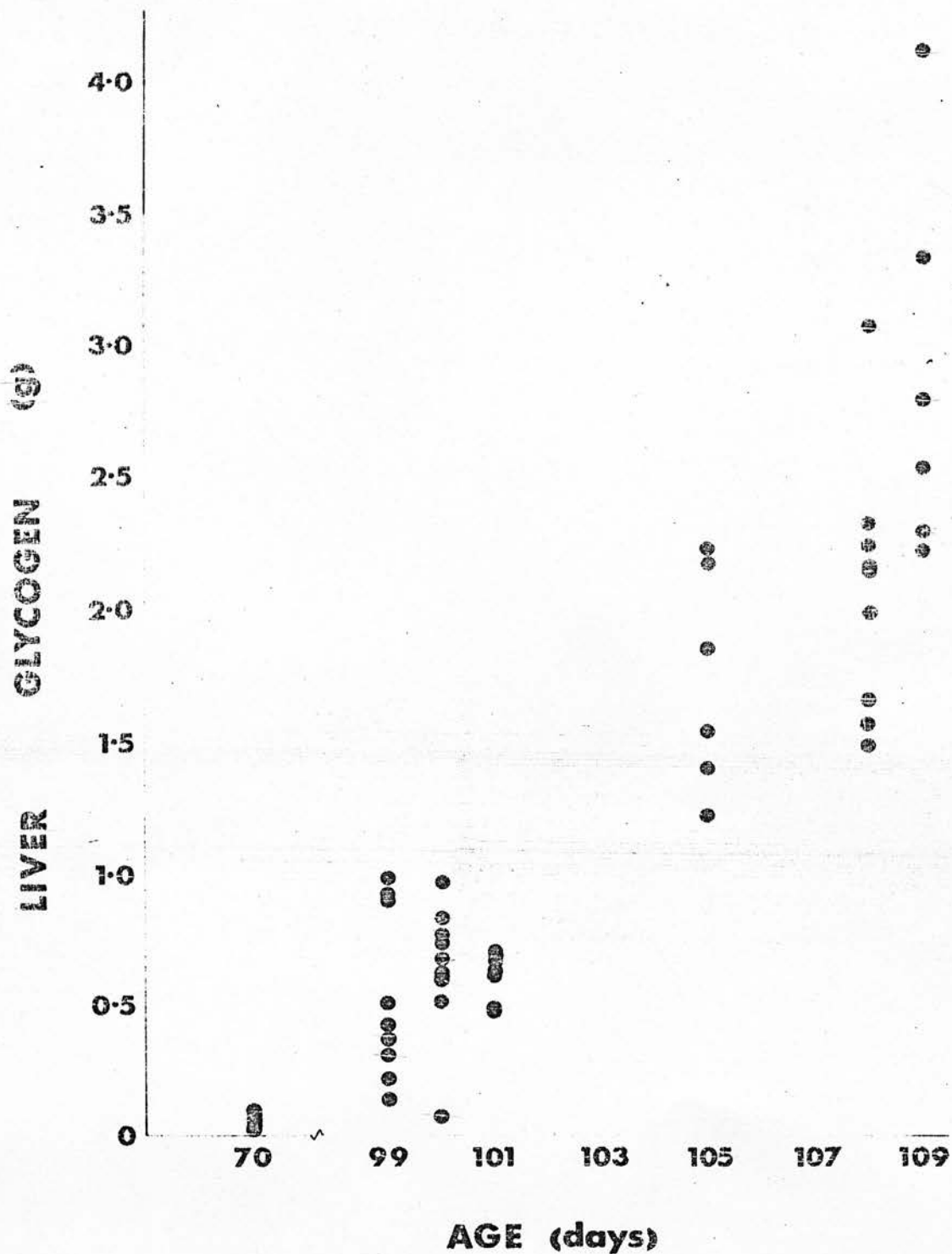


Figure 5.7 The amount of liver glycogen in individual pig foetuses at various stages of gestation between 70 and 109 days.

Total liver glycogen

There was a positive correlation between liver glycogen content and foetal age (figure 5.7). On day 70 of gestation, the liver was found to contain 0.05 ± 0.02 g of glycogen; by day 99 the litter mean had increased tenfold to 0.50 ± 0.10 g, although some foetuses had deposits of almost 1.0 g. Within six days twice as much glycogen was present and three days later, the liver glycogen stores had risen to about 3 g. The individual observations are depicted in figure 5.7, and the estimated means are tabulated below (table 5.5).

Table 5.5 The means for total liver glycogen content in pig foetuses aged between 70 and 109 days of gestation.

Age of foetus (d)	Number of foetuses	Total liver glycogen (g)	s.e. mean
70	6	0.05	0.02
99	10	0.50	0.10
100	11	0.65	0.07
101	7	0.61	0.04
105	6	1.74	0.17
108	10	2.08	0.13
109	6	2.90	0.30

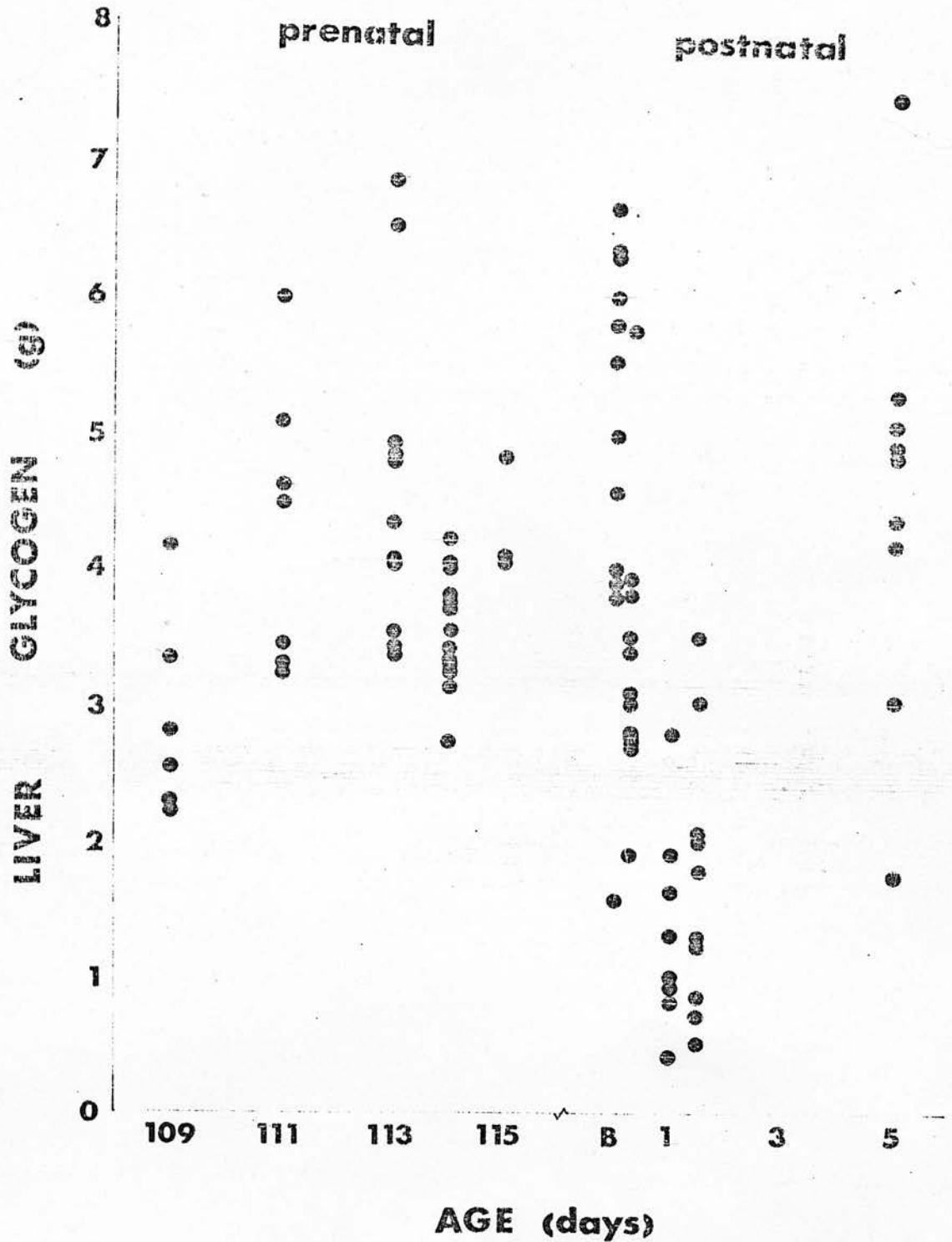


Figure 5.8 The amount of glycogen in the liver of perinatal piglets aged between 109 days gestation and 5 days post partum.

There was some variability between litters at the end of gestation; 4.60 ± 0.35 g was measured in the litter at 113 days and 4.30 ± 0.39 g and 4.30 ± 0.25 g on days 111 and 115 respectively. The quantities recorded for the foetuses analysed on day 114 gave a mean liver glycogen content of 3.54 ± 0.10 g. The changes which occurred in liver deposits of glycogen following birth are summarised in table 5.6 and depicted in figure 5.8.

Table 5.6 The means for total liver glycogen content in neonatal piglets at birth and between 6 and 120 hours post partum.

Age of Piglet (h)	Number of piglets	Total liver glycogen (g)	s.e. mean
0	7	5.23	0.31
0	6	4.79	0.81
6	12	3.29	0.27
24	8	1.38	0.25
36	10	1.71	0.31
120	9	4.54	0.52

Following an initial rapid decline in liver glycogen content, by the end of the fifth day post partum glycogen was again stored in the liver in amounts comparable to those found at birth.

Between litter regression analyses by the method of least squares have indicated that not only was litter mean liver glycogen content influenced by foetal age, but also by litter mean foetal body weight and the product of age and weight. The relationship may be summarised by the equation -

$$Y = 503 (\pm 232) - 5.04 (\pm 2.75)A \\ + 21.27 (\pm 4.87) AW - 2047 (\pm 618)W$$

where Y = litter mean total liver glycogen (g)

A = foetal age (d)

W = litter mean foetal body weight (kg)

(the analysis of variance is shown in appendix 7).

The total quantity of glycogen deposited in the total liver tissue of each litter was calculated and tabulated together with litter size (table 5.7).

Table 5.7 The total liver tissue glycogen from litters containing different numbers of foetal or new born piglets.

Age of foetus (d)	Number of piglets in the litter	Total litter liver glycogen (g)
70	7	0.35
105	6	10.5
111	7	30.1
115	4	17.2
100	11	7.2
101	11	6.7
113	11	50.6
114 (birth)	11	57.6
119 (birth)	11	52.7
99	13	6.5
108	15	31.3
109	14	40.6
114	15	53.0

Approximately the same total quantities of liver glycogen were accumulated in utero almost irrespective of the size of litter, although very small litters would seem to be exceptions to such a generalisation. The difference between the values for day 70 and days 99-101

was 6.5 g which represented an increase of about 0.2 g per day. Between days 99-100 and the end of gestation, there was an increase in glycogen content of 46.7 g which represented a more rapid daily accumulation rate of about 3.3 g. In respect of the variation within litters, the results failed to reveal any evidence to support the notion that there exist regions of the uterine horns more favourable to foetal development than others; liver glycogen did not accumulate either earlier or in larger quantities according to foetal position in utero. Similarly, the order of birth appeared to have no effect on the rate at which liver reserves were mobilised.

A value of 0.08 g for liver glycogen was recorded at 100 days of gestation in the case of a foetus weighing only 320 g when its siblings weighed around 760 g and the litter had a mean liver glycogen content of 0.65 ± 0.07 g; the small foetus bore a closer resemblance to 70 day old fetuses with respect to body size and liver glycogen content than it did to piglets at 100 days of gestational age. At 109 days the foetus weighing 1.34 kg had a liver containing 4.15 g of glycogen in contrast to the rest of the litter analysed which weighed 0.98 kg and had liver glycogen contents of about 2.60 g. However, no general pattern emerged from within

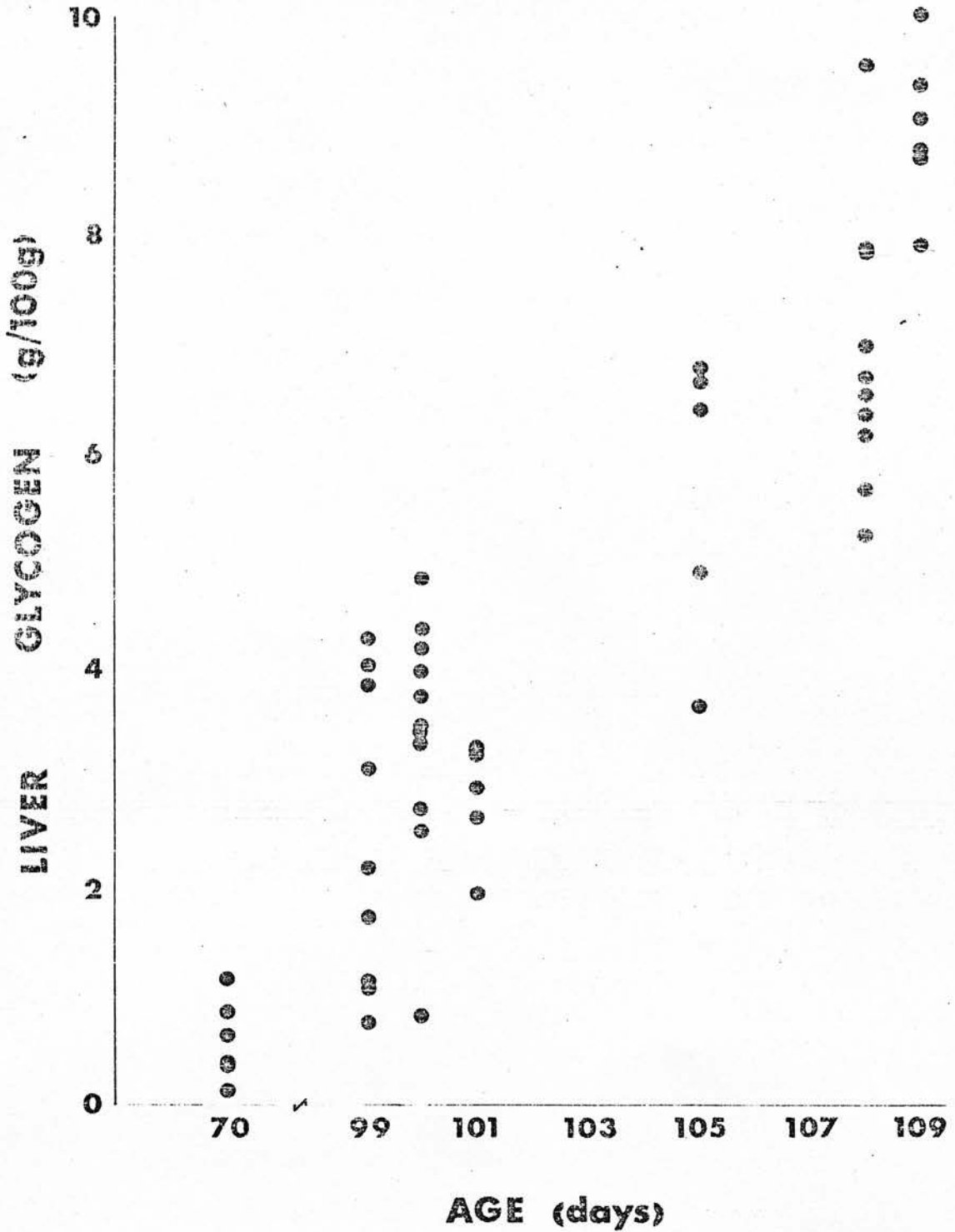


Figure 5.9 The concentration of glycogen in the individual foetal piglet liver between the 70th and 109th day of gestation.

each litter to relate liver glycogen content to body weight.

Liver glycogen concentration

During the latter part of gestation glycogen increased as a component of foetal liver weight from 0.53 ± 0.17 g/100 g at day 70 to 2.43 ± 0.42 g/100 g by day 99 and 8.98 ± 0.29 g/100 g on day 109 (figure 5.9). The results are summarised in table 5.8.

Table 5.8 The litter mean concentration of glycogen in liver from piglet foetuses aged between 70 and 109 days of gestation.

Age of Foetus (d)	Number of foetuses	Concentration of glycogen (g/100g)	s.e. mean
70	6	0.53	0.17
99	10	2.43	0.42
100	11	3.41	0.33
101	7	2.85	0.18
105	6	5.55	0.51
108	10	6.89	0.40
109	6	8.98	0.29

No difference was present in liver glycogen composition between days 111, 113 and 114, when 8.57 ± 0.76 g/100 g, 8.73 ± 0.43 g/100 g and 9.04 ± 0.23 g/100g respectively were recorded. On day 115 a lower value of 7.36 ± 0.77 g/100 g was found.

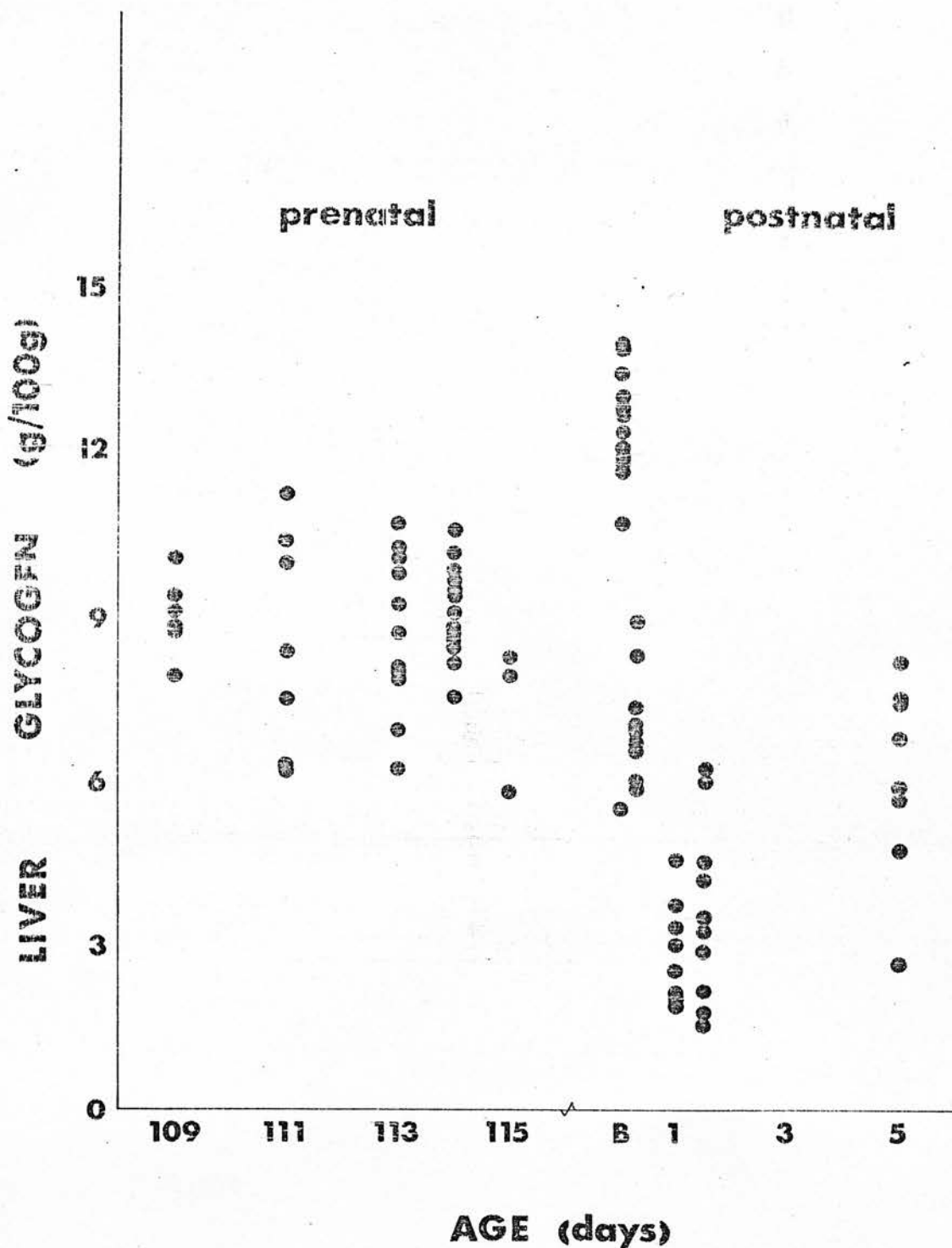


Figure 5.10 The concentrations of glycogen in liver of near term foetal and young post-natal piglets.

