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Ultrastructure of the myocardium during development from early fetal life to adult life in sheep

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INTRODUCTION

The heart during fetal and neonatal development performs at a level which strikingly exceeds that of the mature heart; for example, cardiac output is 2–4 times greater in lambs than in adult sheep (Heymann, Iwamoto & Rudolph, 1981). The extraordinary level of performance of the developing heart poses many challenging questions in relation to the structural and functional mechanisms which support it. These are made more intriguing by ultrastructural studies which have shown a striking immaturity of the myocardium during fetal development and at birth (Hirakow & Gotoh, 1975; Legato, 1975; Chacko, 1976; Sheldon, Friedman & Sybers, 1976; Anversa, Olivetti & Loud, 1980; Olivetti, Anversa & Loud, 1980).

This study examines the ultrastructure of the developing fetal lamb myocardium in a series of animals from 29 days of gestation to term, and compares them with newborn and adult animals. Studies employing sheep have provided most physiological data, and these provide the basis for most current concepts of fetal cardiac function. However, descriptions of the ultrastructural development of fetal myocardium have been limited to the period from 90 days of gestation to term in the sheep (Sheldon *et al.* 1976) or have been performed in other species. Ultrastructural maturity of the heart varies greatly between species, approximately in relation to the relative behavioural maturity at birth (Zak, Kizu, & Bugaisky, 1979), and therefore the development of cardiac function and structure should be compared in the same species.

MATERIALS AND METHODS

Animals

Myocardium was taken from Border–Leicester cross sheep with accurately known mating dates (± one day). A total of 14 fetuses, 2 lambs and 6 adult sheep was studied. Fetal lambs were of varying ages: 29 days gestational age (number of animals, $n=2$), 51 days ($n=4$), 81–90 days ($n=3$) and 131–146 days ($n=5$). Postnatal lambs were 50–52 days of age ($n=2$), and the adult sheep were 1–2 years old ($n=6$).

Tissue collection

Pregnant ewes were anaesthetised with 5 % thiopentone (0·5 ml/kg) followed by 1 % chloralose (2·5 ml/kg). The ewe was ventilated with air (Harvard respirator) and the fetus was delivered via an abdominal incision. Adult sheep and lambs were similarly anaesthetised. Perfusion fixation at physiological levels of arterial pressure was performed in 6 animals: 4 fetuses, 1 lamb and 1 adult. Young fetuses (51 days of

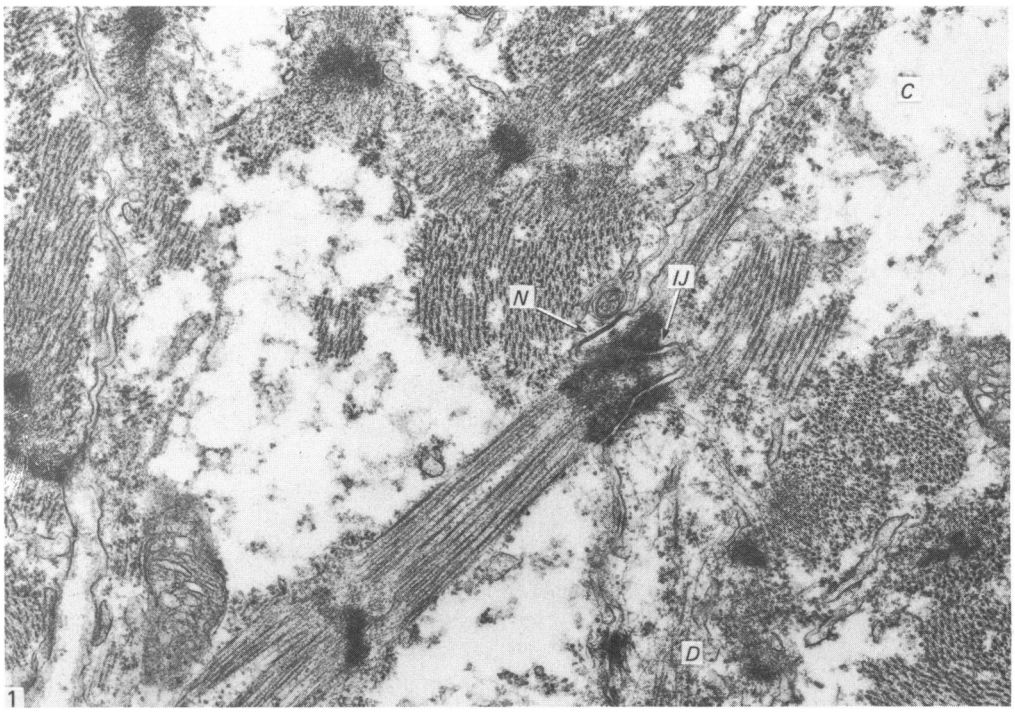


Fig. 1. Right ventricle, 29 days of gestation. *C*, caveolae; *N*, nexus; *IJ*, intermediate junction; *D*, desmosome. × 17 740.