The glycogen component of liver composition was higher at birth than at preceding stages in utero (figure 5.10). The litter delivered after 114 days of gestation had a mean glycogen content of 13.10 ± 0.28 g/100 g of liver and the litter born 119 days post coitum registered a value of 10.71 ± 1.06 g/100 g. Six hours after birth 7.28 ± 0.32 g/100 g was the proportion of glycogen and this had fallen to 2.92 ± 0.34 g/100 g following 24 hours of postnatal life. A recovery in liver glycogen composition seemed to occur thereafter since 3.63 ± 0.52 g/100 g was present 36 hours following birth, and 6.23 ± 0.56 g/100 g was detected by the end of the 5th day (120 hours).



Liver histology

There was always so much glycogen in the foetal liver that it was not possible to identify histologically any particular stage of gestation as having more or less glycogen than another stage. Generally the hepatic cells were either filled with stained material or almost glycogen-free (figure 5.11). The giant polynuclear cells were glycogen free.

Figure 5.11 Section of lobule from the right lateral lobe of the liver of a male foetus at 98 days of gestation. Glycogen is shown as patches of dark stain, apparently filling some cells while others remain essentially glycogen-free (x 325).

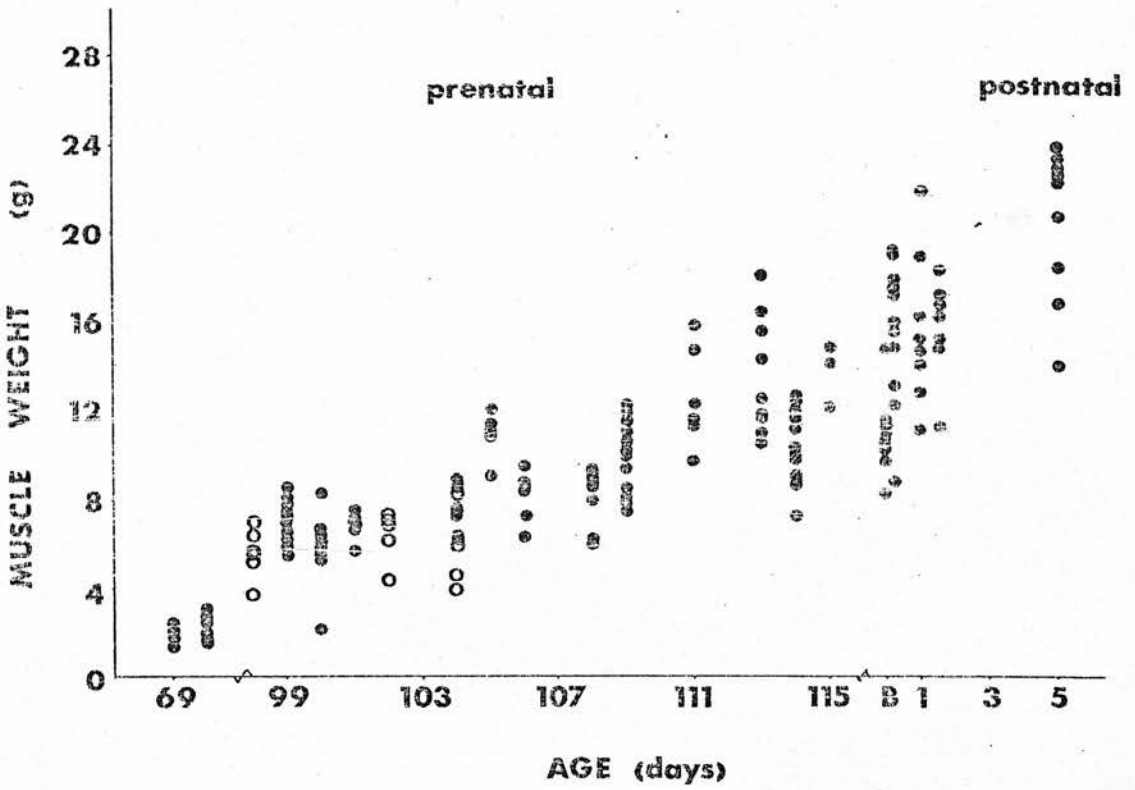


Figure 5.12

Individual foetal and neonatal right extensor carpi radialis skeletal muscle weights of piglets aged between 69 days post coitum and 5 days post partum (open circles represent data from similar piglets in a related unpublished study).

Skeletal muscle weight

As the foetus grew in size the mean weight of the right extensor carpi radialis muscle increased from 0.19 ± 0.012 g at 69 days of gestation to 0.71 ± 0.024 g by day 99, and 0.96 ± 0.045 g by the 109th day (figure 5.12). On days 113 and 115 the mean weights were 1.36 ± 0.076 g and 1.37 ± 0.081 g respectively, although lower weights were recorded on day 114 (1.02 ± 0.045 g) and at birth, (1.09 ± 0.067 g). During the 36 hours following birth the litters sampled had muscle mean weights of about 1.55 g, whereas the mean weight after 120 hours of postnatal life was 2.05 ± 0.113 g.

Several regression models were formulated to describe muscle weight in terms of body weight, age of piglet and the product of age and body weight. It was found that the best fit to both pre - and postnatal observations was given by the line -

$$\log Y = 1.069 - 0.1120 \log W$$

Where Y = right extensor carpi radialis muscle weight (g)

W = body weight of the piglet (kg)

r = 0.969 (n = 282)

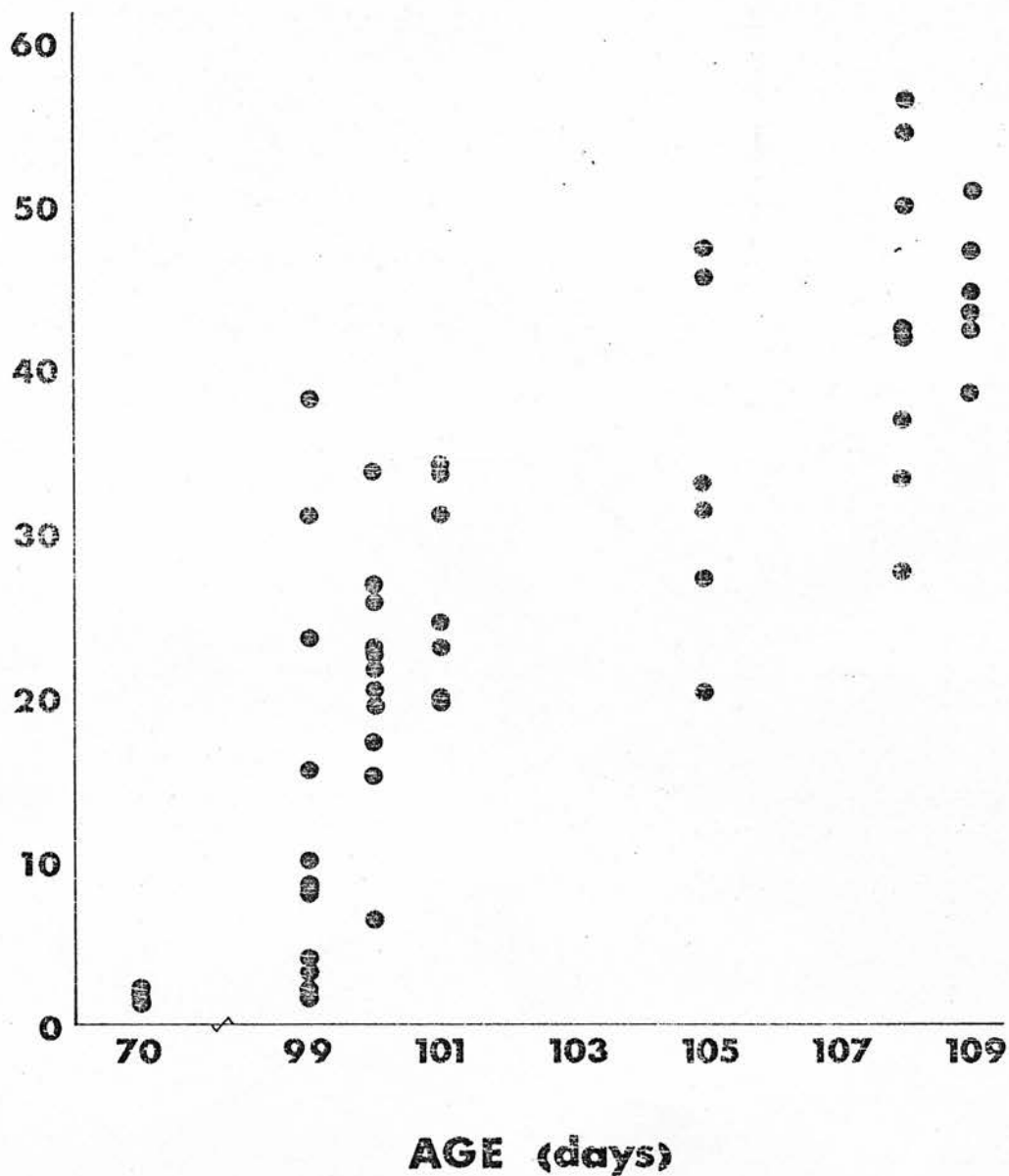


Figure 5.13 The amount of glycogen in the right extensor carpi radialis muscle of individual piglet fetuses aged between 70 and 109 days gestation.

Skeletal muscle total glycogen

As with liver, there was a positive correlation between skeletal muscle glycogen content and foetal age (figure 5.13). The litter mean for the quantity of glycogen in the extensor carpi radialis muscle on day 70 of gestation was 1.7 ± 0.3 mg, which had risen to 13.0 ± 3.5 mg by day 99 and was larger still at 21.2 ± 2.1 mg on day 100. Twice the day-99 litter mean glycogen content was found on day 101 (26.8 ± 2.4 mg).

Table 5.9 summarises the observations made between day 105 and birth.

Table 5.9 The litter mean glycogen content of right extensor carpi radialis skeletal muscle in foetal piglets during the last 10 days of gestation.

Age of foetus (d)	Number of foetuses	Skeletal muscle glycogen content (mg)	s.e. mean
105	6	34.4	4.3
108	10	42.0	3.0
109	6	44.8	1.7
111	7	51.8	5.7
113	11	51.9	4.4
114	14	50.0	2.5
115	3	55.4	4.6
Birth (114)	7	67.6	4.2

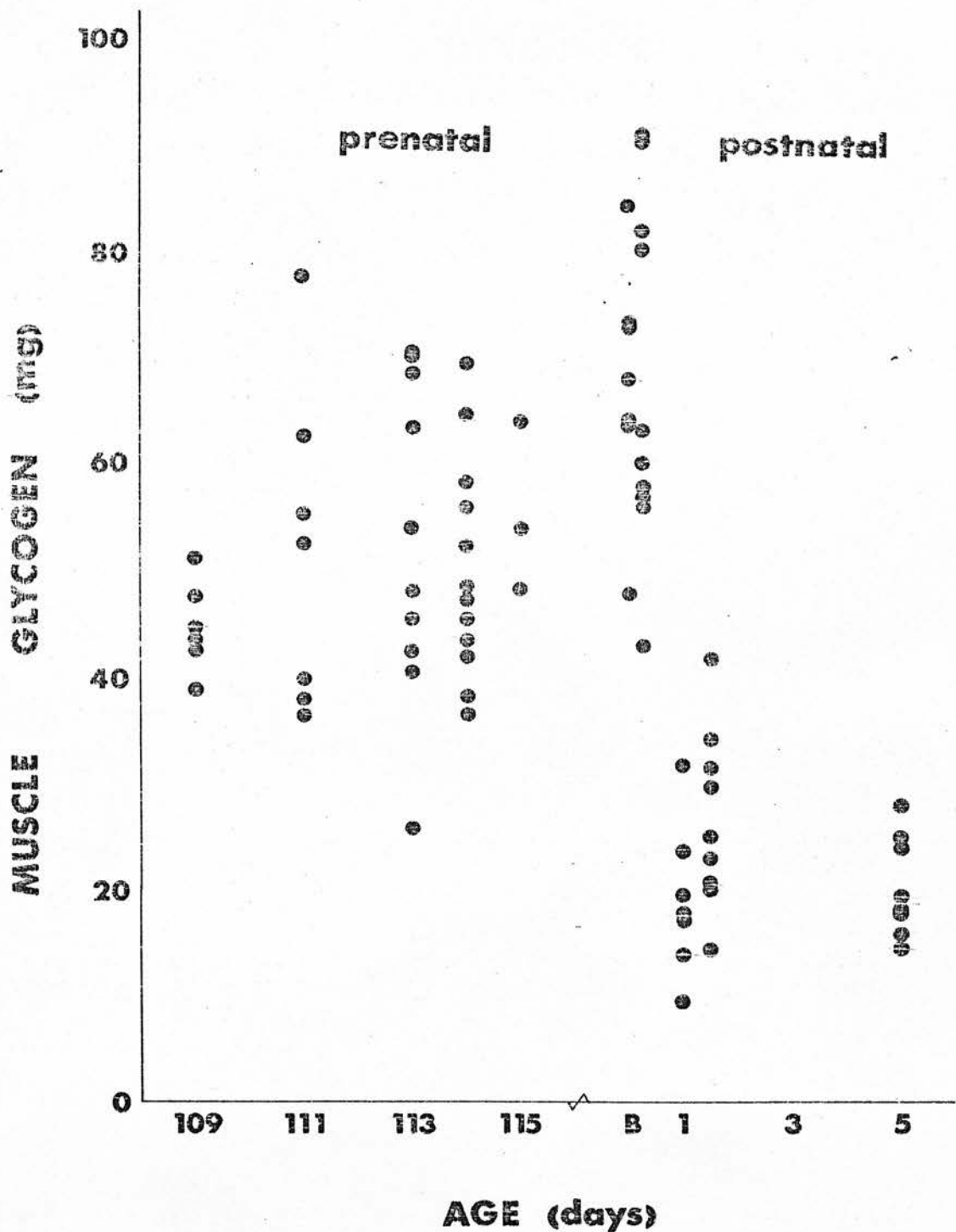


Figure 5.14 The amount of glycogen in the right extensor carpi radialis muscle of individual piglet fetuses and neonates aged between 109 days post coitum and 5 days post partum.

The litter mean values calculated for the end of gestation (days 111, 113, 114 and 115) showed little variation between one another, ranging between 50 and 55 mg (figure 5.14). The differences between the means on days 111, 113 and 115, and the mean at birth (67.6 ± 4.2 mg) were not significant ($P > 0.05$) whereas the mean glycogen content recorded on day 114 of gestation was significantly ($P < 0.01$) less than the litter mean at birth.

The prenatal results were found to be best summarised by the regression equation -

$$Y = 0.0274 (\pm 0.0338)A + 0.1555 (\pm 0.0599)AW \\ - 15.37 (\pm 7.59)W - 0.729$$

where Y = litter mean right extensor carpi radialis muscle glycogen content (mg)

A = age of foetus (d)

W = mean litter foetal body weight (kg)

(the analysis of variance is shown in appendix 8).

The glycogen content of the muscle six hours after birth was 68.0 ± 5.2 mg which was the same amount as was measured at birth. Thereafter, however, the

glycogen content fell to 18.9 ± 2.4 mg at the end of the first day (figure 5.14) and did not rise significantly ($P > 0.05$) after 36 hours and 120 hours post partum (26.1 ± 2.6 mg) and (21.4 ± 1.7 mg) respectively.

Skeletal muscle glycogen concentration

The concentration of glycogen in skeletal muscle increased during the second half of gestation (figure 5.15). Glycogen represented only 1.0 ± 0.3 g/100 g of muscle wet weight at 70 days gestation and at 99 days, 9 of the 12 fetuses sampled had right extensor carpi radialis muscles with less than 2 g/100 g of glycogen (litter mean was 1.8 ± 0.4 g/100 g). The three fetuses with values greater than 2 g/100 g lay within the spread of results for litters aged 100 and 101 days which had mean glycogen concentrations of 3.6 ± 0.2 and 3.8 ± 0.3 g/100 g respectively (figure 5.15).

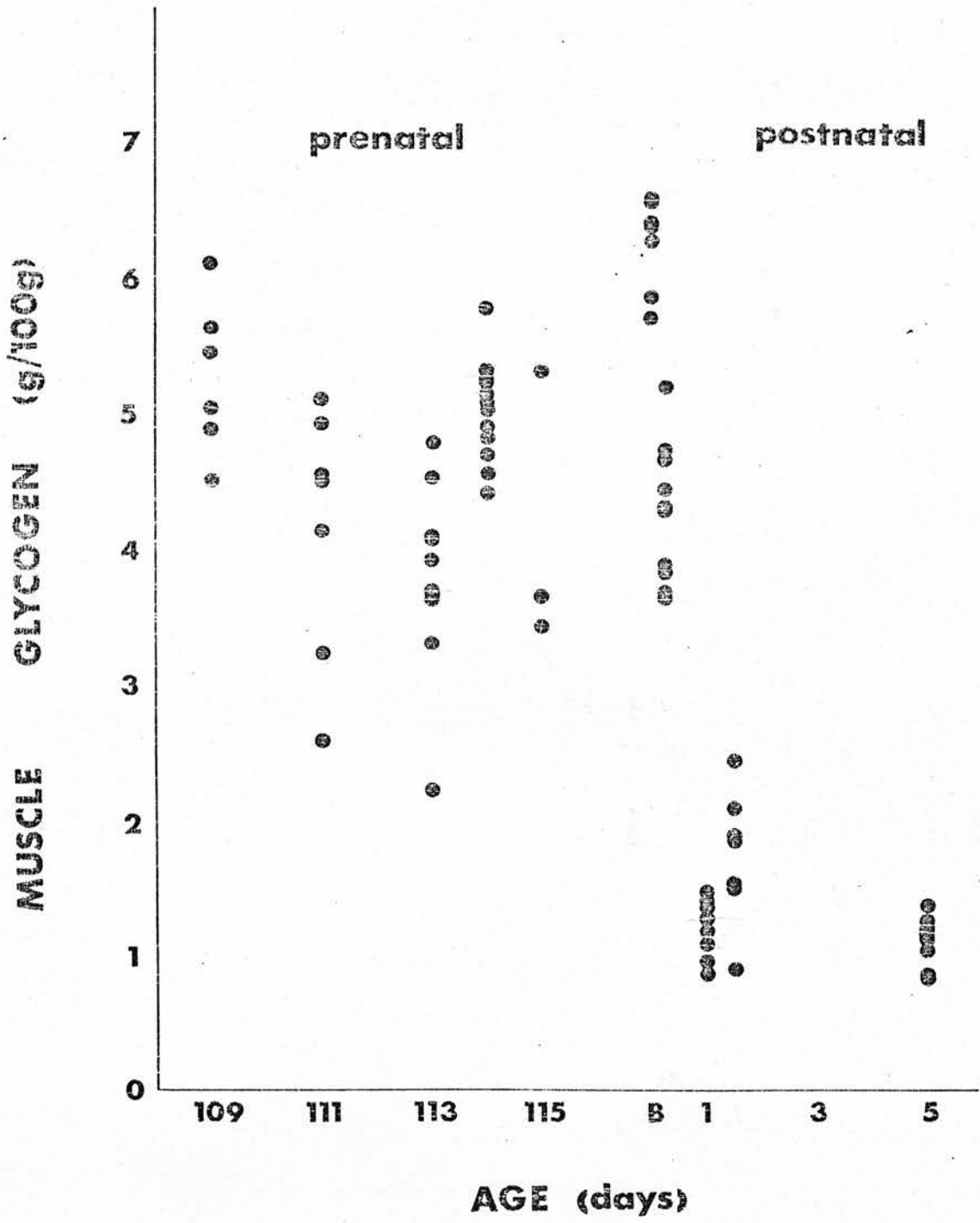


Figure 5.16 The glycogen concentration of right extensor carpi radialis muscle of near term foetal and young post-natal piglets.

The litter mean values of foetuses between day 105 of gestation and birth are shown in table 5.10.

Table 5.10 The concentration of glycogen in the right extensor carpi radialis muscle of late gestation foetal and new born piglets.

Age of foetus (d)	Number of foetuses	Skeletal muscle glycogen con- centration (g/100 g)	s.e. mean
105	6	3.2	0.4
108	10	5.1	0.3
109	6	5.2	0.2
111	7	4.1	0.3
113	11	3.8	0.2
114	14	5.0	0.1
115	3	4.1	0.6
Birth (114)	7	6.2	0.1

The increasing glycogen concentration observed between days 99 and 109 was not sustained during the last five days of gestation (figure 5.16).

At birth the muscle glycogen concentration was 6.2 ± 0.1 g/100 g but after 6 hours of postnatal life, it was 4.3 ± 0.2 g/100 g and had fallen to 1.2 ± 0.1 g/100 g by the end of the first day post partum. A slightly higher glycogen content was present 36 hours after birth (1.7 ± 0.2 g/100 g), but 120 hours following parturition, the same glycogen concentration was detected ($1.0 \pm$

0.1 g/100 g) as was found on the 70th day of gestation (figures 5.15 and 5.16). A number of models were unsuccessfully investigated in an attempt to find a suitable relationship between muscle glycogen concentration and stage of gestation, body weight of the foetus and the product of age and body weight. Body weight of the foetus accounted for only a small proportion of the variation between individual foetuses with respect to muscle glycogen composition.



Skeletal muscle histology

Glycogen was located within the cytoplasm of the primary myofibres and to a lesser extent in secondary myofibres. Smaller amounts appeared to be present in the smooth muscle of the arteries and veins, and almost none could be detected in the interstitial space (figure 5.17). Like liver tissue, it was impossible clearly to identify histologically the stage of gestation because of the intensity of staining in every section made.

Figure 5.17 Transverse section of the right extensor carpi radialis muscle from a 99 day old foetal pig illustrating the glycogen filled cytoplasm of primary and secondary myofibres (x 650).

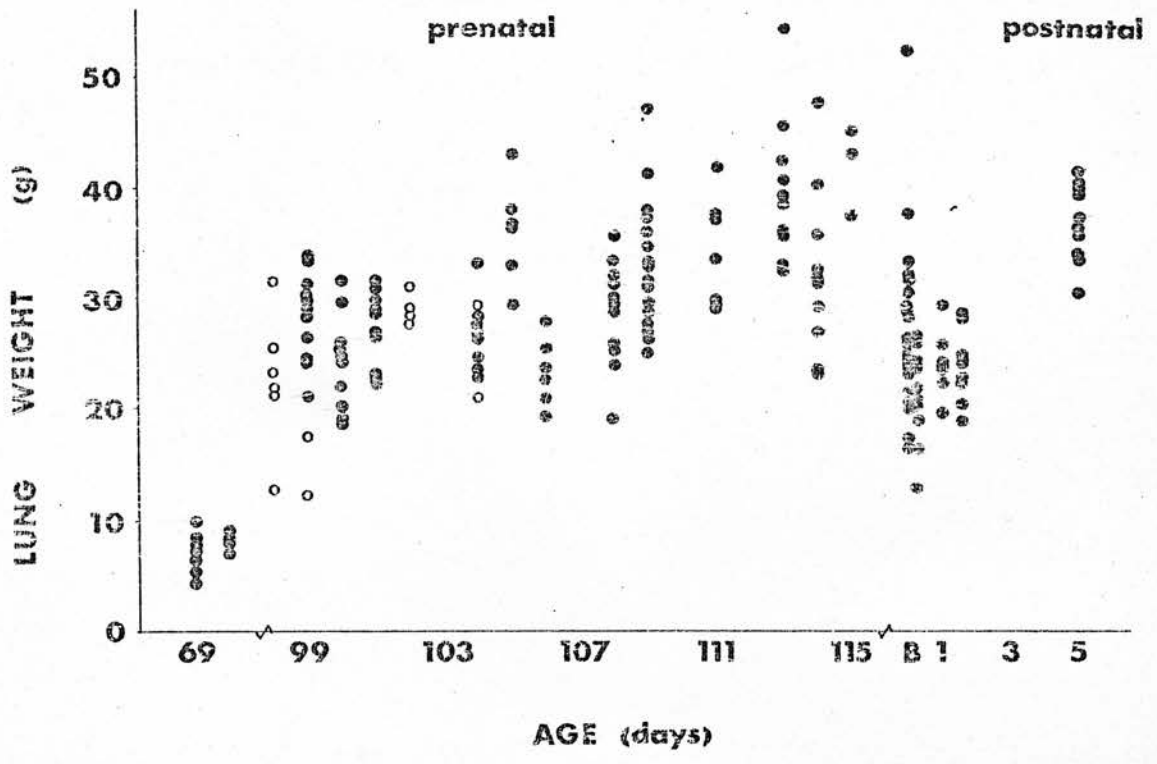


Figure 5.18 The relationship between individual piglet lung weights and stage of gestation, or age post partum.

Lung weight

Lung increased in weight from 7.3 ± 0.41 g and 8.3 ± 0.26 g at days 69 and 70 respectively of gestation to 22.7 ± 1.99 g on day 100. Marked variation occurred however, between litters of approximately equivalent age; lung weights on days 99, 101 and ~ 101 of gestation were 28.2 ± 1.06 g, 27.1 ± 0.99 g and 24.7 ± 0.84 g respectively, and similarly on days 104, 105 and 106 the litter mean lung weights were 26.8 ± 0.87 g, 36.2 ± 1.84 g and 24.2 ± 1.18 g respectively (figure 5.18). From day 108 until term, there was a general increasing trend in lung weight; 28.5 ± 1.08 g and 34.1 ± 1.50 g were recorded on days 108 and 109, 34.1 ± 1.86 g, 39.7 ± 1.84 g and 41.9 ± 2.31 g were the litter mean weights of lung on days 111, 113 and 115; on day 114, 31.5 ± 1.74 was the mean weight.

After birth the lung weights recorded were very much lower than those in late gestation; 31.1 ± 3.56 g, 26.1 ± 1.62 g and 24.1 ± 1.21 g were the litter mean weights found immediately following birth, 21.4 ± 1.06 g six hours later, 24.0 ± 1.00 g after 24 hours and 23.6 ± 0.89 g by the time the piglet was 36 hours post partum. Lung weight had increased to 36.8 ± 1.11 g in piglets aged 120 hours.

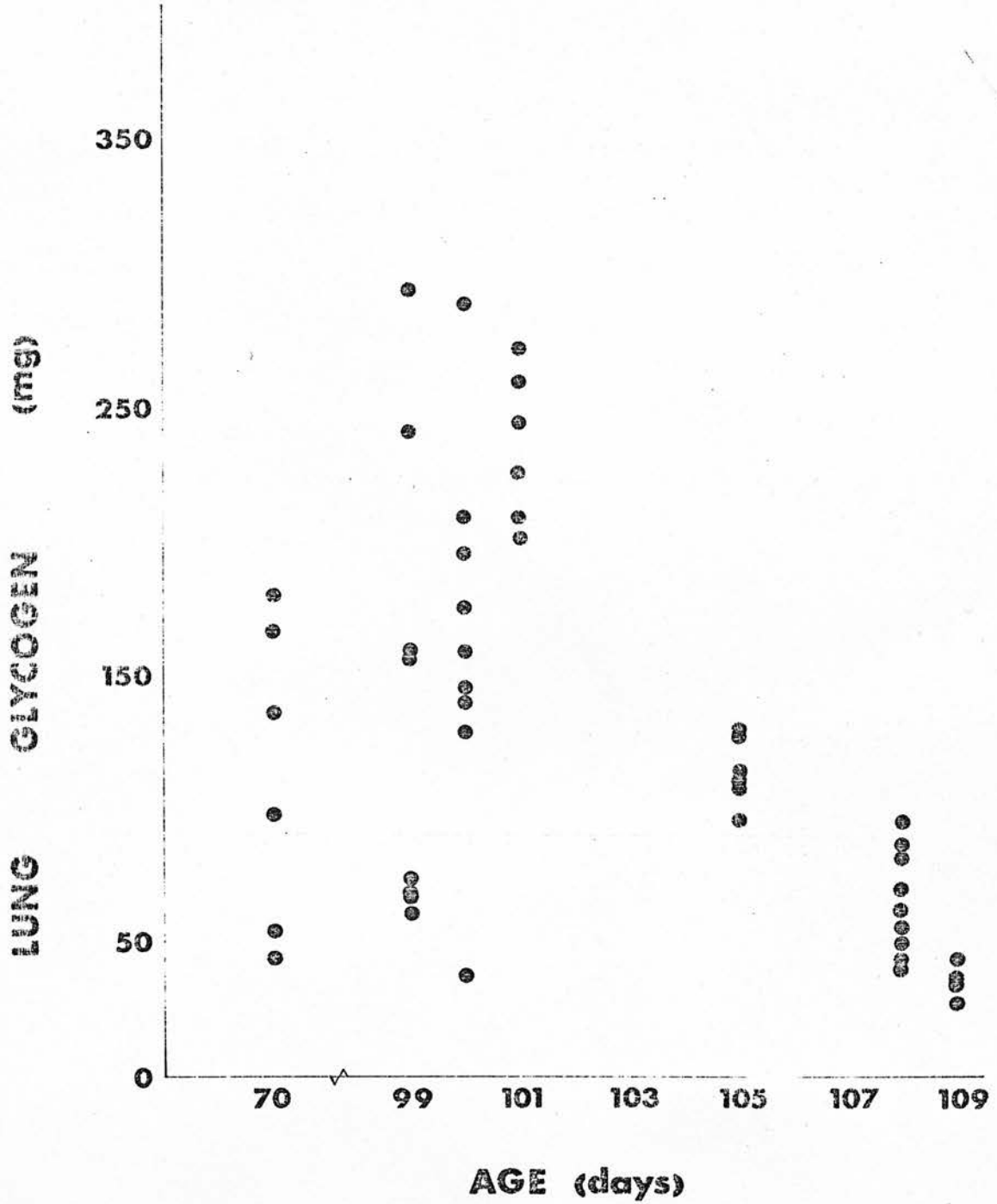


Figure 5.19 The amount of glycogen in the lungs of individual piglet fetuses aged between 70 and 109 days of gestation.

Total lung glycogen

The lungs from 70 day old foetal piglets contained relatively large quantities of glycogen, the litter mean being 0.114 ± 0.023 g. The values for the litter mean glycogen content on days 99 and 100 were not significantly higher ($P > 0.05$) at 0.142 ± 0.027 g and 0.165 ± 0.027 respectively, although almost twice as much (0.226 ± 0.014 g) was present on day 101. The amount of glycogen declined after day 101 (figure 5.19) although the quantities of glycogen in the lung during the last five days in utero were generally more than two times as much as were present on day 109 (table 5.11).

Table 5.11 The litter mean amount of glycogen in the lungs of foetal piglets aged between 101 days of gestation and term.

Age of foetus (d)	Number of foetuses	Lung glycogen content (g)	s.e mean
101	7	0.226	0.014
105	6	0.115	0.005
108	10	0.065	0.006
109	6	0.036	0.002
111	7	0.095	0.012
113	11	0.058	0.004
114	14	0.072	0.004
115	3	0.085	0.004

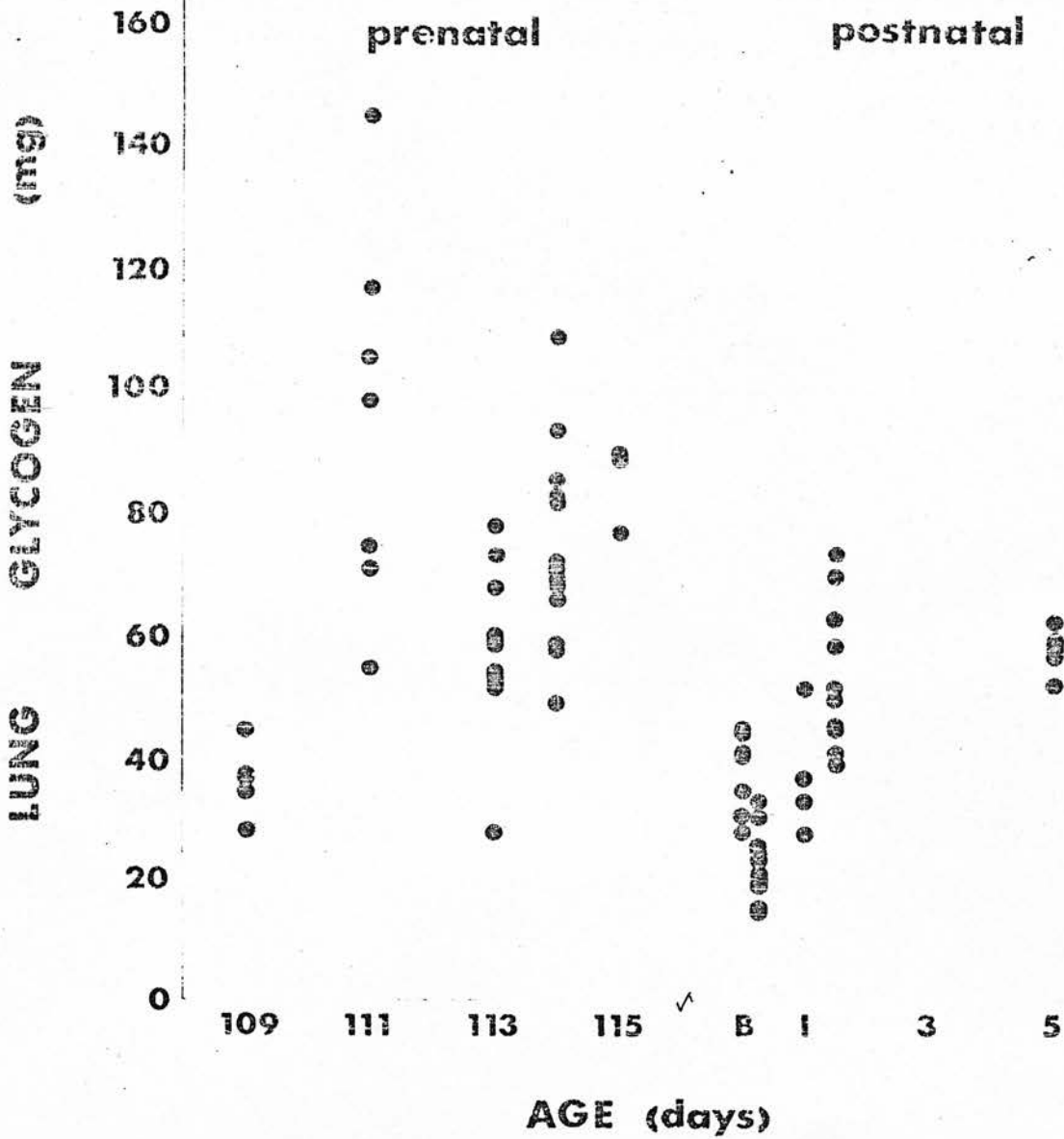


Figure 5.20 The amount of glycogen in the lungs of individual near term prenatal piglets, and young postnatal piglets.

There was a highly significant ($P < 0.001$) reduction in total lung glycogen following birth when only 0.038 ± 0.003 g was detected. The decline appeared to have continued to a value of 0.022 ± 0.002 g by 6 hours post partum and yet reversed within the first 24 hours to reach 0.035 ± 0.004 g (figure 5.20). The amounts present 36 and 120 hours after birth were 0.054 ± 0.004 g and 0.058 ± 0.001 g, respectively were consistent with the suggestion that following an initial post-natal mobilisation of glycogen, the amount increased again towards the levels observed in late gestation.

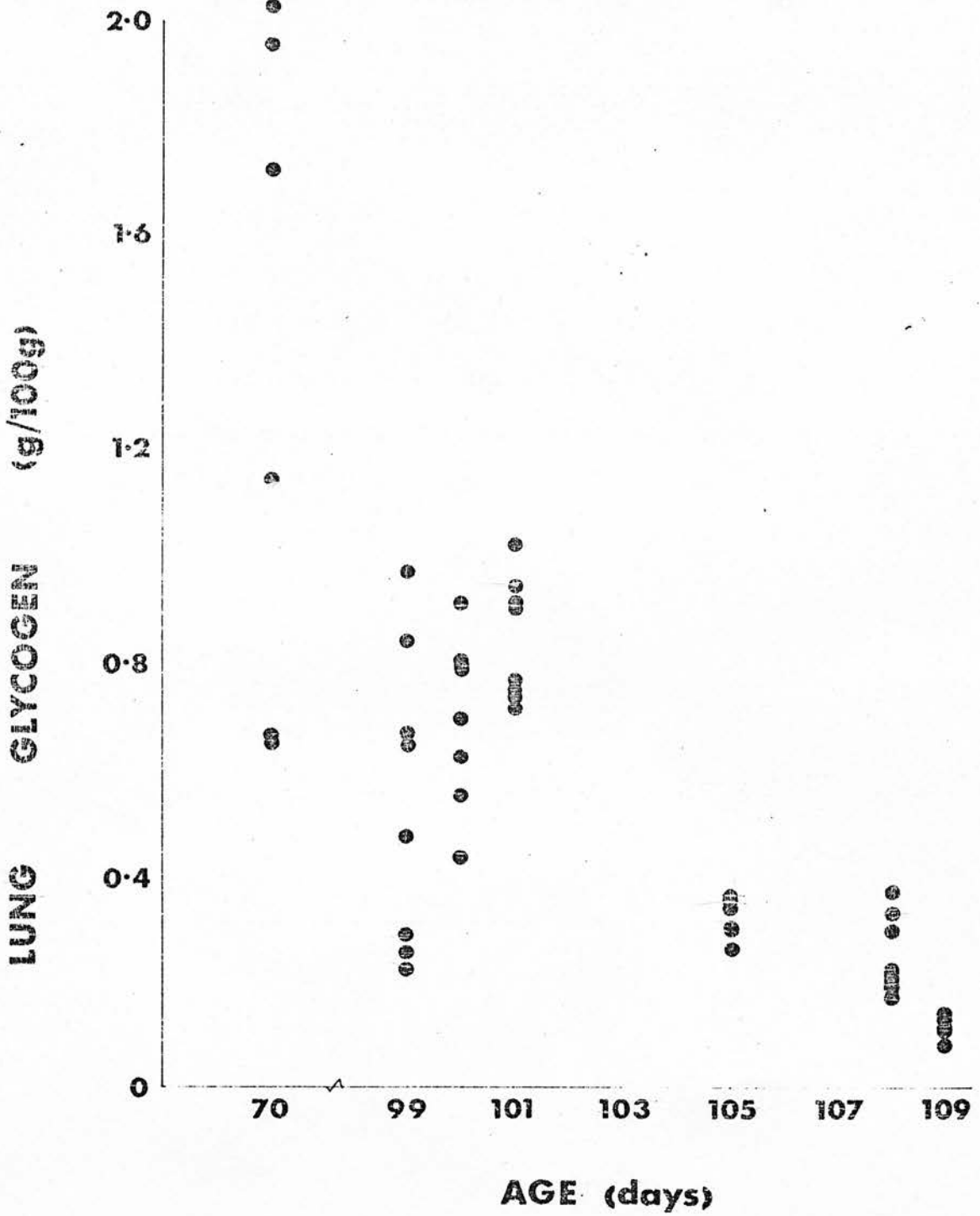


Figure 5.21 The concentration of glycogen in lung tissue of individual fetuses aged between 70 and 109 days of gestation.