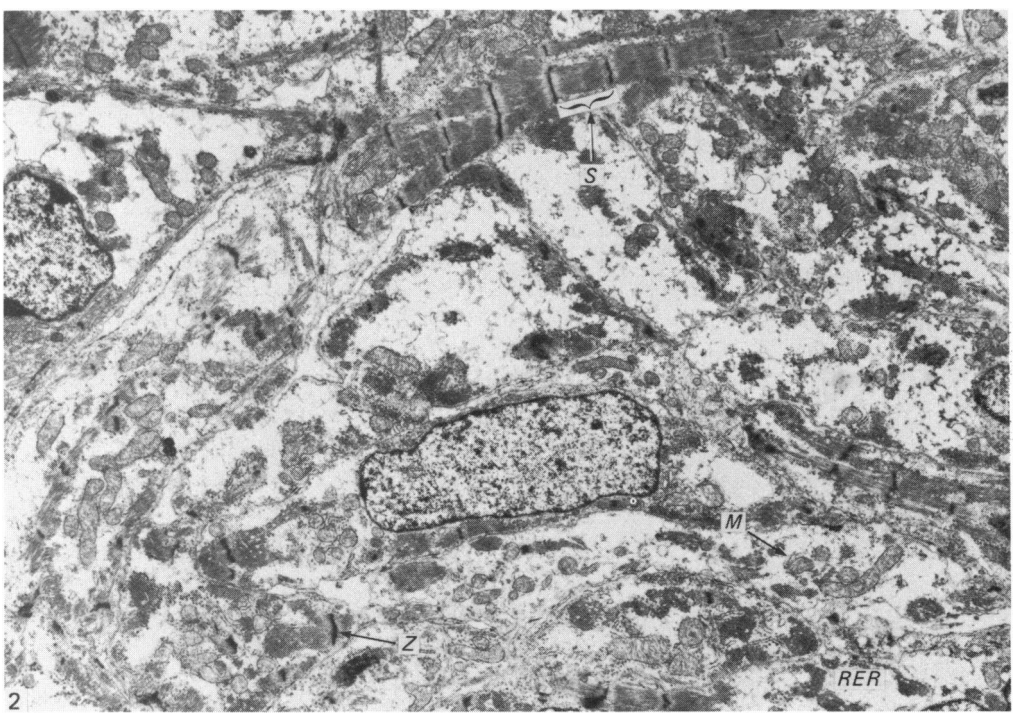


Fig. 2. Left ventricle, 29 days of gestation. *S*, sarcomere; *M*, mitochondria; *Z*, Z line with attached fibrils; *RER*, rough endoplasmic reticulum. × 3920.

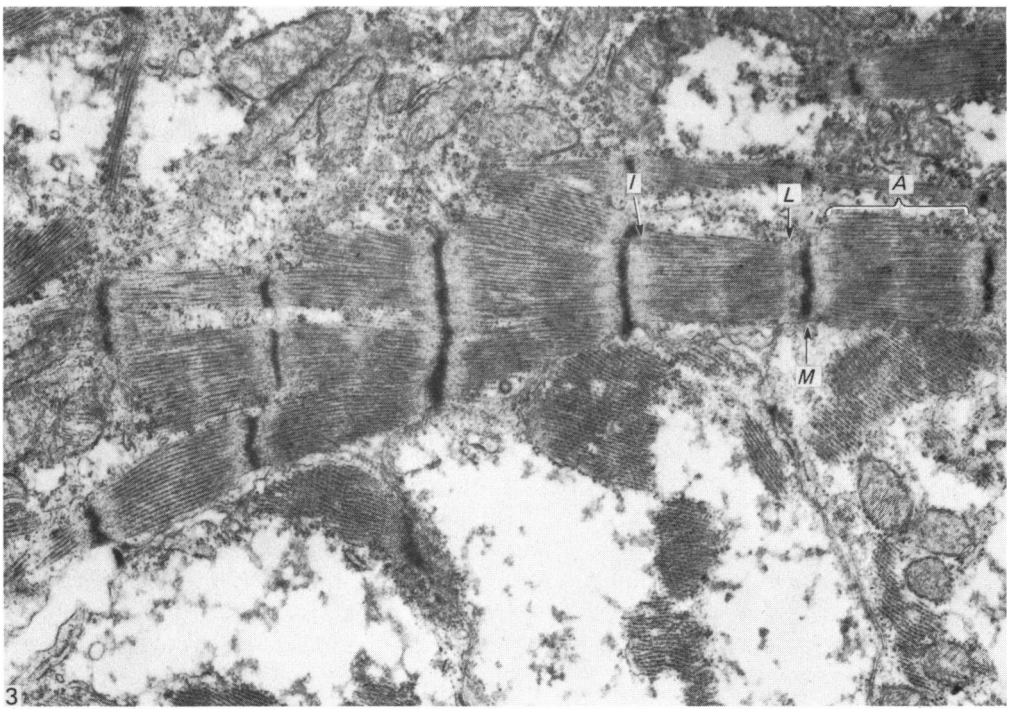


Fig. 3. Left ventricle, 29 days of gestation. Enlargement of upper part of Fig. 2 showing well developed sarcomeres. *I*, I band; *L*, L band; *M*, M line; *A*, A band. × 12000.

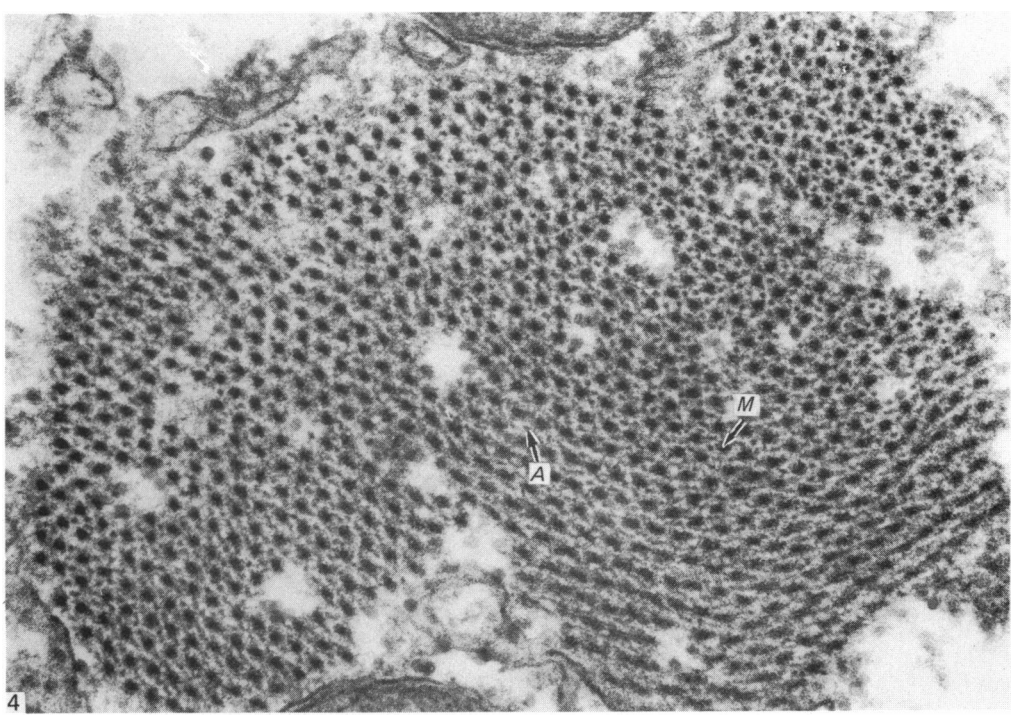


Fig. 4. Transverse section of myofilaments of the right ventricle at 29 days of gestation. *A*, actin filament; *M*, myosin filament. × 83020.

gestation) were delivered within an intact amniotic sac and the umbilical vein was cannulated for perfusion by puncture using a fine catheter tipped with a 25 g needle. In larger fetuses (85 and 144 days of gestation) and in the newborn and adult sheep, perfusion was accomplished via the brachiocephalic artery which was exposed through a thoracotomy, cannulated proximally and tied distally. The right atrium was incised to release fluid and perfusion was initiated with heparinised (100 IU/ml) saline at 4 °C for 15–20 minutes. When the effluent from the atrial incision was free of blood, perfusion was continued with a solution containing 2 % paraformaldehyde and 3 % glutaraldehyde buffered in 0·1 M sodium cacodylate at pH 7·4 (Smolich, Stratford, Maloney & Ritchie, 1977). Since the extent of fixation by perfusion was regionally variable, immersion fixation was employed in 14 studies. The heart was rapidly excised from the anaesthetised animal, weighed, and tissue was dissected from the right and left ventricles at the base of the papillary muscles. Specimens were cut into 1 mm cubes, fixed in 2 % paraformaldehyde and 3 % glutaraldehyde in 0·1 M sodium cacodylate at pH 7·4 (Smolich *et al.* 1977), washed in buffer, and kept for 1–2 hours in 1 % osmium tetroxide at room temperature. After being washed in buffer again, the specimens were kept overnight in uranyl acetate and maleate at 4 °C. The tissues were then dehydrated through a graded series of alcohols and epoxy propane, and embedded in Epon Araldite. Sections, 60–90 nm thick, were stained by immersion for 5 minutes in aqueous uranyl acetate, followed by washing with distilled water and immersed in lead citrate for 5 minutes. Sections were examined in a Jeol 100 S transmission electron microscope.

RESULTS

Myofibrils

During fetal development, the most striking change in the myocyte was a progressive increase in the amount and organisation of contractile material. As seen macroscopically, the heart was fully developed at 29 days of gestation with all chambers present. At the ultrastructural level, myofibrils were seen in various stages of development within a single section. Typically, scattered myofilaments were oriented in longitudinal, oblique and transverse directions (Fig. 1). Sarcomeres at this age were generally characterised by irregular Z lines and apparent disorder of the thick and thin filaments. In particular, discrete A and I bands were not clearly seen and the myofilaments were often loosely packed and did not lie parallel within the sarcomere. In a minority of myocytes, regularly aligned striated myofibrils were sometimes found adjacent to cells containing the more usual irregular contractile material (Fig. 2). Striations seen in these more ordered fibrils were clearly identifiable in longitudinal section as the A, I, and L (pseudo-H) bands and M lines characteristic of the adult myocardium (Fig. 3). Despite an irregular appearance in longitudinal section, such myofibrils cut in transverse section consistently showed a regular hexagonal lattice of thick and thin filaments (Fig. 4).

With further fetal development, progressive production of contractile material resulted in a greater concentration of more regularly aligned myofibrils as seen in longitudinal section at 51–90 days of gestation (Figs. 6, 7). In the fetal heart at term, (Figs. 8, 10) a more advanced development was shown by the closer packed, regularly aligned myofibrils marked with the well defined striations characteristic of mature cardiac muscle (Fig. 12).