Lung glycogen concentration

The evidence suggested that lung glycogen concentration declined during the second half of gestation (5.21). The concentration of glycogen in the foetal pig lung at 70 days of gestation was 1.36 ± 0.257 g but by 99 days the carbohydrate polymer represented only 0.48 ± 0.088 g/100 g of lung tissue. There was a rise in concentration to 0.71 ± 0.050 g/100 g and 0.85 ± 0.045 g/100 g on days 100 and 101, the value on the latter day being significantly higher ($P < 0.01$) than the level observed on day 99. The drop in concentration seen earlier was continued from day 101 to 109 (table 5.12).

Table 5.12 The concentration of glycogen in lung tissue of foetal piglets aged between 101 days' gestation and term.

Age of Foetus (d)	Number of piglets	Concentration of lung glycogen (g/100 g)	s.e. mean
101	7	0.85	0.045
105	6	0.32	0.016
108	10	0.23	0.022
109	6	0.12	0.008
111	7	0.28	0.040
113	11	0.15	0.012
114	14	0.22	0.009
115	3	0.21	0.019

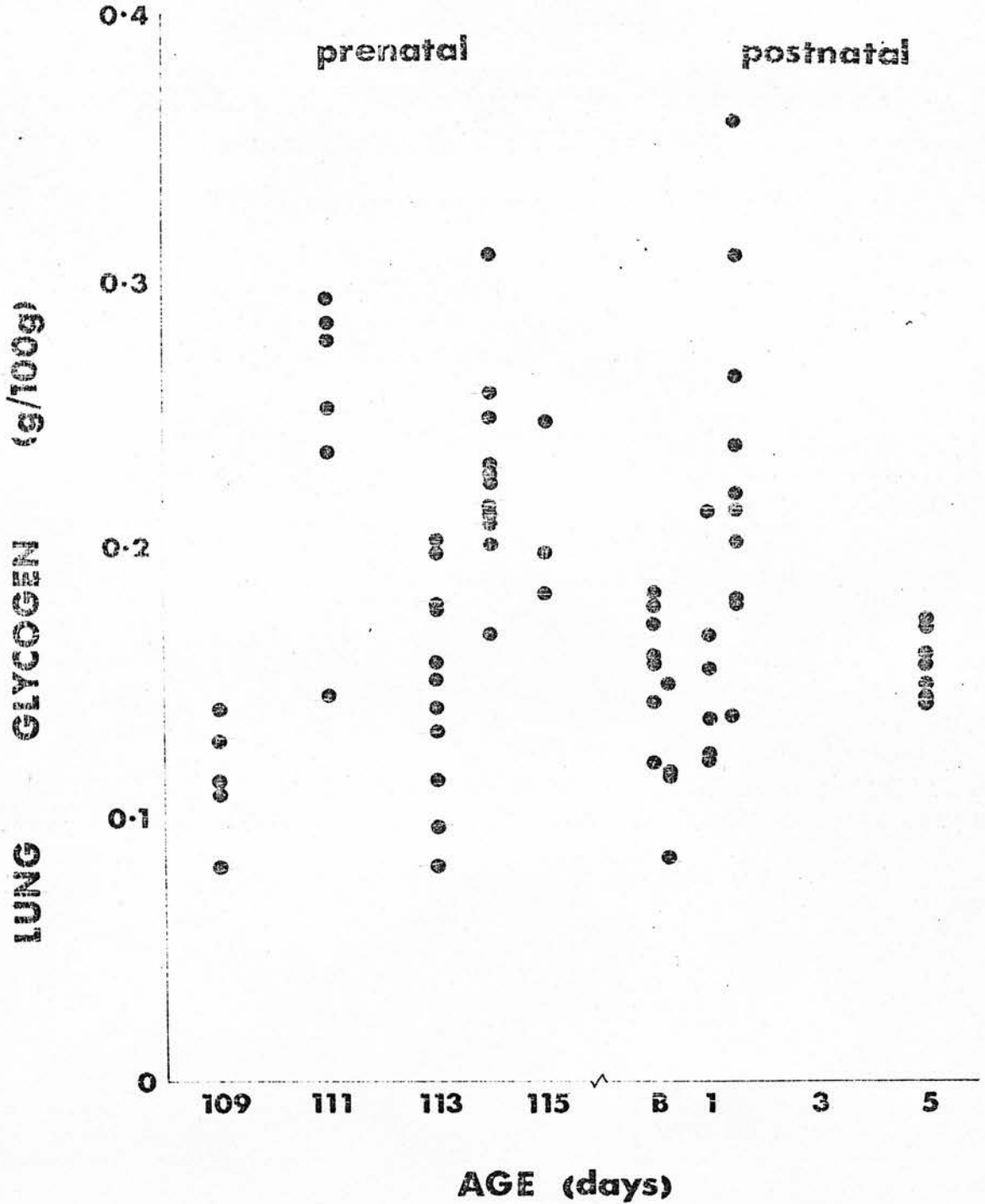
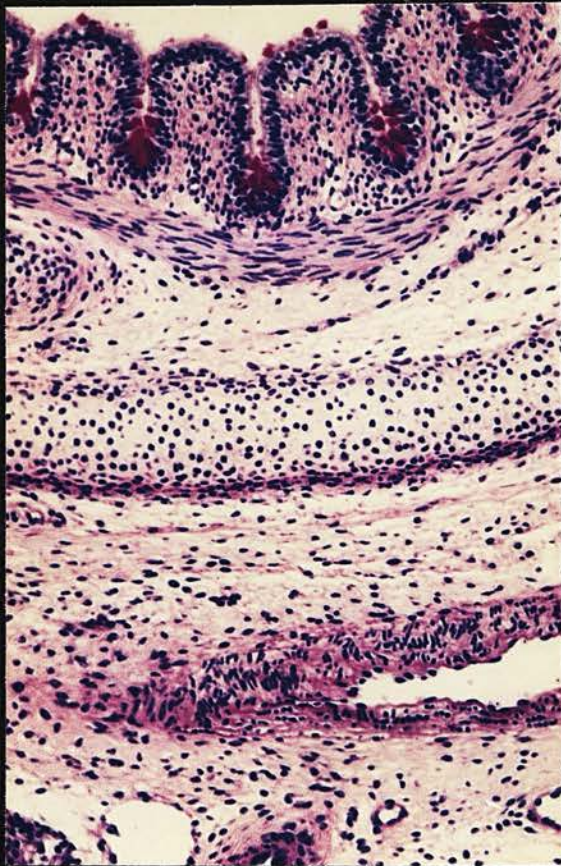
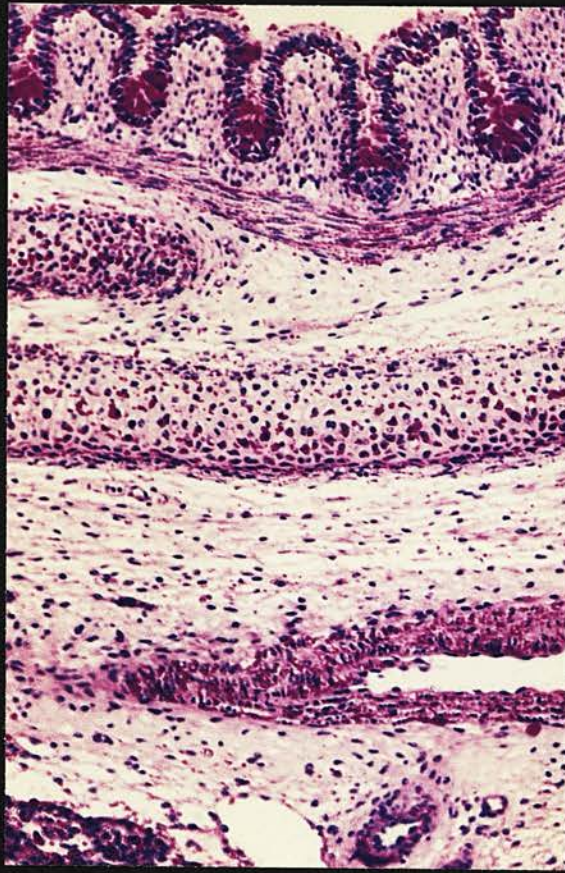


Figure 5.22 The concentration of glycogen in the lungs of individual near term foetal piglets and young postnatal piglets.

Most of the individual values for the concentration of glycogen in the foetal lung during the remaining prenatal period lay above 0.20 g/100 g (figure 5.22) with the low total glycogen values recorded on day 113 being reflected in low estimates of glycogen concentration (table 5.12). The concentration of glycogen at birth was 0.16 ± 0.008 g/100 g whereas 6 hours later it was 0.11 ± 0.008 g/100 g, significantly ($P < 0.002$) less than at birth. The increase in lung glycogen composition seen 24 and 36 hours post partum (figure 5.22) when litter mean concentrations were 0.15 ± 0.014 g/100 g and 0.23 ± 0.021 g/100 g respectively was not apparent 120 hours after birth when glycogen concentration was 0.16 ± 0.005 g/100 g.

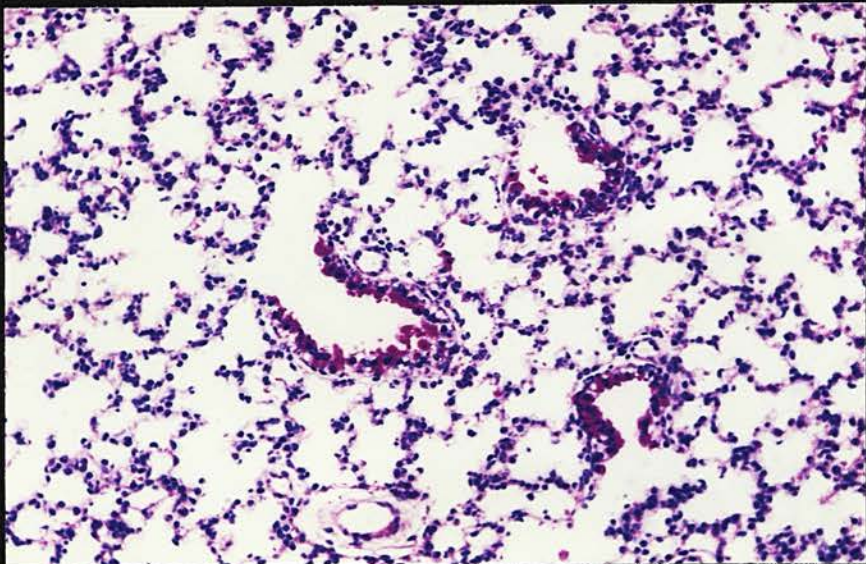
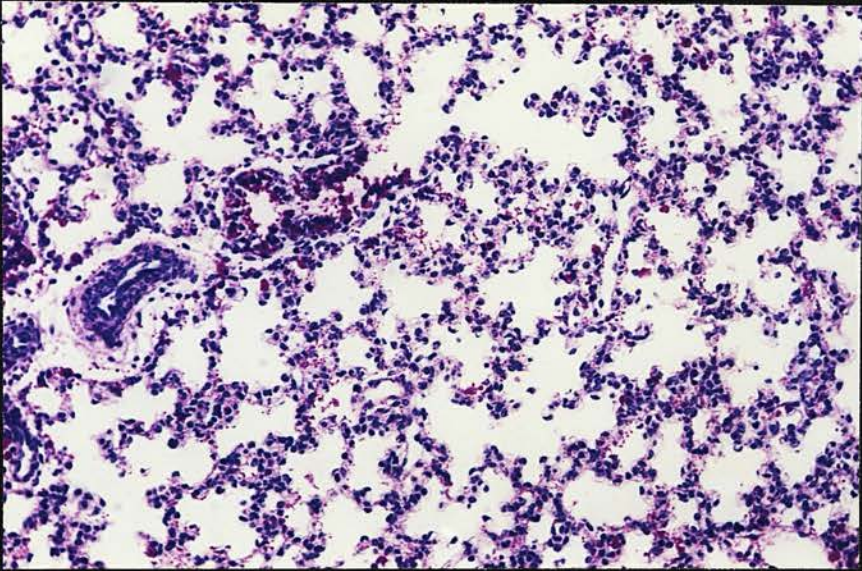
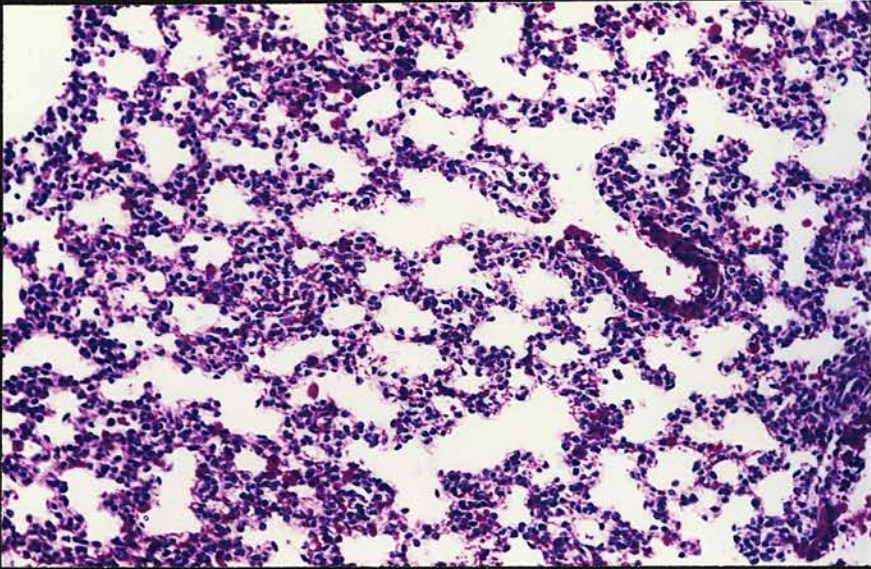


Lung histology

The larger bronchi showed positive staining for glycogen in the columnar ciliated epithelium, in the cytoplasm lying beneath the nuclei (5.23). Goblet cells full of PAS positive diastase resistant material were noted at the bases of the longitudinal folds (figure 5.24). Glycogen was present in the smooth muscle encircling the bronchus as well as in arterial and venous smooth muscle. Only a few particles of glycogen positive stain could be seen in the tissue parenchyma supporting the ciliated epithelia, whereas larger denser staining areas were found associated with the nuclei of the bronchial cartilage (5.23).

Figure 5.23 Transverse section of a small bronchus removed from a 450 g female foetus at 98 days' gestational age. Glycogen is present as red stain in the subnuclear region of the ciliated epithelium, the cytoplasm of the bronchus and vascular smooth muscle, in the perinuclear area of bronchial cartilage and to a lesser extent in the supporting tissue matrix (x 160).

Figure 5.24 Serial transverse section of that shown in figure 5.23 but digested with diastase to demonstrate which stained regions were due to glycogen and which were mucopolysaccharides. Goblet cells filled with red stained material may be seen in the folds of the bronchiolar epithelium (x 160).



The glycogen present in the lung of a 98 day old foetus was distributed in the terminal bronchiolar region as shown in figure 5.25. The epithelial tissues were heavily stained as were the smooth muscle cells. The respiratory portion of the lung changes in appearance from the relatively heavily stained appearance at day 99 (figure 5.26) to the almost glycogen-free state seen on day 109 of gestation (figure 5.27). The epithelial cells of the terminal bronchioles remained heavily stained throughout the last two weeks before birth, but there was a significant change in the morphology and histochemistry of the respiratory epithelia. There appeared to be less glycogen filled cuboidal cells after day 99 and there was a suggestion that the density of staining in the alveolar cells and septa declined towards term.

- Figure 5.25 Section of lung taken from a male aged 99 days showing glycogen as red stain in the epithelia of the respiratory bronchioles, alveolar ducts and alveoli (x 160).
- Figure 5.26 Section of lung from a male foetus aged 104 days demonstrating the reduced presence of glycogen in the epithelia of the alveoli but continuance of red stain in the smaller bronchi (x 160).
- Figure 5.27 Section of lung from a 109 day old female foetus illustrating the red stain filled bronchial epithelium but only scattered fragments of stain throughout the alveoli (x 160).

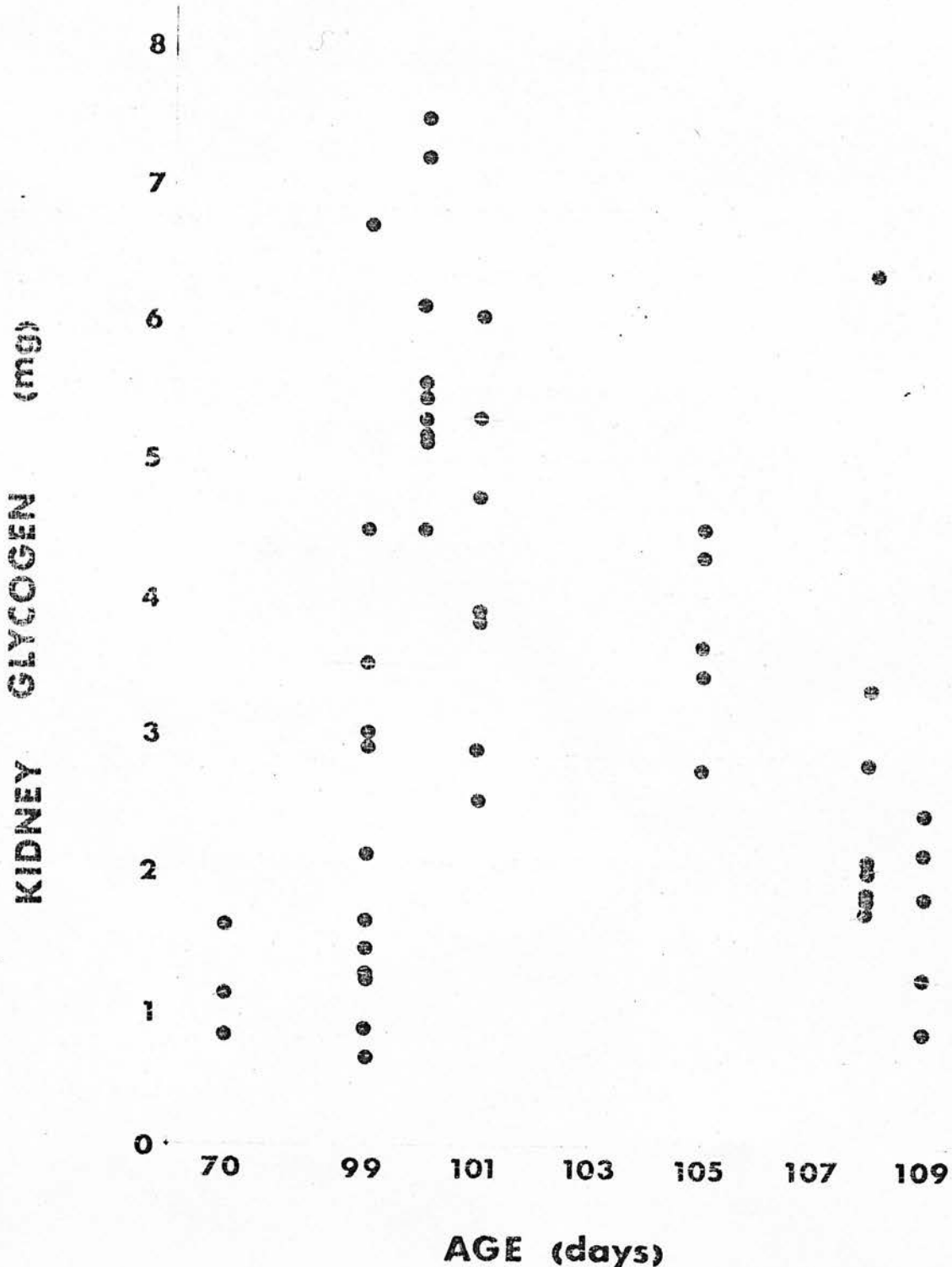


Figure 5.28 The amount of glycogen in the kidneys of individual fetuses aged between days 70 and 109 of gestation.

Total kidney glycogen

Very small quantities of glycogen were found in the kidneys of both foetal and neonatal piglets (figures 5.28 and 5.29). Between 0.8 and 1.6 mg (mean 1.2 ± 0.17 mg) was present on the 70th day of gestation and although the mean content on day 99 was 2.5 ± 0.51 mg the difference between means for days 70 and 99 was statistically not significant ($P > 0.05$). The litter mean kidney glycogen content on day 100 was greater ($P < 0.001$) than on day 99, as well as being higher ($P < 0.05$) than the value recorded on day 101 of gestation (table 5.13). The decline in glycogen content continued on days 105, 108 and 109 of gestation as depicted in figure 5.28 and summarised in table 5.13.