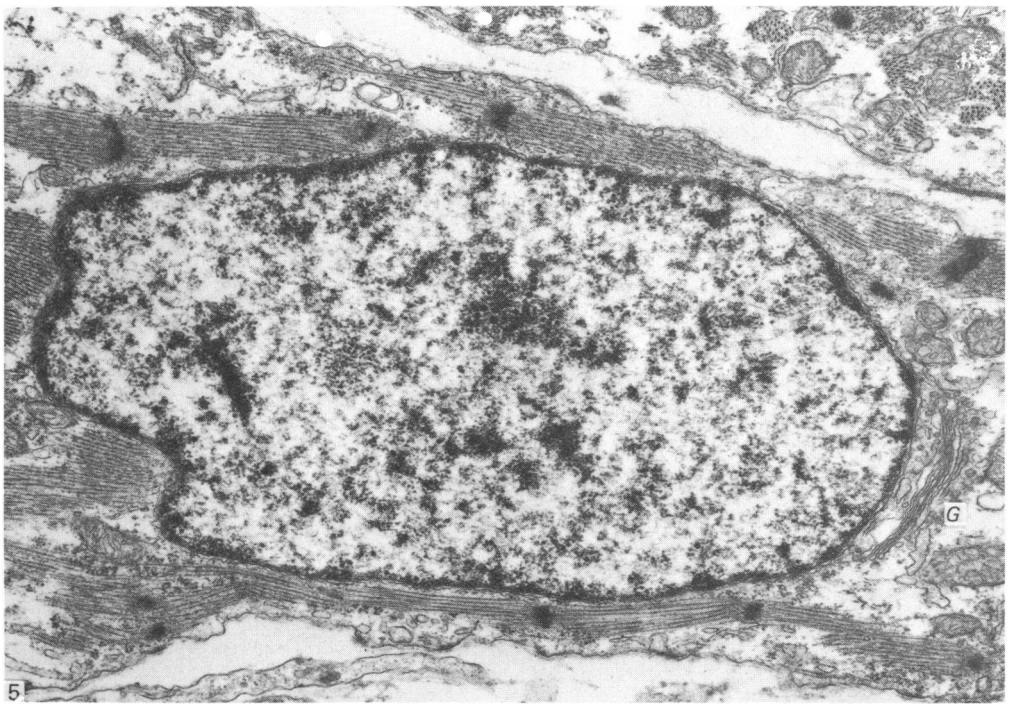


Fig. 5. Myocyte from the right ventricle at 29 days of gestation showing Golgi apparatus (*G*). ×11630.

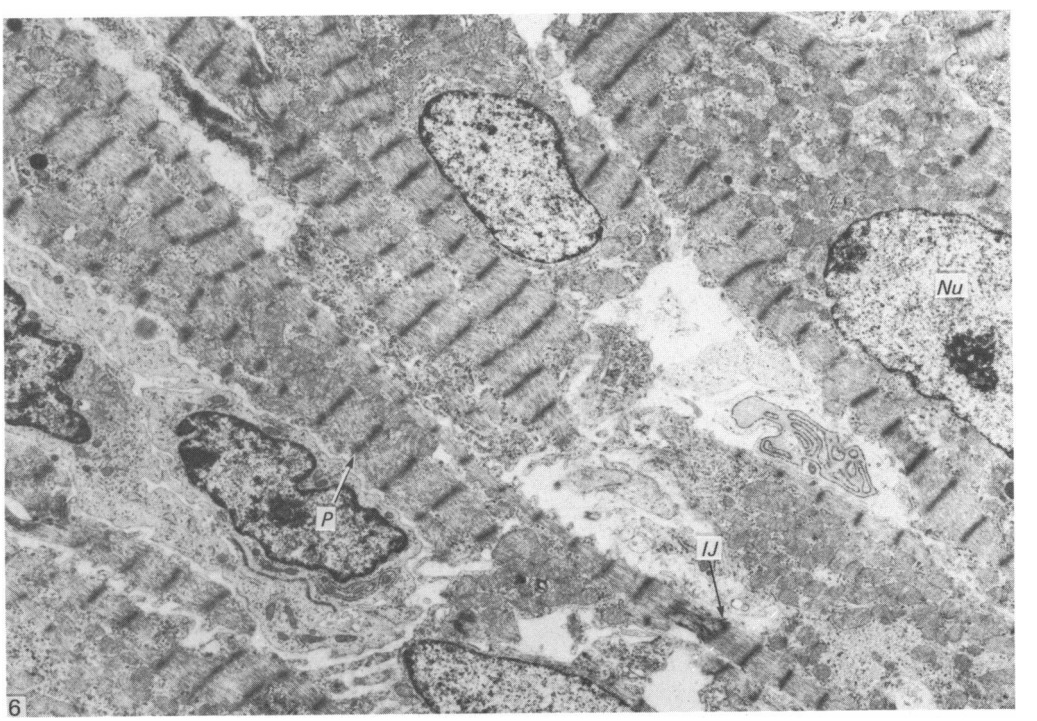


Fig. 6. Right ventricle, 51 days of gestation. *P*, plasmalemma associated with Z lines; *IJ*, intermediate junction; *Nu*, nucleolus. ×4300.

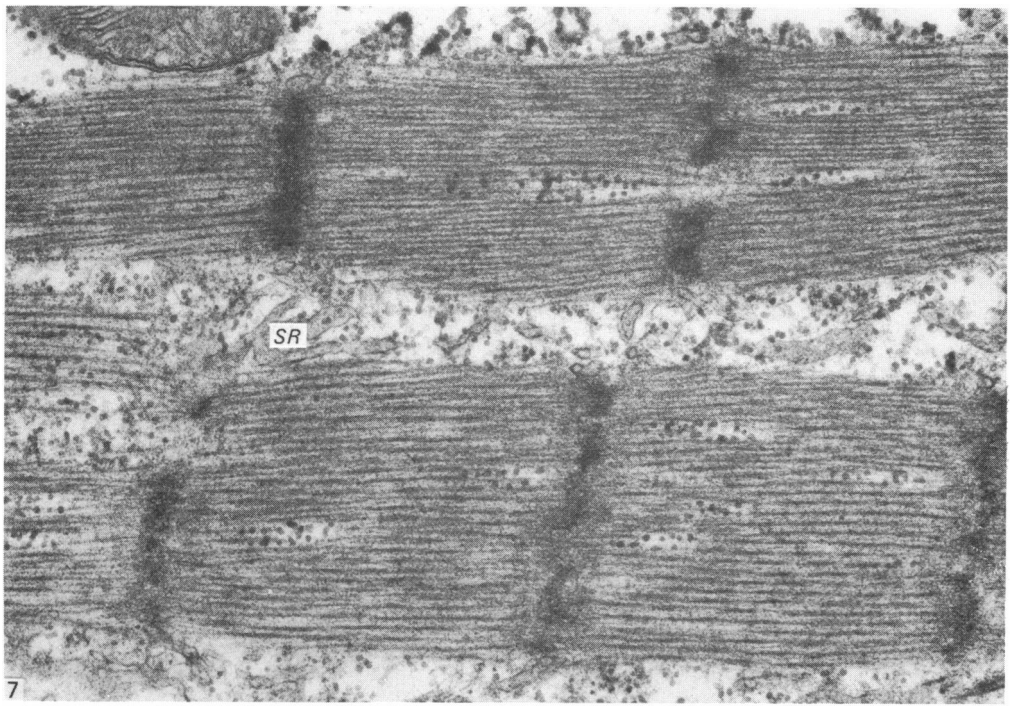


Fig. 7. Myofibrils from the right ventricle at 51 days of gestation. *SR*, sarcoplasmic reticulum. ×37000.