Table 5.13 The litter mean quantities of glycogen in kidneys of fetuses aged between 70 and 109 days of gestation.

Age of foetus (d)	Number of foetuses	Kidney glycogen content (mg)	s.e. mean
70	4	1.2	0.17
99	12	2.5	0.51
100	10	5.7	0.30
101	7	4.2	0.48
105	6	3.7	0.26
108	10	2.6	0.45
109	6	1.6	0.25

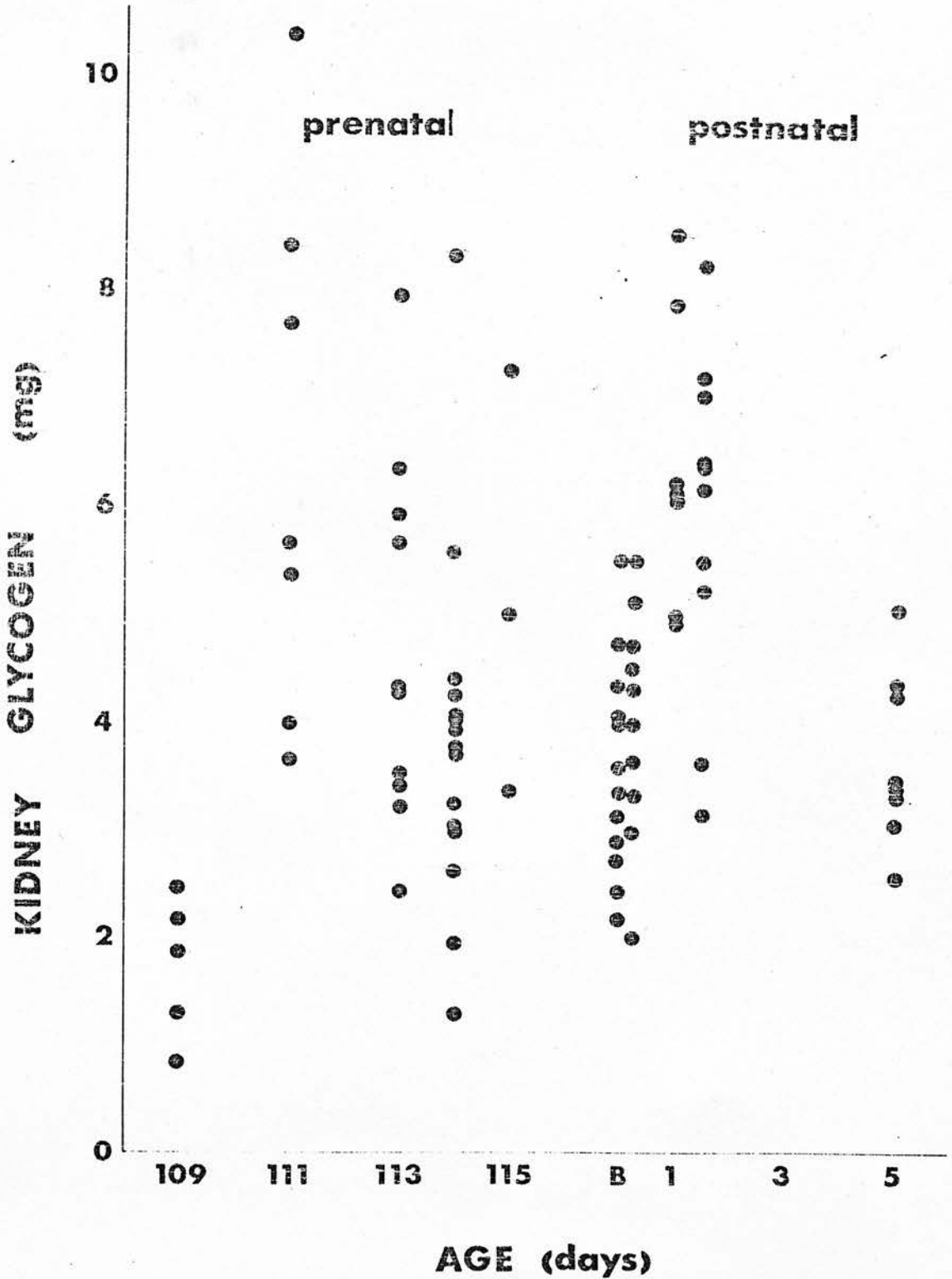


Figure 5.29 The amount of glycogen in the kidneys of individual near term foetal and young postnatal piglets.

The higher values recorded after day 109 may have been anticipated by one foetus on day 103 which had a kidney glycogen content of 6.4 mg, approximately the same amount as the litter mean found on day 111 (table 5.14).

Table 5.14 The litter means for total glycogen in both kidneys of near-term foetal and young postnatal piglets.

Age of piglet	Number of piglets	Kidney glycogen content (mg)	s.e. mean
111d	7	6.5	0.93
113d	10	4.7	0.54
114d	15	3.8	0.42
115d	3	5.2	1.12
Birth (B) 114d	7	3.2	0.29
Birth (B) 119d	6	3.9	0.44
B + 6h	12	4.0	0.30
B + 24h	8	6.9	0.70
B + 36h	10	5.9	0.50
B + 120h	9	3.6	0.28

The amounts of glycogen present at birth and 6 hours following birth were comparable with values at the end of gestation (figure 5.29) and (table 5.14). Slightly larger quantities of glycogen were found 24 and 36 hours post partum whereas 120 hours after birth 3.6 ± 0.28 mg was the litter mean glycogen content recorded.

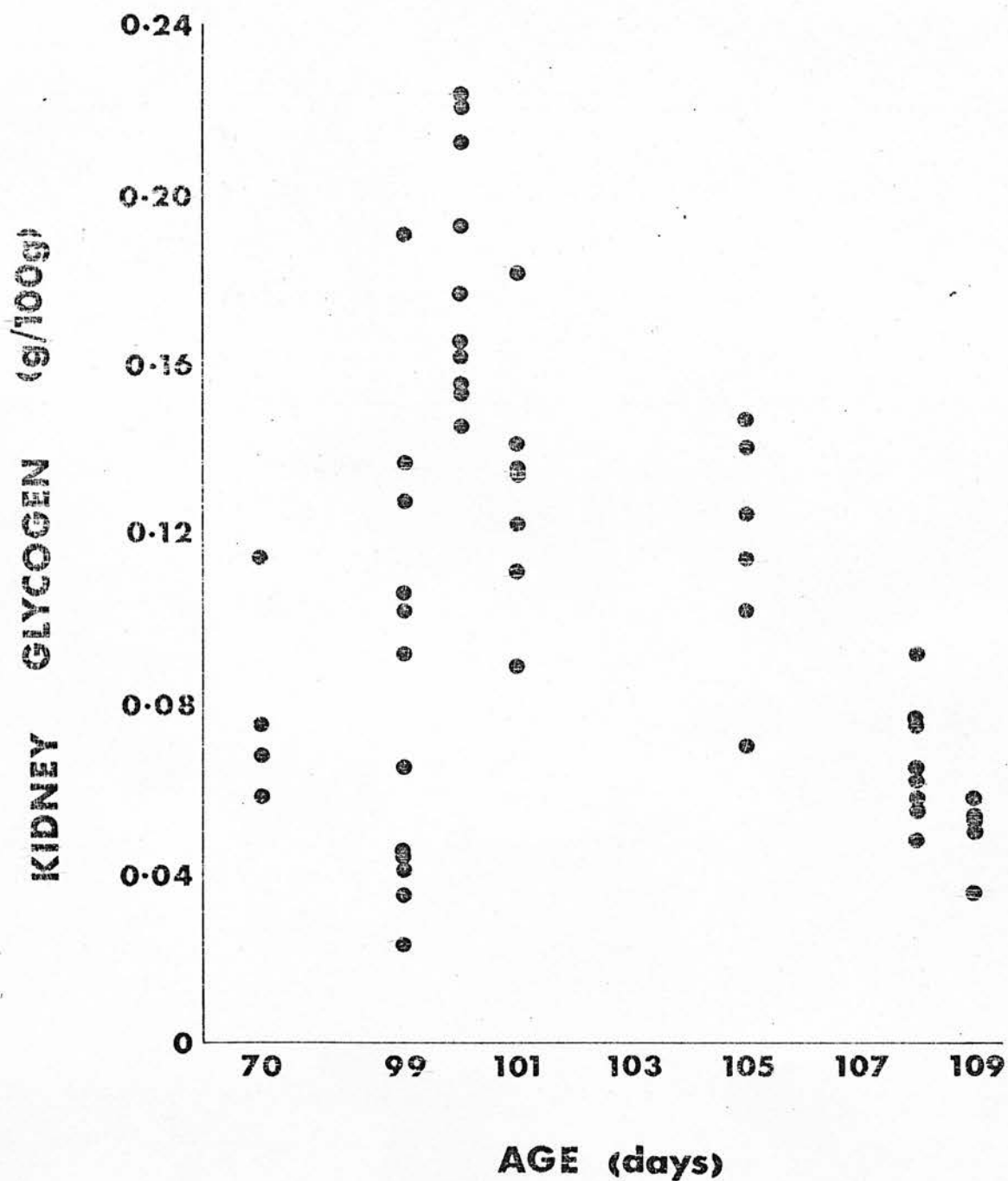


Figure 5.30 The concentration of glycogen in the kidneys of individual pig foetuses aged between 70 and 109 days of gestation.

Kidney glycogen concentration

When the results were expressed in terms of renal glycogen concentration, the variations which occurred with time were very similar to those noted above. Glycogen contributed only very slightly to the weight of the organ, ranging from 0.02 to 0.22 g/100 g over the time period 70 to 109 days of gestation (figure 5.30). The observations are summarised in table 5.15.

Table 5.15 The litter mean concentration of glycogen in the kidneys of foetal pigs aged between 70 and 109 days of gestation.

Age of foetus (d)	Number of foetuses	Concentration of kidney glycogen (g/100 g)	s.e. mean
70	4	0.08	0.012
99	12	0.08	0.015
100	10	0.18	0.009
101	7	0.13	0.011
105	6	0.12	0.011
108	10	0.07	0.009
109	5	0.05	0.004

The pattern of change in glycogen concentration during the last few days in utero and during the first hours following birth (figure 5.31) was similarly analogous to the changes seen when the total glycogen content was plotted against stage of gestation and time post partum (figure 5.29).

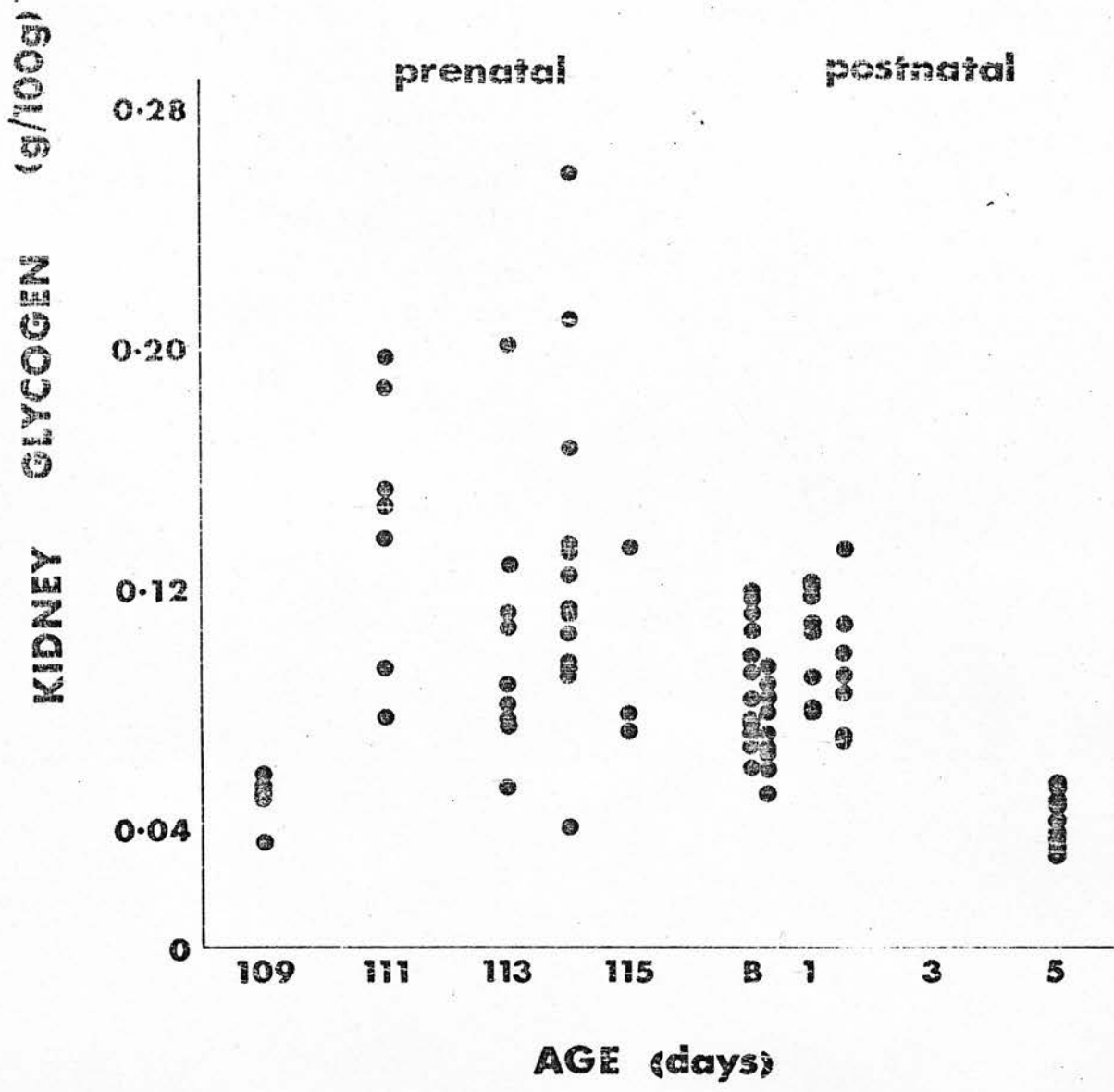


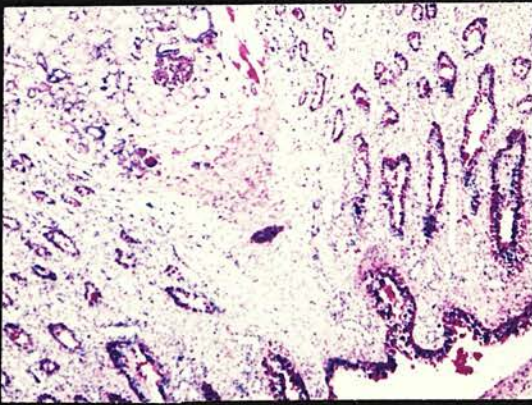
Figure 5.31 The concentration of glycogen in the kidneys of individual near term foetal and young postnatal piglets.

The results have, therefore, been produced in tabular form (table 5.16).

Table 5.16 The litter mean glycogen concentration in the kidneys of piglets at the end of gestation and during the 120 hours following birth.

Age of piglet	Number of piglets	Concentration of kidney glycogen (g/100 g)	s.e. mean
109d	5	0.05	0.004
111d	7	0.14	0.017
113d	10	0.10	0.013
114d	14	0.13	0.014
115d	3	0.09	0.020
Birth (B) 114	7	0.09	0.008
Birth (B) 119	6	0.09	0.008
B + 6h	12	0.07	0.003
B + 24h	8	0.10	0.006
B + 36h	10	0.10	0.010
B + 120h	9	0.04	0.003

There was a greater degree of variation within the litters just prior to birth than was detected following parturition.



Kidney histology

Very small quantities of PAS positive staining material could be seen distributed throughout the tissues of the foetal kidney. The nephrons did not contain glycogen, the PAS positive material being found to be diastase resistant (figure 5.32). High concentrations of glycogen were, however, located in the epithelium of the collecting tubules (figure 5.33 and 5.34), but absent from the epithelium of the loop of Henle (figure 5.34). The smooth muscle fibres in the renal pelvis and vascular smooth muscle were seen to contain glycogen.

No detectable difference in glycogen content with respect to age could be observed histologically.

Figure 5.32 Transverse section of the right kidney removed from a female foetus at 98 days gestation illustrating the structural morphology of the organ (x 40).

Figure 5.33 Same section as is shown in figure 5.32 but demonstrating the red stained glycogen deposited in the epithelia of the collecting ducts (x 50).

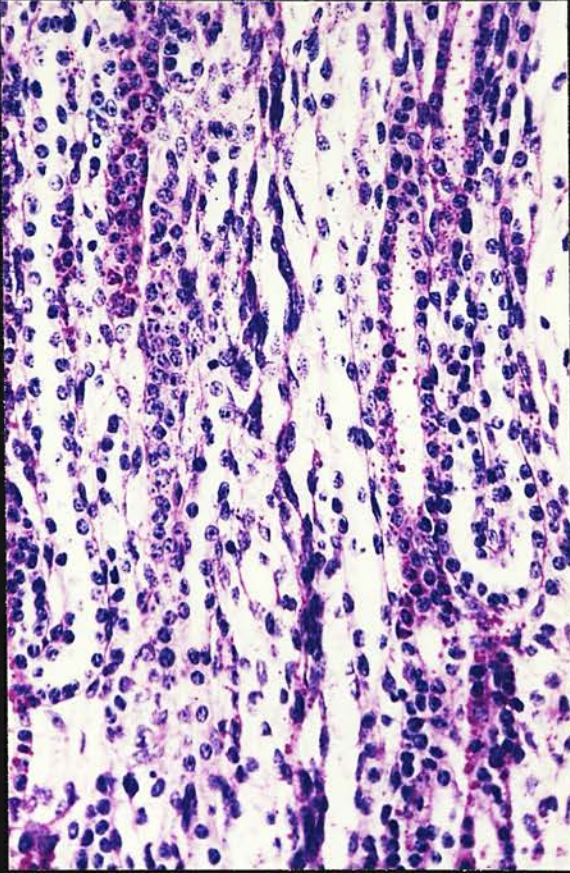


Figure 5.34 The same section as shown in figure 5.32 but illustrating the presence of glycogen in the epithelial cells of the collecting ducts and the cytoplasm of the vascular smooth muscle and the absence of stain from the cells of the loop of Henle (x 320).

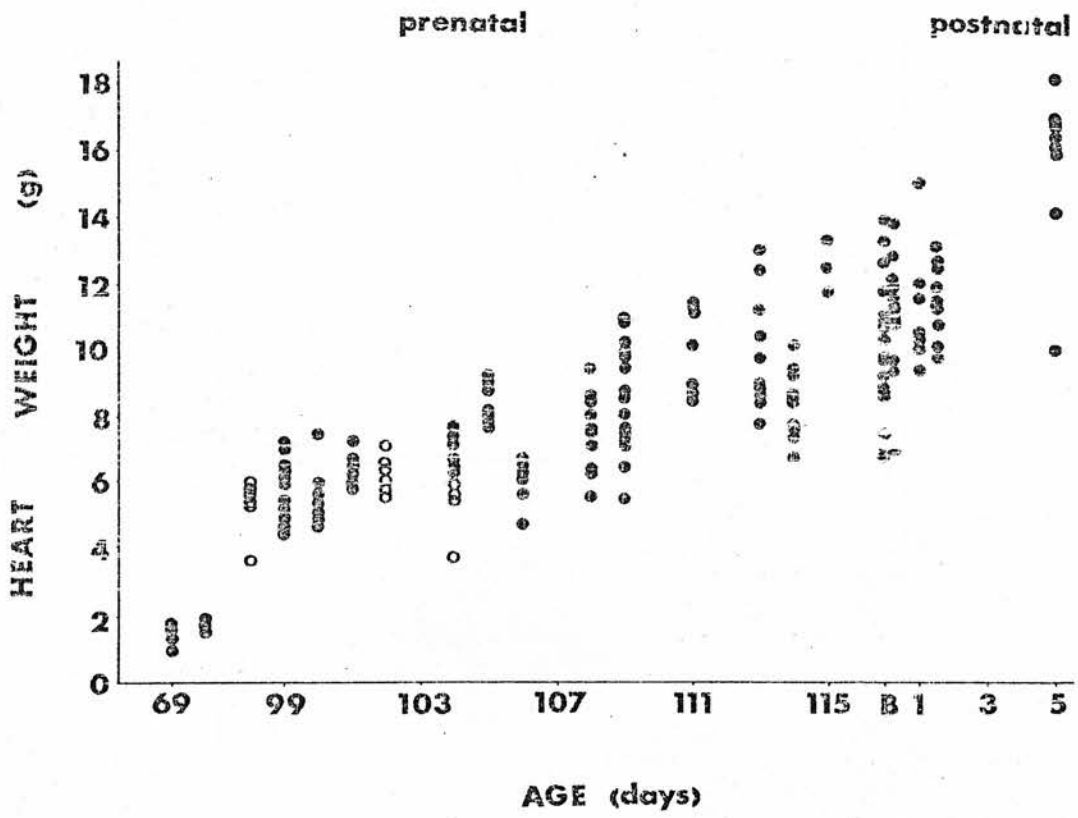


Figure 5.35 The relationship between individual piglet heart weights and stage of gestation or age post partum.