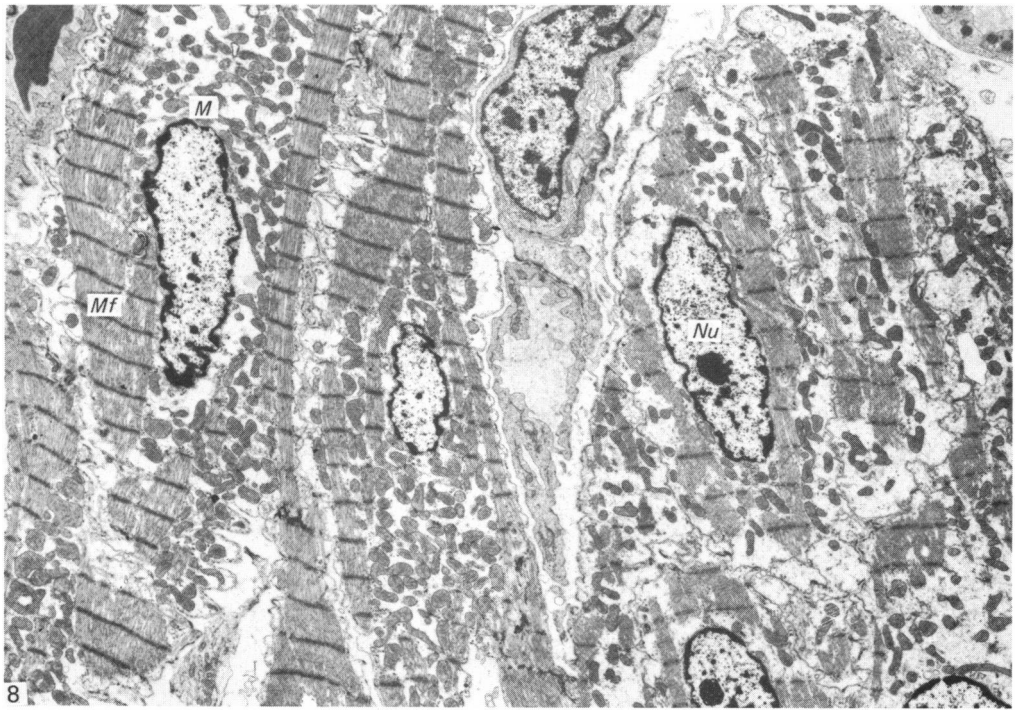


Fig. 8. Right ventricle, 131 days of gestation. *Mf*, myofibrils; *M*, mitochondria; *Nu*, nucleolus. ×3170.

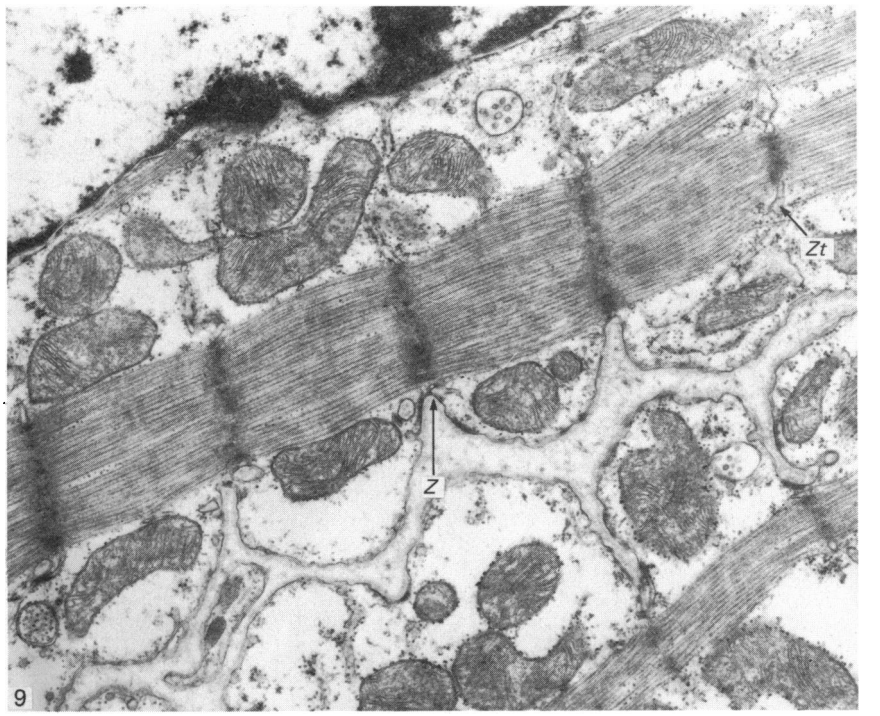


Fig. 9. Right ventricle, 145 days of gestation (term). *Z*, Z groove; *Zt*, Z tubule. × 15 810.

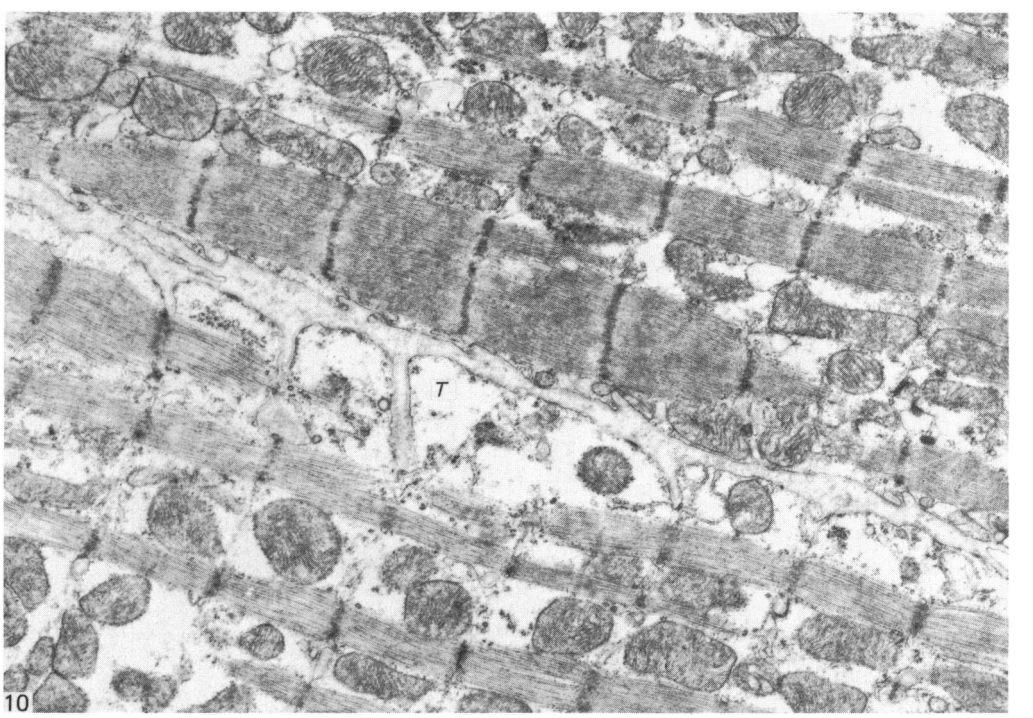


Fig. 10. Right ventricle at 145 days of gestation (term) showing T tubules (*T*). × 9660.

Nucleus

The ultrastructural appearance of nuclei was largely unchanged throughout the period of development from early fetal to adult life. At all ages, chromatin was both scattered throughout the nucleus and condensed around the nuclear periphery. Nucleoli could sometimes be seen (Figs. 6, 8). In general, nuclei became more elongated and indented between 29 days (Fig. 2) and late gestation (Fig. 8). Golgi apparatus, in close apposition to the nucleus, was well developed as early as 29 days gestational age, as seen in Figure 5 where it consisted of five saccules lying close to one pole of the nucleus, separated from it by a layer of vesicles.

Sarcoplasmic reticulum

Sarcoplasmic reticulum was difficult to define in early gestational tissue, but was evident at 51 days of gestation (Fig. 7). Later in fetal life, at 145 days of gestation, tubules which resembled the Z tubules of sarcoplasmic reticulum were associated with the Z line (Fig. 9), and lay in similar configuration to postnatal structures (Fig. 11).

Cell membrane

By 29 days gestation the cell membrane was clearly delineated, with the extracellular surface covered by an electron-dense laminar coat, and caveolae invaginating into the cell (Fig. 1). As myofibrils became more densely packed and aligned in the developing myocyte, the plasmalemma lay in parallel with the contour of the outermost myofibrils, and was closely associated with the Z lines by 51 days of gestation (Fig. 6). This was more obvious later in fetal development when, by 145 days of gestation, the contour of the cell membrane as seen in longitudinal section was clearly scalloped, forming deep grooves adjacent to the Z lines, and sometimes blended with the Z line material (Fig. 9). Transverse tubules (T tubules) were also seen to arise from the plasmalemma and extended into the interior of the cell (Fig. 10).

Intercalated discs

At 29 days of gestation intercalated discs were present and three specialised components were clearly defined (Fig. 1). The desmosome could be distinguished as an electron-dense spot, and this appearance did not change throughout development. The nexus was identified where the paired membranes came closest together and were aligned parallel to the long axis of the myofibrils. It was easily recognised at this early stage of gestation and remained unchanged in appearance during further fetal and postnatal development. On the other hand, the intermediate junction was considerably modified during early fetal life. Identified where it passed transversely across the developing myofilaments, the intermediate junction was slightly convoluted at the 29 days stage (Fig. 1), but became more tortuous at 51 days of gestation (Fig. 6). Subsequent differentiation after 51–90 days was minor, and the appearance at these gestational ages resembled that of intermediate junctions in adult tissue (Fig. 12).

Mitochondria

At all stages of fetal development mitochondria varied considerably in size and shape, the shape ranging from circular to elongated (Fig. 2). At 29 days gestation, mitochondria were scattered throughout the myocyte (Fig. 2), but with subsequent