Heart weight

The litter mean heart weight on days 69 and 70 of gestation were approximately 1.5 g and by about day 101 it had risen to 6.32 ± 0.221 g (figure 5.35). Towards the end of gestation, the litter mean heart weight approached 10 g, although the large litter on day 114 (15 foetuses) had a mean heart weight of 8.28 ± 0.226 g. By way of contrast, the small litter on day 115 (4 foetuses) had a mean heart weight of 12.52 ± 0.448 g. Three litters observed at birth gave heart weights of 8.37 ± 0.424 g, 9.82 ± 0.238 g and 10.57 ± 0.849 g; 6 hours after birth there was an increase to 10.87 ± 0.479 g. The mean heart weight 120 hours after birth was 15.74 ± 0.711 g (figure 5.35). Regression analysis by the method of least squares indicated that the prenatal observations were well described by the equation -

$$Y = 6.576 (\pm 0.672)W + 0.0219 (\pm 0.0195)A - 1.41 (\pm 1.47)$$

where Y = litter mean heart weight (g)

W = litter mean body weight (kg)

A = age of foetuses (d)

(See appendix 9 for analysis of variance)

However, when the prenatal and postnatal litters were taken together as a single population of piglets,

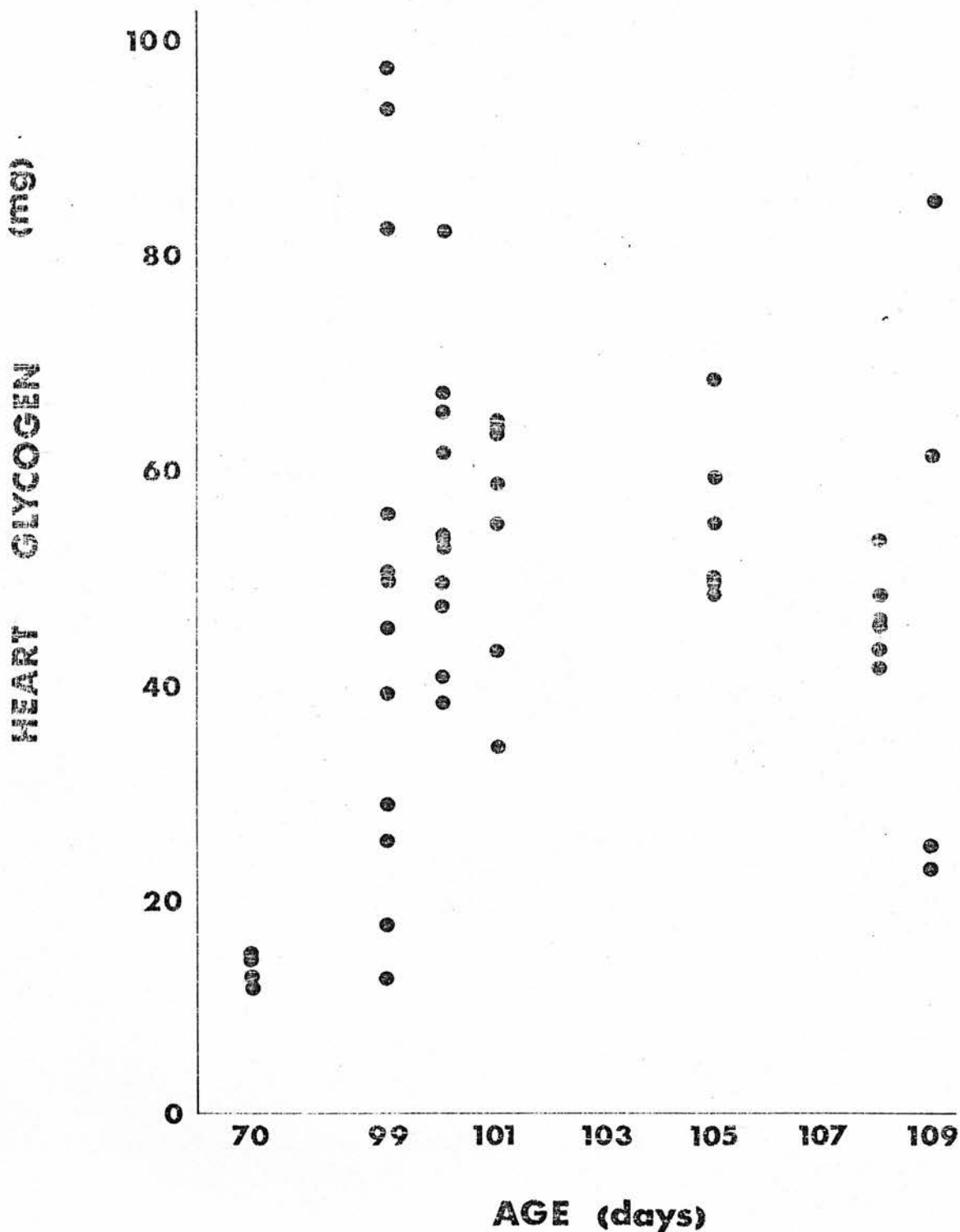


Figure 5.36

The amount of glycogen in the hearts of individual foetal piglets aged between 70 and 109 days of gestation.

the equation -

$$\text{Log } Y = 1.989 (\pm 0.006) + 1.056 (\pm 0.013) \text{ Log } W$$

where Y = heart weight of piglet (g)

W = body weight of piglet (kg)

accounted for the major proportion of the variance (appendix 10).

Total heart glycogen

The small amounts of glycogen present on day 70 of gestation (13.3 ± 0.72 mg) had increased to 49.1 ± 7.63 mg by day 99. There was less variation about the mean on day 100 (55.7 ± 3.80 mg) than on day 99, and the quantity of carbohydrate polymer seemed to remain steady until day 108 (figure 5.36 and table 5.17).

Table 5.17 The litter mean amount of glycogen in the hearts of pig foetuses aged between days 70 and 109 of gestation.

Age of foetus (d)	Number of foetuses	Heart glycogen content (mg)	s.e. mean
70	4	13.3	0.7
99	13	49.1	7.7
100	11	55.7	3.8
101	7	54.8	4.4
105	6	55.2	3.2
108	6	46.6	4.1
109	6	111.3	46.8

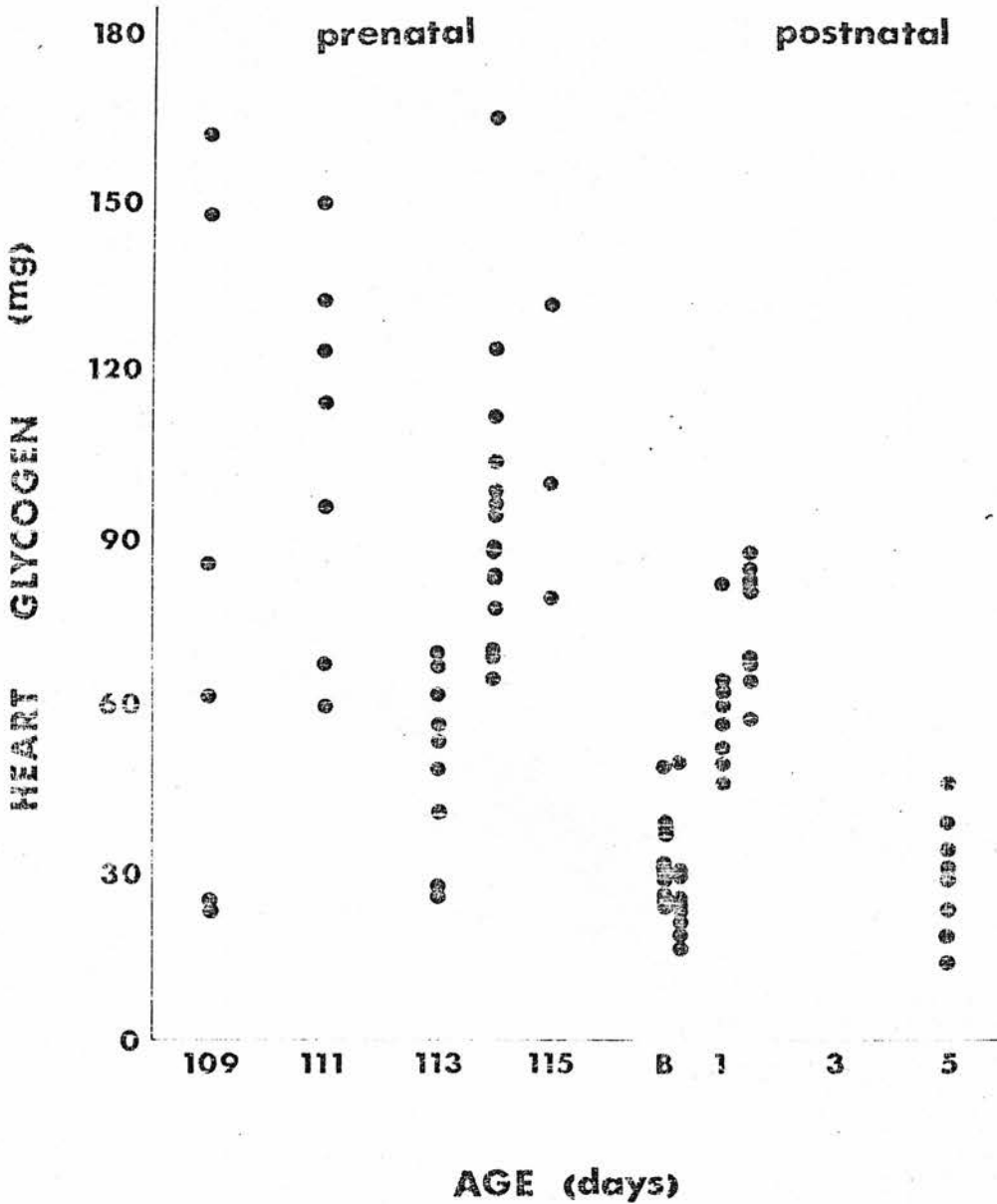


Figure 5.37 The amount of glycogen in the hearts of individual near term foetal and young postnatal piglets.

On day 109 of gestation one foetus had a heart which seemed to contain 325 mg of glycogen; the rest of the litter had less than 180 mg (litter mean 111.3 ± 46.75 mg). This increased variation between foetuses within litters continued on days 111, 114 and 115 (105.8 ± 12.57 mg, 94.3 ± 6.5 mg and 103.5 ± 15.31 mg respectively) together with a two-fold increase in the heart glycogen content (figure 5.37). The amounts present on day 113 (50.7 ± 4.33 mg), however, more closely represented the quantities present on day 108. Only small amounts of glycogen were present in the heart immediately following birth (30.1 ± 1.55 mg and 33.9 ± 4.13 mg) and 6 hours post partum (25.7 ± 2.47 mg). Despite this, the glycogen content seemed to recover 24 and 36 hours after birth when 58.9 ± 3.94 mg and 73.8 ± 3.28 mg were detected; the low level of 30.3 ± 3.44 mg was found 120 hours after birth (figure, 5.37).

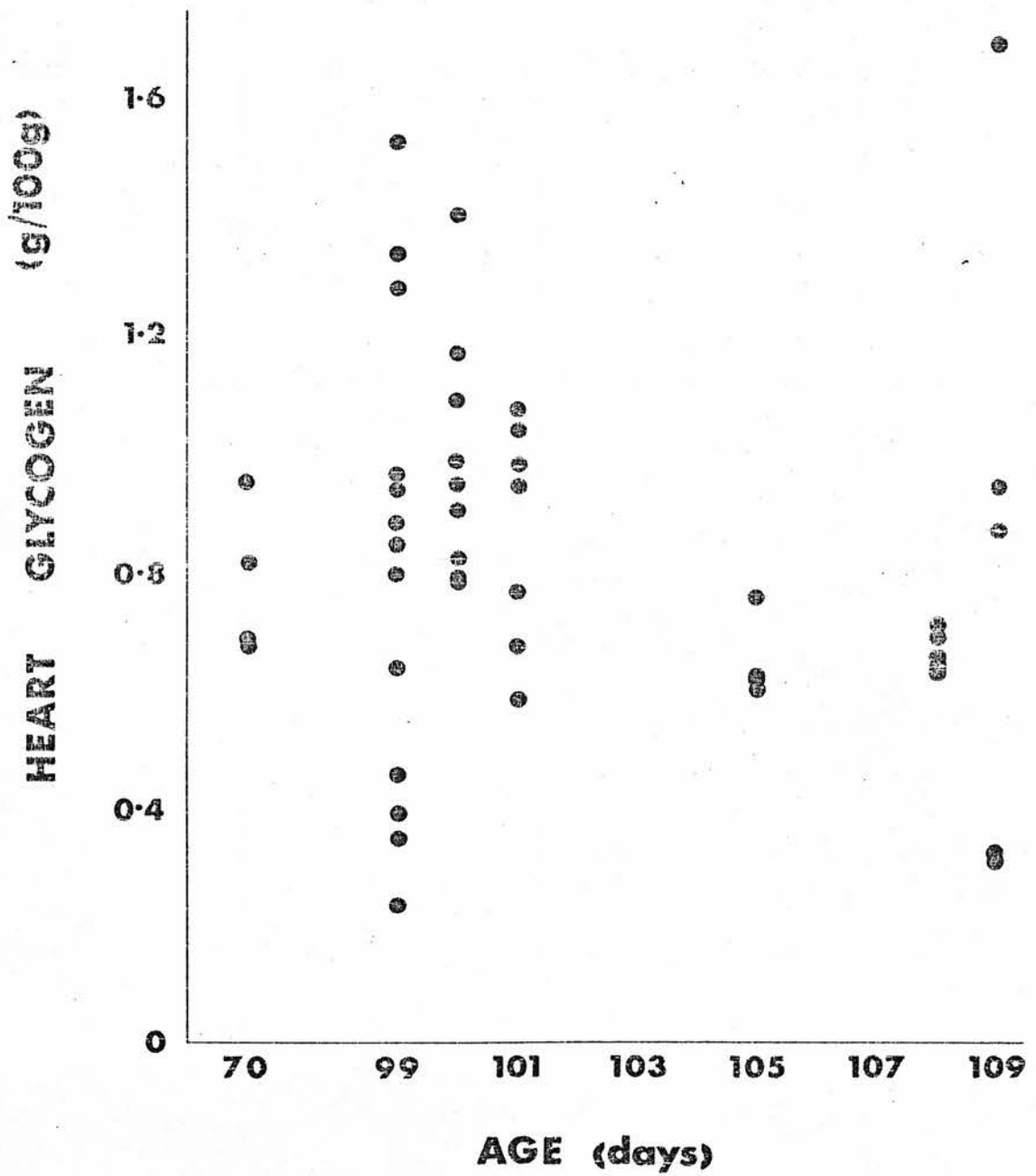


Figure 5.38 The concentration of glycogen in the hearts of individual pig foetuses aged between days 70 and 109 of gestation.

Heart glycogen concentration

The mean concentrations of glycogen in the hearts of 70, 99, 100, 101, 105 and 108 days old foetuses were very similar to one another and could be summarised as shown in table 5.18.

Table 5.18 The mean heart glycogen concentration of litters of foetal piglets aged between 70 and 108 days.

Age of foetus (d)	Number of foetuses	Concentration of heart glycogen (g/100g)	s.e. mean
70	4	0.78	0.07
99	13	0.82	0.11
100	11	0.98	0.06
101	7	0.87	0.07
105	6	0.67	0.03
108	6	0.68	0.01

There was considerable within litter variation on day 99, but this declined thereafter until day 108 (figure 5.38). The wide spread of glycogen content values observed on day 109 was also apparent when the concentration of glycogen in the heart was calculated (1.42 ± 0.62 g/100 g). The remaining litters at the end of gestation had hearts with lower proportions of glycogen; on days 111, 114 and 115, 1.13 ± 0.16 g/100 g, 1.14 ± 0.08 g /100 g and 0.83 ± 0.15 g/100 g were found,

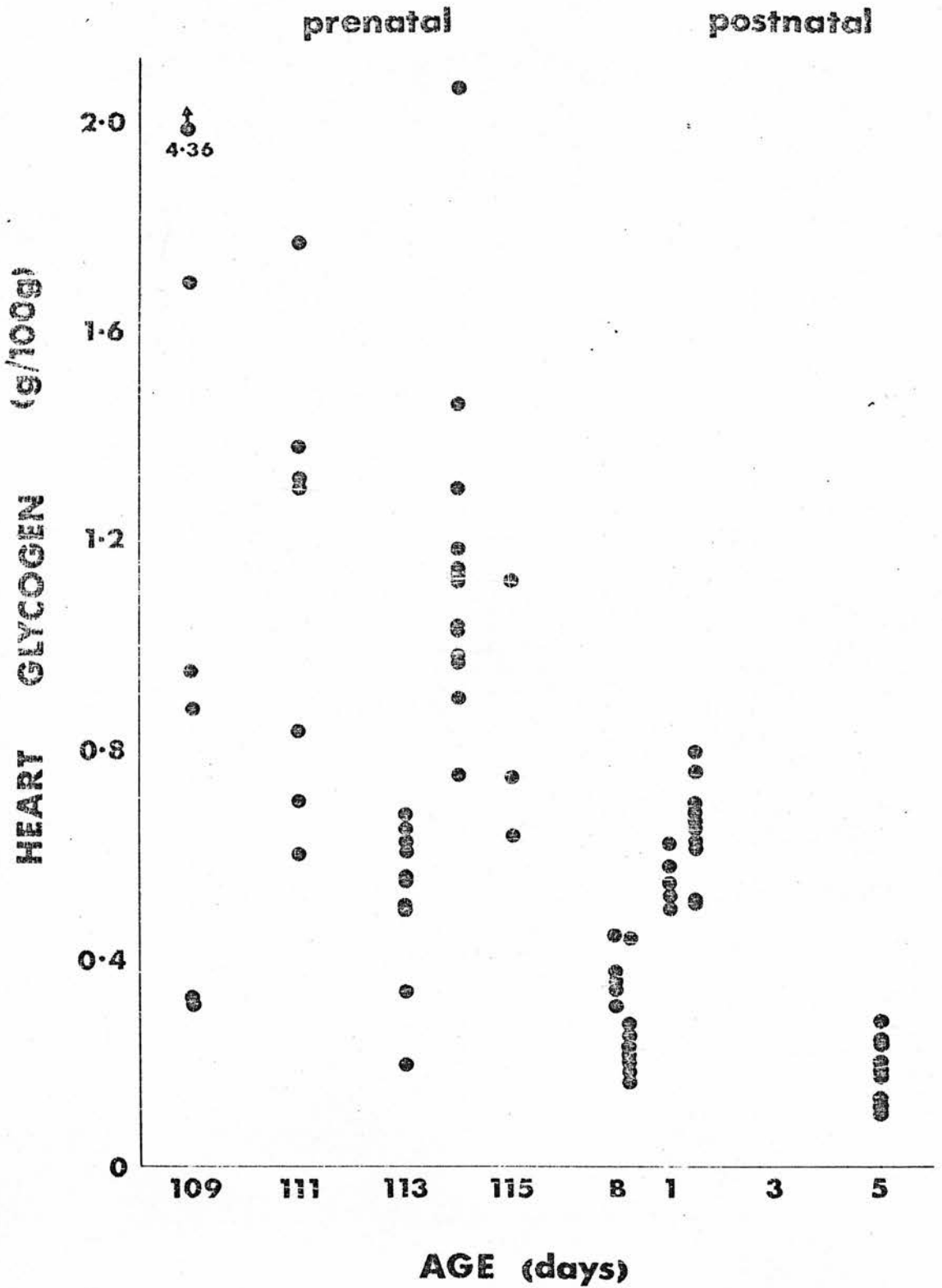


Figure 5.39 The concentration of glycogen in the hearts of individual near term foetal and young neonatal piglets.

and on day 113 of gestation 0.53 ± 0.04 g/100 g was the estimated glycogen concentration (figure 5.39).