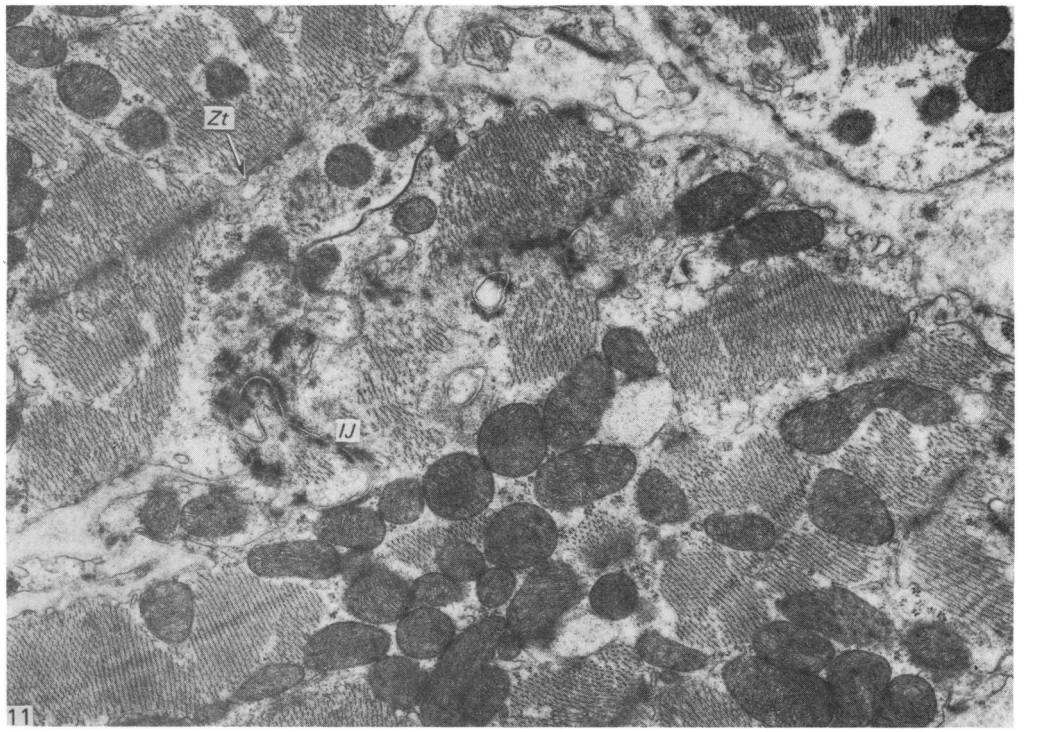


Fig. 11. Right ventricle, 52 days postnatum. *Zt*, Z tubule; *IJ*, intermediate junction. ×13080.

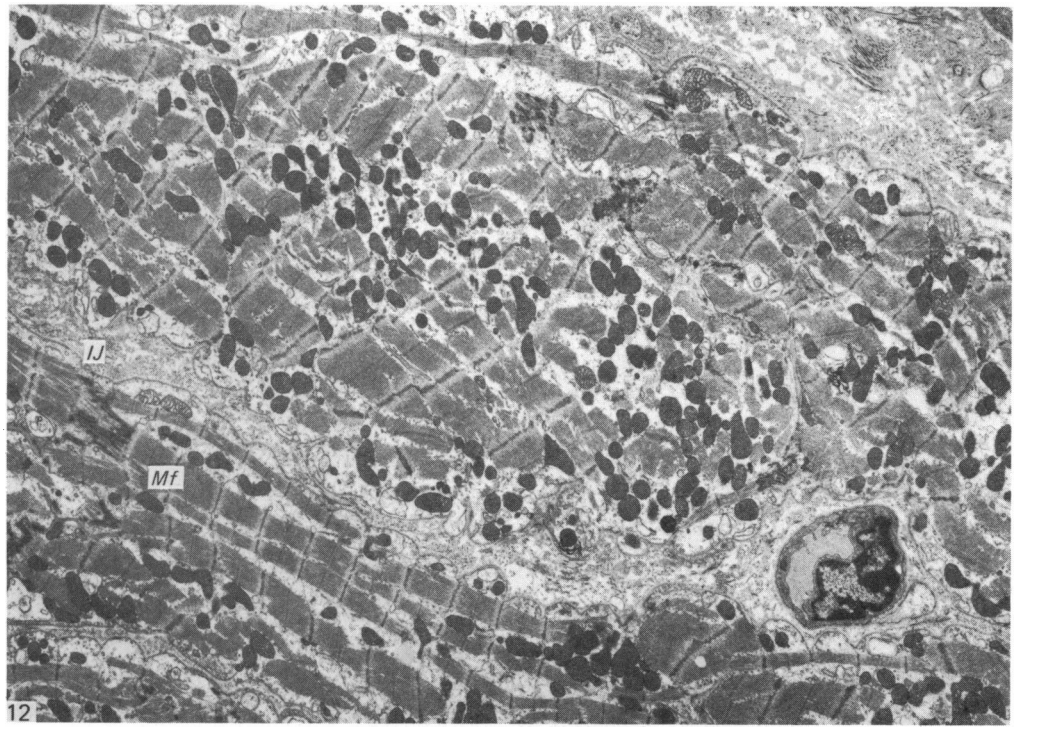


Fig. 12. Right ventricle, adult sheep. *IJ*, intermediate junction; *Mf*, myofibrils. ×3790.

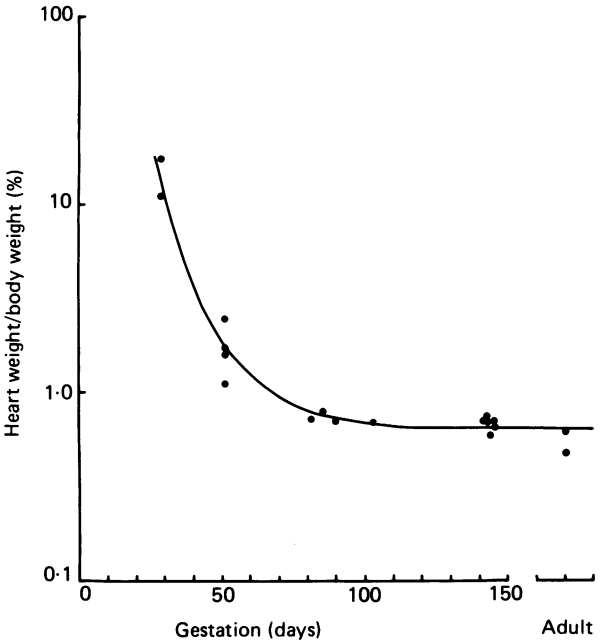


Fig. 13. Relationship between heart weight and body weight during fetal and postnatal development in the sheep.

increase in density of myofibrils, they became aggregated more around the poles of the nucleus and between the myofibrils (Fig. 8).