The mean heart concentrations 0.33 ± 0.01 g/100 g and 0.36 ± 0.02 g/100 g glycogen detected at birth, were found in litters born following 119 and 114 days of gestation respectively. Six hours after birth the glycogen concentration had fallen to 0.24 ± 0.02 g/100 g, but seemed to have increased to 0.53 ± 0.02 g/100 g and 0.65 ± 0.03 g /100 g by 24 and 36 hours post partum. At 0.19 ± 0.02 g/100 g, the litter mean concentration of glycogen 120 hours after birth was very low (figure 5.39).

Effects of anoxia

The direct effect of anoxia on foetal heart glycogen concentration was tested in a gilt which was 108 days pregnant. The blood flow to the uterus was stopped (maternal cardiac arrest) after six foetuses had been sampled. Observations continued on the following three foetuses and it was found that the cardiac glycogen contents of the piglets sampled after the cessation of maternal blood flow were progressively lower than the mean of foetal cardiac tissue from the foetuses sampled earlier (table 5.19).

Table 5.19 The heart glycogen content and concentration before and after cessation of uterine flow.

Sampling time after arrest of uterine blood flow	Total heart glycogen (mg)	Heart glycogen concentration (g/100 g)
0 *	47	0.68
17	25	0.28
22	18	0.21
28	10	0.13

* Value for litter mean before stoppage of blood flow.



Heart histology

At all stages of gestation studied there was considerably less glycogen in the cardiac muscle (figure 5.40) than in liver tissue, with the muscle of the left ventricle apparently containing more stained material than the musculature of the right ventricle. Purkinje fibres were more heavily stained than the adjacent muscle, but vascular smooth muscle were conversely less heavily stained.

Figure 5.40 The section was taken from the cardiac left ventricle of a foetus at 99 days of gestation. Glycogen was distributed throughout the cytoplasm, but may also be seen associated with the Z line region of the myofibres.

D I S C U S S I O N

Liver

The results show that between days 70 and 100 of gestation, liver glycogen accumulates in the foetus at the rate of 10 mg/d whereas between day 100 and birth, 311 mg was the calculated average daily rate of liver glycogen storage. The observations made by Padalikova et al. (1972) suggested similarly slow (21 mg/d) glycogen storage in the liver prior to day 94 and quicker accumulation thereafter (141 mg/d) to day 114, and yet less than 50% of the rate suggested by the present series of results.

The explanation for the discrepancy lies partly in the differences between the amounts found at the end of gestation; Padalikova et al. (1972) estimated that 3.32 ± 0.251 g of glycogen were present on day 114 of gestation in contrast to 5.23 ± 0.313 g and 4.79 ± 0.811 g recorded at birth by the author. The fact that the same value for glycogen content (0.50 g) was recorded on day 94 by the Czechoslovakian workers and 5 days later in the present investigation also contributed to the lower rate of accumulation found. However, the major proportion of the difference between the two sets

of results may be ascribed to differences in liver weights. The amount of glycogen found by Padalikova et al. (1972) at 114 days gestation represented 10.9 ± 0.57 g/100 g of liver which indicated that foetal liver weights were distributed about a mean of 30.45 g. Foetal piglets at the end of gestation in the present study had mean liver weights in excess of 39.0 g.

The concentration of glycogen in the livers of neonates estimated by Anderson and Wahlstrom (1970) and Swiatek et al. (1970) are in good agreement with the results found by the author, in contrast to the very high concentrations published by Mersmann (1971), Mersmann and Houk (1971), Mersmann et al. (1972) and Seerley et al. (1974). It is difficult to reconcile the glycogen concentrations of 20 g/ 100 g or more found by the latter groups with the observations that water constitutes between 72 and 81 g/100 g of new born liver weight (Widdowson and Dickerson, 1960; Brooks and Davis, 1969), and that protein concentration is in the proportion 13 g/100 g of liver weight (Brooks and Davis, (1969). Moreover, between 2 and 4 g of water have been shown to associate with each gram of liver glycogen deposited (Puckett and Wiley, 1932; Fenn, 1939). In view of the speed with which the hepatic stores of glycogen are mobilised after birth, from about 5.0 g at

birth to less than 1.5 g only 24 hours later, it is not surprising that low concentrations of liver glycogen were reported at 'birth' by Morrill (1952a), McCance and Widdowson (1959) and Elneil and McCance (1965); in each instance some time elapsed between the time the piglets were born and time of liver removal for analysis; Morrill (1952a) indicated that his 'new born' pigs were killed 1 to 15 hours after farrowing. The present series of estimates did not suffer from such sources of error.

The litter mean glycogen contents at birth represented 90 ± 5.4 kJ (after 114 days gestation) and 82 ± 13.9 kJ (after 119 days gestation); 24 hours after birth only 24 ± 4.3 kJ remained. The mobilisation from the liver of about 65 kJ of energy in the form of glucose must be viewed in the context of an estimated loss of about 1437 kJ from the new born piglet during the first postnatal day (Mach and Princ, 1968). It would seem likely that such a relatively small amount of energy will be used to nourish those organs and tissues which depend on glucose as the major source of their energy; for example brain, renal medulla, erythrocytes, lymphocytes and retina (Krebs, 1972) being tissues closely linked to the animals ability to survive the change from

an aquatic, totally dependent environment to one in which it must breathe air and respond to a range of sensory stimuli. The fact that the amount of glycogen in the liver seems to begin to rise 36 hours after birth would indicate that the hepatic stores are necessary only for the short period elapsing between birth and the establishment of suckling behaviour.

Numerous attempts have been made to gain an understanding of how the deposition of glycogen in the foetal liver is initiated and the subsequent accumulation controlled (see reviews by Dawkins, 1965; Walker, 1968, 1971, and Greengard, 1970). The availability of glucose as a substrate undoubtedly plays a role. It was believed as a consequence of the work of Cahill, Ashmore, Earle and Zottu (1958) that glucose entered the liver cell by free diffusion. Recent work would suggest that glucose penetrated the liver cell wall by a very fast active transport system (Williams, Exton, Park and Regens, 1968). In vitro studies have indicated that by elevating the glucose concentration in the medium perfusing isolated rat liver, glycogen synthesis was promoted by the conversion of glycogen synthase (E.C.2.4.1.11) to its active form and the transformation of glycogen phosphorylase (E.C.2.4.1.1.) to the inactive form. Studies of the pattern of enzyme development in the foetal pig

remain less complete than those in laboratory species. Nevertheless, Zhivkova (1968) has detected phosphorylase in piglet livers as early as the 36th day of gestation and Swiatek et al. (1970) substantiated the presence of the enzyme in late gestation, but found that the activity of the active form of the enzyme was low.

Total glycogen synthase levels are high 10 days before birth in the pig (Mersmann et al. 1972) but Swiatek et al. (1970) found that the active form of the enzyme had low prenatal activities. Mersmann et al. (1972) also found low activities when the active form of the enzyme was analysed, and noted that the activity on day 104 of gestation was higher than on day 110 or birth.

Non-circadian 'high frequency' rhythms of liver glycogen content have been found in chick embryos (Sollberger, 1964) and the possibility, therefore, exists that rapid release of glucose from hepatic glycolysis into the foetal blood stream may occur in utero during a period of net glycogen anabolism (Shelley, 1969). Glycogen turn-over studies have been made on the rat foetus, the results of which indicate that total liver

glycogen is mobilised and replaced rapidly (Goldwater and Stetten, 1947).

It would seem probable that the lower quantities of glycogen found in the liver at the end of gestation may be explained on this basis, particularly when it is recalled that the preoperative treatment included for surgical reasons, a 24 hour fast.

Skeletal muscle

The prenatal increase in skeletal muscle glycogen content followed a pattern which was consistent with the view that during gestation this tissue constituted a 'sink' for foetal piglet blood glucose; it is known that skeletal muscle lacks the enzyme glucose-6-phosphatase and therefore, is unable to convert glucose-6-phosphate back to glucose (Newsholme and Start, 1973). The fact that the concentration of glycogen in skeletal muscle did not follow the same type of consistently increasing trend as the weight of muscle glycogen indicated that another component of skeletal muscle mass was varying. The possibility that it was changes in water content which may have occurred can be deduced from both the work of Greenleaf, Olsson and Saltin (1969) and Olsson and Saltin (1970), who were able to demonstrate

that 3 to 4 g of water is bound to each gram of glycogen in skeletal muscle, and from the observation that the skeletal muscle of the foetus includes large but decreasing amounts of extracellular fluid (Dickerson and Widdowson, 1960). It is likely that the fluctuations found in foetal skeletal muscle glycogen concentration reflect the integration of an increase in intra-cellular glycogen-associated water and a decline in extracellular fluid. In order to present estimates of the total quantity of glycogen in the skeletal musculature of the piglet, it is necessary to make several assumptions; firstly that the muscle glycogen measurements made during the present investigation represent good estimates of the composition of the whole piglet musculature; secondly, that the muscle tissue represents 38% of the foetal body weight and 34% at birth. The concentration of glycogen in extensor carpi radialis muscle at birth was the same as the glycogen composition of semimembranous muscle (Dalrymple, et al. 1973) and the unspecified muscle tissue analysed by Elneil and McCance (1965), higher than the amount in quadriceps femoris (Holub, Padalikova and Filka, 1961) and trapezius muscle (Dalrymple, et al. 1973), but lower than the concentrations in gluteus maximus and longissimus dorsi muscles (Curtis et al., 1966; Seerley et al., 1974)

semitendinosus (Anderson and Wahlstrom, 1970) and the unspecified muscles studied by McCance and Widdowson (1959). Although variations in glycogen concentration between different muscles have been recognised for almost a century (Cramer, 1887), it is unlikely that this alone accounts for the diversity seen between the values published. The present investigation employed (unlike the other studies) the glucose specific enzymic method of analysis, thereby avoiding the problems of cross reaction which result in elevated values when 'reducing substance' methodology is used (Hugget and Nixon, 1957). Very few observations have been reported on the developmental stages of foetal and neonatal skeletal tissue weight in the pig (Macdonald, 1971). McMeekan (1940) found that 29% of the body weight of new born piglets was comprised of skeletal muscle, whereas the dissection studies undertaken at the Rowett Institute led Fowler (1974) to suggest a higher figure, within the range 32 to 35% of the birth weight. There was between 37 and 44% 'flesh' in the bodies of piglets at the end of gestation (Pomeroy, 1960) which would agree with the proportion of 'flesh' found in the lamb foetus at comparable stages of gestation (Wallace, 1948). In view of the fact that unlike the lamb foetus there are no large accumulations of fatty tissue in the prenatal or new born piglet (McMeekan, 1940; Wallace, 1948; Pomeroy, 1960) the foetal 'flesh' percentage value was thought likely to

Table 5.20 Estimated litter mean glycogen content of the musculature of foetal and neonatal pigs.

Stage of gestation (d)	70	99	100	101	105	108	109	111	113	114	115	Birth
Litter size	7	13	11	11	6	15	14	7	11	15	4	11
Litter mean body weight (g)	275	767	723	834	1118	1023	1183	1293	1395	1133	1613	1144
Muscle percentage	38	38	38	38	38	38	38	38	38	38	38	34
Litter mean body musculature (g)	104	292	275	317	425	389	449	491	530	431	613	435
Litter mean muscle glycogen (g/100 g)	1.0	1.8	3.6	3.8	3.2	5.1	5.2	4.1	3.8	5.0	4.1	6.2
Litter mean total muscle glycogen (g)	1.0	5.3	9.9	12.0	13.6	19.8	23.4	20.1	20.2	21.5	25.12	27.2

represent the percentage of skeletal muscle tissue plus associated ligaments. In the present study the muscle tissue was not dissected free of tendon and therefore, the value 38% was chosen from Pomeroy's results as representative of the amount of such tissue during the second half of gestation. When the data given by McMeekan (1940) were reanalysed to include an estimate of the intramuscular fat, tendon and the moisture lost during dissection the resulting figures were similar to the value 34% chosen for the muscle of new born pig.

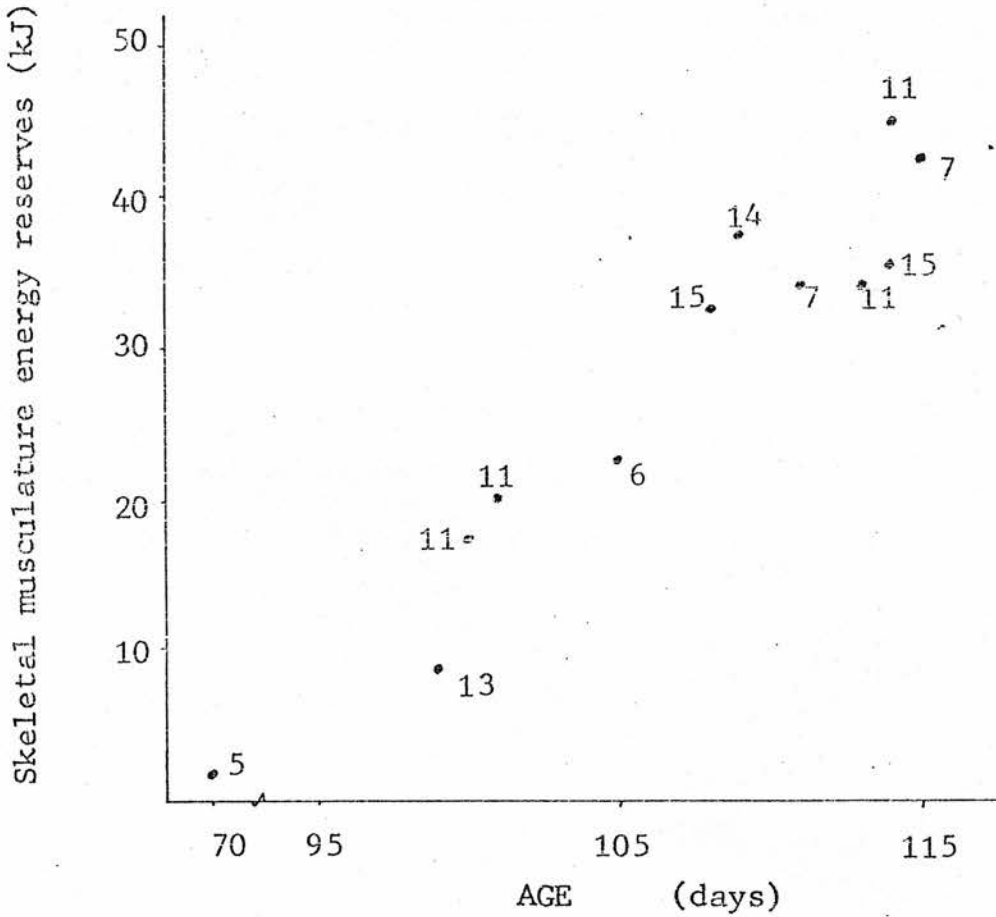


Figure 5.41 The relationship between foetal and neonatal total skeletal muscle, energy reserves (glycogen) and stage of gestation. (Number of foetuses sampled as shown in figure).

The calculated values for the weight of glycogen present in the musculature of the foetus and new born piglet are shown in table 5.20. These results would suggest that a greater amount of glycogen than the 13.4 g estimated by Holub et al. (1961) may be found stored in the muscle tissue of the newly born pig. The quantity 27.2 g glycogen represents about 470 kJ of stored muscular energy available at birth; the same calculation carried out on the value for the litter mean 100 day pig gave a value of 170 kJ for stored energy. These values, along with those representing other stages of gestation, were plotted against gestational age (figure 5.41), and the linear regression equation calculated by the method of least squares had the form -

$$Y = 18.3 X - 1668.8$$

where Y = energy stored as body muscle glycogen (kJ)

X = stage of gestation (d)

Analysis of variance indicated that the line gave a good fit to the glycogen estimates ($P < 0.001$). From the slope of the line it may be suggested that each day 18.3 kJ of energy was being accumulated in the muscle, which in terms of weight of glycogen deposited represented 1.1 g/d.

The limitations of the calculation prevented consideration of the estimated differences with respect to muscle glycogen content between foetuses within the same litter; Pomeroy (1960) found that there were inconsistent differences in the proportion of muscle between large and small siblings. The lack of observed rather than estimated values for muscle as a percentage of body weight at different times during the first few days after birth reduced the amount of confidence which could be placed in the estimates of glycogen content based thereon. Reductions in body weight after birth which will occur as a consequence of decreasing total body water content (McCance and Widdowson, 1959; Manners and McCrea, 1963) are confounded by increases in alimentary tract contents (from ingested milk) and the weight of fatty tissue (Manners and McCrea, 1963; Elsley, 1964). The figure chosen to represent the proportion of muscle in the body of the pig 24 hours after birth (30%) was therefore, based on McMeekan's results, adjusted in the light of advice given by Fowler (1974) and Elsley (1974), and the observations published by Manners and McCrea (1963). This estimate led the author to suggest that 5.5 g of glycogen remain in the musculature of the foetus 24 hours post partum, a decline from the amount present at birth of about 20 g glycogen, representing 343 kJ of

energy.

Whether such a drop would occur may be questioned in the light of recent work by Schiaffino and Hanzlikova (1972). They were able to show that in the neonatal rat, glycogen depletion is relatively slow in the immature leg muscles (extensive deposits were found still present 24 hours after birth), in contrast to the more developed diaphragm muscle from which glycogen stores were almost completely mobilised in the first six hours after birth.

Lung and Kidney

The present study suggested that the glycogen concentration in the lung of the piglet at mid gestation was higher than towards the end of gestation, a finding which has also been made in sheep (Fauré - Fremiet and Dragiou, 1923; Shelley, 1960), rhesus monkey (Shelley, 1961) and man (Szendi, 1936; Villee, 1954). However, Padalikova et al. (1972) reported that 'the glycogen percentage in the lungs shows statistically insignificant variation, ranging about the average value of 2.4%'. Just prior to birth the author observed that glycogen represented less than 0.5 g/100 g of piglet lung tissue, as was reported in other species (Szendi, 1936; Shelley, 1960; 1961); Padalikova et al. (1972) by way of contrast

estimated that 1.7 ± 0.13 g/100 g was the proportion of glycogen present on day 114 of gestation.

The present study demonstrated that pulmonary glycogen is associated with the epithelial cell population thereby confirming for late stages of gestation the localisation pattern observed in pig embryos (Gage, 1906) and neonates (Baskerville, 1970). It is, therefore, noteworthy in this connection, that at the time when the epithelial lining of the bronchioles begins to become discontinuous - approximately 80 to 85 days of gestation (Flint, 1906; Clements, 1938) - Padalikova et al. (1972) detected their highest concentration of glycogen.

It is possible that the discrepancy between the present series of results and those of Padalikova and co-workers may lie in the methods used. The prenatal lung was full of fluid at every stage of pregnancy sampled by the author; it is not clear if the Czechoslovakian workers removed this material prior to assay or analysed the organ intact as in the present study. A definite sequence of cell maturation with respect to glycogen content has been demonstrated among the five lobes of the foetal rabbit lung (Kikkawa et al. 1971), with the upper lobes consistently more mature and with maturation proceeding caudally from the apex to the base, a scheme consistent with the embryological development of the pig

lung (Flint, 1906). Whether the difference between the present study (where the whole lung was analysed) and that of Padalikova et al. (1972) (where it is not clear what tissues or parts were sampled) may be explained on this basis similarly remains unclear.

The results for both lung and kidney revealed a peak of glycogen content and concentration at about days 100 to 101 of gestation with kidney exhibiting the increased amounts of carbohydrate a day earlier than lung tissue. Thereafter, the amounts present in both organs declined. No comparable change in lung glycogen content was noted by Padalikova et al. (1972) since they carried out observations solely on days 94 and 104 of gestation and not at any intervening period. However, they did note that approximately the same concentrations of glycogen were present on each of the days which would indicate that the rise in glycogen concentration commenced at or shortly after day 94 of gestation.

The liver results suggested that glycogen content and concentration had begun to rise at some stage of gestation between days 90 and 100, and extrapolation of the total body muscle regression equation, to the value for the amount estimated to be present on day 70 of

gestation, indicated that the increased rate of glycogen deposition commenced at about day 92. The apparently co-ordinated increase in organ glycogen content suggested that each tissue was responding to an alteration in the overall hormonal status of the foetus.

Hormonal status of the foetus

Over fifty years ago Aron (1920a, b, c, 1922a, b, 1924) drew attention to the correlation he had found between the increase in liver glycogen deposits and the appearance of islets of Langerhans in the foetal pancreas. Subsequent studies by Lockwood and Misbin (1972) would suggest that the pancreas of the 100 day old foetal piglet contained significant amounts of insulin, although on the 104th day of gestation, plasma insulin levels of less than 4μ units/ml were reported (Swiatek et al., 1970). No measurements appear to have been made at an earlier stage of foetal development in the pig; however, between 5 and 20μ units/ml were recorded in unfed new born piglets 1 hour after birth (Gentz, Persson, Kellum, Bengtsson and Thorell, 1971).

Although insulin was present in the pancreas of the rabbit foetus prior to day 20 (Milner, 1969), on day 24

of gestation, a peak of plasma insulin concentration was noted (Kervran, Jost and Rosselin, 1972) which coincided with the start of glycogen accumulation by the liver (Szendi, 1936). Infusion of insulin antibodies into the foetal rat decreased the incorporation of ^{14}C -glucose into liver glycogen (Manns and Brockman, 1969), thereby suggesting that insulin plays an important role in controlling foetal liver glycogen synthesis in that species.

Aron (1922a) had suggested that the foetal liver was responding to a purely foetal stimulus and studies recently carried out would tend to support such a thesis, the placentae of various species apparently having only a limited permeability to insulin (Goodner and Freinkel, 1961; Josimovich and Knobil, 1961; Alexander, Britton, Cohen and Nixon, 1972). The possibility that a comparable state holds for the pig placenta remains to be tested.

The possibility that the foetal adrenal also exerted a controlling influence over liver glycogen arose out of the studies of Long, Katzin and Fry (1940), Jost and Jacquot (1955) and Jacquot (1959); in adrenalectomised maternal rats, foetal liver glycogen on day 21

of gestation was normal at about 7 g/100 g whereas in the livers of fetuses decapitated on or before day 18 the concentration of glycogen remains very low (less than 1 g/100 g). It was soon noted, however, that substantial species differences existed with respect to the foetal response to corticosteroids administered after decapitation; in the rat rapid liver glycogen accumulation follows cortisol injection, although such was not the case in the foetal rabbit (Jost and Jacquot, 1955; Jacquot, 1959).

Some of the discrepancy between results from different species was resolved when it was appreciated that maternal adrenocortical hormones could reach the rat foetus (Jacquot, 1959) whereas in rabbits, although the doe was not adrenalectomised, maternal hormones could not compensate for foetal decapitation (Jost and Picon, 1970).

No comparable studies seem to have been carried out on the pig. Nevertheless, results of recent investigations of maternal blood plasma corticoid levels detected a peak concentration over days 98 and 99 of pregnancy (Killian, Garverick and Day, 1973; Ash, Challis, Harrison,

Heap, Illingworth, Perry and Poyser, 1973) which would seem to match the rises seen in lung and kidney glycogen content in the present study. Foetal competence to produce adrenal 17 - hydroxycorticosteroid has been demonstrated on day 103 of gestation (Dvorak, 1972) but appears not to have been sought earlier. The ability of the pig placenta to transfer foetal and maternal hormones also seems either to have escaped attention or technically is not easy to study. It is now appreciated as a result of in vitro studies with organ cultures of foetal rat liver explants that both glucocorticoids and insulin have important roles in the development of glycogen synthesis in that organ. Studies by Schwartz and Rall (1973) and Eisen, Goldfine and Glinsmann (1973) have produced results which support the hypothesis that the increase in liver glycogen synthesis requires glucocorticoids for the induction of glycogen synthase and insulin for the activation of the enzyme. However, the precise effects that adrenal and pancreatic hormones have on glycogen in foetal lung, kidney and skeletal and cardiac muscle still remain to be elucidated (Alescio and Dani, 1972; Litwack and Singer, 1972).

Heart

The present study provided support for the concentration of cardiac glycogen (0.34 g/100 g) reported at birth by Elneil and McCance (1965), and in failing to detect levels greater than 0.45 g/100 g was therefore unable to substantiate the value of 1.5 g/100 g published earlier by McCance and Widdowson (1959). The concentrations measured in the new born guinea-pig were comparable (Shelley, 1961) whereas rat, rabbit and rhesus monkey all showed higher proportions of glycogen (Dawes and Shelley, 1968).

The similarity between the pig and guinea-pig in this regard may be extended with the observation that newly born members of both species were found to be unable to survive asphyxia for more than 3 or 4 minutes (Miller and Miller, 1965), whereas rat and rabbit could survive over five times longer (Dawes, 1968). It seemed possible to Dawes et al. (1959) that there was a causal relationship between the animal's ability to survive asphyxia and the concentration of glycogen in the heart, a suggestion compatible with the belief that maintenance of the circulation may be the limiting factor determining survival.

It was noted in the present series of foetal studies after removal of the heart that the time to the last gasp was of the same length as when new born piglets were asphyxiated (Miller, Miller and Westin, 1964); Miller, Zakhary and Miller, 1964). In a separate study undertaken by the author it was noted that when a 99 day old foetus was delivered enclosed in its foetal membranes into a warm (37°C) bath of saline, the time to last gasp was prolonged in comparison with litter mates whose hearts had been removed for glycogen analysis. In view of the observation that the concentration of glycogen following 99 days of gestation is almost three times the concentration at birth, these results would tend to support the notion that the piglets' inability to survive prolonged asphyxia at birth arises as a consequence of the low concentrations of glycogen to be found in the heart.

There was evidence to suggest that the litter sampled on day 114 of gestation was about to be born. However, the concentration of glycogen in their hearts was over four times the concentration found in new born piglets' hearts. These observations suggested that the cardiac reserves of carbohydrate were mobilised during

the birth process itself. Support for the suggestion came from the finding in a gilt 108 days post coitus that 17 minutes after the arrest of maternal blood flow, less than 50% of the cardiac glycogen concentration present before cardiac arrest remained. Further declines in glycogen concentration occurred until approximately 20% of the former amount was left 30 minutes following the stoppage of maternal blood flow. Jones (1966) and Randall (1968) have shown that the interval between the delivery of individual piglets was about 15 minutes. Taken together with the observations made on other species (reviewed by Dawes, 1968) and (Dawes and Shelley, 1968), the carbohydrate stored in the heart of the foetus at the end of gestation may be regarded as providing energy to maintain the circulation during a transient period of hypoxia in the life of the piglet. That the amounts stored in the heart may not always be completely adequate is suggested by the finding that piglets born with a ruptured umbilical cord all scored below 6/10 when assessed using an Apgar type viability score (Randall, 1971).

Nutrition and energy storage

The amount of glycogen present in the body of the pig foetus at term has been estimated to be in excess of

30 g according to the measurements made on liver, lung, kidney, skeletal and cardiac muscle during the present investigation. This amount is larger than the estimates of body carbohydrate reserves given by Holub et al. (1961), McCance and Widdowson (1959) and Elneil and McCance (1965) but agrees with the findings of Mitchell, Carroll, Hamilton and Hunt (1931); the latter group analysed foetuses at term and discovered that approximately 37 g of dry matter could not be accounted for when the crude protein, ether extract and ash were summed. In view of the large amount of skin (~ 200 g) possessed by the foetal piglet (Pomeroy, 1960) and the concentration of glycogen (2.8 ± 0.25 g/100 g) estimated to be present in this tissue (Padalikova et al., 1972), it would appear that the difference between the weight of glycogen found by the author and the 'lost' weight reported by Mitchell et al. (1931) could be largely accounted for as skin glycogen.

The susceptibility of foetal glycogen reserves to maternal energy intake was demonstrated in 1935 by Stuart and Higgins. The livers of foetal rats from pregnant animals fasted for 22 hours were found to contain less than half of the glycogen that was present after a fast of 10 hours. The foetal cardiac stores of glycogen have also

been shown to decline following a period of maternal starvation (Shelley, 1961). It was suspected that the livers of piglets in the present series were retaining less glycogen than could be normally anticipated and that this was a result of the pre-surgical routine of starving the gilt for 24 hours. Efforts to increase foetal liver glycogen content by infusing solutions of glucose into pregnant rabbits failed, although there appeared to be an increase in cardiac glycogen (Gelli, Enhorning, Hultman and Bergstrom, 1968). Anderson and Wahlstrom (1970) found that gilts with high pregnancy weight gain produced piglets having a higher liver glycogen content at birth than piglets from gilts which gained less during pregnancy. They also noted that dichlorvos (2,2-dichlorovinyl dimethyl phosphate) fed to the gilt produced an apparent increase in foetal liver glycogen concentration. However, Batte, Robinson and Moncol (1968) found no effect on liver glycogen content at birth when dichlorvos was fed.

The present author fed two gilts for the last five weeks of pregnancy on a diet containing 50% less energy than the pellets given to the other experimental gilts. It was found at birth that the piglets from the under-

nourished animals had litter mean liver glycogen contents of 4.03 and 4.33 g of glycogen in contrast to the levels of 4.79 and 5.23 g of glycogen in the livers of neonates from well-fed mothers. Less glycogen was also found in skeletal and cardiac muscle.

There would appear, therefore, to be some evidence to support the view that foetal glycogen reserves can be influenced by changes in the level of maternal nutrition.

C O N C L U S I O N S

The observations made on foetal and neonatal tissues with regard to glycogen content led to the formulation of the following conclusions:

1. The foetal liver began to accumulate glycogen at a more rapid rate, on or shortly after day 94 of gestation.
2. The amount of glycogen in the liver of the new born piglet is to some extent dependent on the nutrient intake of the mother.
3. Approximately the same total quantities of liver glycogen were accumulated in utero almost irrespective of the number of fetuses in the litter; very small litters (less than 6 fetuses) would seem to be exceptions to the generalisation.
4. The glycogen concentration in the liver at birth lay between 10 and 15 g/100 g whereas muscle glycogen concentration was approximately 6 g /100 g.

5. Calculations indicated that an average 27 g of glycogen were stored in the skeletal musculature of the new born pig.
6. Approximately 1.1 g of glycogen was accumulated in the skeletal musculature of the foetal piglet during the last 14 days of gestation.
7. On average about 20 g of glycogen were mobilised from the skeletal muscle of the new born piglet during the first 24 hours post partum.
8. Lung glycogen is associated with the epithelial cell population of the lung.
9. The concentration of glycogen in the lung is higher at 70 days gestation than during the last 14 days.
10. Both lung and kidney exhibit a peak of glycogen at about day 100 and 101 of gestation.
11. There is evidence to suggest that foetal insulin and adrenal cortex hormones may be

responsible for the increased accumulation of foetal tissue glycogen.

12. The concentration of glycogen in the heart of the term foetal pig was over four times the concentration found in new born piglets' hearts (0.35 g/100 g).
13. The heart glycogen reserves were adequate for maintaining the circulation of the anoxic piglet over a short period of time only.
14. Energy reserves of the new born piglet which are stored as glycogen amount to about 37 g.

SUGGESTIONS FOR
FURTHER STUDIES

The present study indicated that the changes in tissue glycogen content occurring between days 90 and 105 of gestation are worthy of closer study. Similarly, the differences between litters containing the same, and different numbers of foetuses has been poorly investigated. Little is known about patterns of change in glycogenic and gluconeogenic enzyme activities in the tissues of the foetal pig at different stages of gestation, and the influence of endogenous or exogenously applied hormones is almost completely unknown. Studies in this area may assist in promoting earlier, and perhaps, therefore, larger deposits of foetal energy reserves.

The role that glycogen may play in foetal water and mineral metabolism is another aspect of foetal physiology worthy of study. The role of neural stimuli on the energy metabolism of the pig foetus similarly deserves attention.

6.

DISCUSSION

The development of the foetal pig was studied from a number of different but related points of view. As a consequence, it was possible to obtain a better appreciation of growth as the process resulting from the integration of a large number of sometimes conflicting changes in the physiological state of the animal.

It seemed reasonable to focus attention first on the relationship between the foetal and maternal circulations, as this anatomical region was for the foetus the source of all nutrients and the ultimate site for all waste disposal. The changes which were observed in the morphology of the vascular relationship between the maternal and foetal pig, at different times of gestation, raised questions with regard to the stimuli which caused the two circulations to be aligned in the way found. It was anticipated that the surface area of contact between the two circulations would increase in order to contribute to the increased nutritional and waste excretion requirements of the growing foetus. However, it was not immediately apparent why the capillaries should be so arranged that the maternal and foetal blood appeared to flow in parallel, as was suggested by both the anatomical and respiratory gas tension evidence.

Such a pattern has been calculated to be less efficient than countercurrent blood flow when transfer of heat or nutrients between the two streams were considered (Faber, 1969, 1973; Bartels, 1970). It is possible that the uterine blood flow is large enough to compensate for any loss of efficiency resulting from such an arrangement. It is also possible that the placental area of the pig foetus could increase during the second half of gestation, but only at the "cost" of a less efficient placental blood flow pattern. The answers still remain to be found.

Many of the deficiencies in the methods used by the author to study the distribution and pattern of placental blood flow were appreciated at the outset (Macdonald, 1972), but they were employed nevertheless in order to provide the preliminary evidence and experience upon which could be based a more thorough investigation, which will be carried out when the necessary resources become available.

The integration of the observations made on respiratory blood gas tension and blood glucose concentration gave evidence to suggest that glucose was supplying less than 70% of the energy metabolism of the

pig foetus. That the foetus was metabolising amino acids to supply energy was suggested by the results tabulated in appendix 4. Although no reliable quantitative evidence of energy supplied by lipid metabolism was obtained in the present study, it is known from the work of Feaster, Neal and Wallace (1966) that fatty acids can be transferred across the placenta of 85 day old pig foetuses.

A number of technical difficulties remain to be overcome before it will be possible to measure with accuracy the substrates metabolised by the foetus in utero. Steps have nevertheless, been taken to gain an impression of overall foetal metabolic rate (Needham, 1931, 1942; Brody, 1938, 1945; Moustgaard, 1962; Zotin, 1972). The evidence would suggest that the metabolic rate of the foetus (expressed as O_2 ml/h/g) declines during the second half of gestation. The results of a large series of experiments on the metabolic rate of the new born pig are consistent with such a view (Mount, 1968; Mount and Stephens, 1970; Studzinski, 1972).

It may be imagined that there would be a higher requirement for nutrients per gram of foetal tissue at

those times during gestation when the morphology and internal organs of the foetus are undergoing structural remodeling. It is also likely that during those stages of gestation when the energy reserves of the foetus are being accumulated, there will be a larger difference between substrate concentrations in umbilical artery and vein blood than may be explained solely on the basis of foetal energy metabolism.

Calculations based on the results of the present series of glycogen studies indicated that approximately 2 g of tissue glycogen was being deposited each day during the last two weeks of gestation which represented the removal of between 1.5 and 2 mg of glucose from the foetal circulation each minute. The glucose concentration difference between umbilical venous and arterial blood was found to lie between 0 and 12.5 mg/100 ml. However, present ignorance of the rate of umbilical blood flow in the pig thwarted all attempts to estimate the proportion of glucose in the blood which is removed and stored as glycogen by the foetus.

The information available would lend support to the suggestion that substrates other than glucose were

providing substantially more than 30% of the energy of the foetal piglet. How much is provided by amino acid and free fatty acid catabolism still remains to be quantified. That the initiation of glycogen deposition in the foetal tissues of the pig might be triggered by the same stimulus as the process leading to the termination of pregnancy was an attractive idea, not least because it could conceivably simplify the co-ordination of foetal and maternal preparturition development. The results of work done on other species have indicated that foetal pituitary and adrenal hormones play an important role in the induction of parturition (Liggins, 1973) and there is some evidence to suggest that a similar situation pertains in the pig (North, Hauser and First, 1973). There is also fragmentary evidence of foetal pituitary hormone release at about day 95-100 of gestation (Bascom and Osterud, 1927; Smith and Dortzbach, 1930) although at that stage of gestation the trigger may possibly be maternal rather than foetal (Dixon and Marshall, 1924).

The results obtained in lung and kidney tissue during the present study would suggest that there had been an endocrine stimulus to glycogen deposition at about the time of pituitary hormone release noted by

Smith and Dortzbach (1930). Furthermore, measurements of the corticosteroid concentration in foetal urine and allantoic fluid gave suggestive evidence that foetal adrenal cortex activity had occurred at about the same stage of gestation (Macdonald and Cook, 1974. Preliminary observations).

Although there is no direct evidence at present to prove the point, it would appear reasonable to speculate that whatever neural mechanism controls the secretion of pituitary hormones at birth, may also be, or be linked to, the stimulus controlling glycogen deposition. It was natural to extend the speculation further by suggesting that the same mechanism may control oestrus cycle length, and that it was a modification by the foetus of a fundamental maternal periodicity which caused gestation to be extended to the precise length found. Such a concept is not, however, new (Mende, 1821; Tyler Smith 1849). What may be new is the suggestion that the oestrus cycle length is of a learned periodicity experienced by each generation in utero.

With respect to the stores of foetal glycogen it appeared to the author that these could be looked upon as reserves of potential energy used to drive the 'life'

of the piglet from the uterine environment to an independent existence ex utero. In view of the fact that the piglet is made up of a number of systems designed specifically for postnatal life (e.g. lung for respiration, skeletal muscle for movement and brain for co-ordination), the failure of any one of these systems as a consequence of insufficient energy stored in the appropriate place would constitute per se an example of insufficient potential energy.

In terms of thermodynamics the stored energy of the foetus may be viewed as a necessary increase in the entropy of the piglet in order that it may surmount a thermodynamic 'hurdle' in attaining successful postnatal life. However, it also occurred to the author that birth could be regarded as one small thermodynamic event in a much larger trend in which the entropy of molecular information in the form of nucleic acid is very slowly but steadily increasing. Regarded in this light the energy necessary to drive life forward may not be restricted to foetal reserves alone. The mother constitutes potential energy available to the foetal and new born animal. Some of this energy is expressed at ovulation and rather more during foetal growth. Most, however, is supplied between birth and weaning in the

form of milk. A further extension of the idea led the author to the suggestion that the nutrients transferred across the placenta from "generation 1" to "generation 2" in utero are not only to supply potential energy of a short term nature to "generation 2", but also to begin the accumulation of potential energy for the still un-conceived "generation 3".

Growth was thus exposed as a phenomenon which could be studied not only in terms of the foetal development occurring within the life time of a single member in one species of animal, but as a multi-generation, inter-species process; its driving force was conceived by the author to be the attainment of higher states of entropy with regard to the information coded on the molecular nucleic acid. The concept appealed as one eminently suitable for reflection and further study as it appeared to lie on the very border-line between the fundamental questions 'How?' and 'Why?'.