Relationship between heart weight and body weight

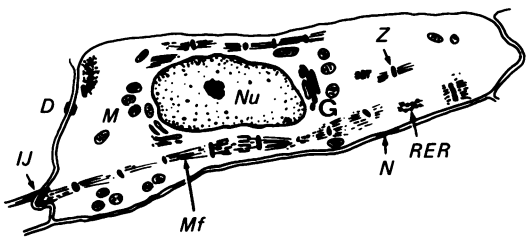
Heart weight expressed as a percentage of body weight (Fig. 13) was reduced twentyfold during development, the ratio falling from 14 % at 29 days gestation to 0·7 % at term, and to 0·5 % after birth.

DISCUSSION

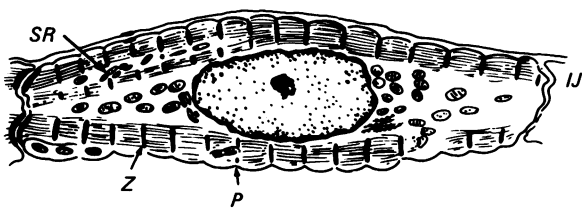
This morphological study of developing sheep myocardium presents descriptions of myocyte ultrastructure from early fetal life (29 days of gestation) to adulthood, and provides information previously unavailable for this species. All major ultrastructural features which characterise the adult myocyte are to be found in early fetal life (Fig. 14) although with considerably different degrees of development of specific features, notably myofibrils, intercalated discs and mitochondria. By contrast the plasmalemma, nucleus and other cell organelles (sarcoplasmic reticulum, Golgi apparatus, rough endoplasmic reticulum) are not changed remarkably after approximately 50 days.

Increasing convolution of the intermediate junction component of the intercalated disc is a feature of myocardial development in the fetal sheep which has been noted previously in other animal species (Melax & Leeson, 1969; Hirakow & Gotoh, 1975; Legato, 1975). This junctional complexity develops in fetal life in most species studied, with the exception of the rabbit (Muir, 1957). Other organelles found in well developed form in the fetal sheep include the transverse tubular system, which has

(*a*) 29 days gestation



(*b*) 51 days gestation



(*c*) 145 days gestation (term)

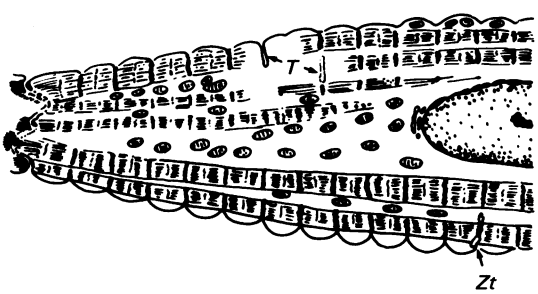


Fig. 14 (*a–c*). Diagrammatic summary of the development of the sheep ventricular myocyte. (*a*) 29 days of gestation. Myofilaments are few in number, irregular in their orientation within the myocyte and are not well organised within the sarcomere. The intercalated disc is present, consisting of desmosome, nexus and slightly convoluted intermediate junction. (*b*) 51 days of gestation. Regularly aligned myofibrils are seen in greater concentration. The plasmalemma parallels closely the contour of the outermost myofibrils. The intermediate junction is more convoluted than at 29 days gestation, and sarcoplasmic reticulum is present. (*c*) 145 days of gestation (term). The myofibrils in great concentration are regularly aligned and show well defined striations. T tubules and Z tubules are present. Plasmalemma is regularly scalloped adjacent to the Z lines. *D*, desmosome; *G*, Golgi apparatus; *IJ*, intermediate junction; *M*, mitochondria; *Mf*, myofilaments; *N*, nexus; *Nu*, nucleolus; *P*, plasmalemma associated with Z lines; *RER*, rough endoplasmic reticulum; *SR*, sarcoplasmic reticulum; *T*, T tubule; *Z*, Z line; *Zt*, Z tubule.

been demonstrated at 90 days of gestation (Sheldon *et al.* 1976), and the sarcoplasmic reticulum which the present study demonstrates to be present as early as 51 days of gestation.

Deficits in the amount and organisation of myofibrillar material are striking features of myocyte development which have not been described previously in early gestational development in lambs. At 29 days of gestation, only a small minority of myocytes exhibit myofibrils with regular alignment and well structured striations similar to mature tissue, the majority of cells containing myofibrils which are irregularly aligned along the axis of the cell and lack ordered striations. Thus while there is considerable variation the overall appearance is characterised by a paucity of myofilaments and by irregularity in their arrangement within sarcomeres, features of the immature myocyte which have been described previously in rat and human fetuses (Leak & Burke, 1964; Chacko, 1976). With advancing gestation in the fetal sheep, the contractile elements at first undergo an increasing density of myofilaments, and subsequently develop well defined striations. Thus, at term, the sarcomere structure of fetal tissue is not substantially different from that of the adult myofibril.

These observations of contractile tissue paucity and disorder are difficult to reconcile with physiological data which show an extraordinary pumping performance of the fetal heart *in vivo* (Heymann, Iwamoto & Rudolph, 1981). This presents an outstanding challenge in perinatal physiology: of the many possible avenues of investigation, two problems merit special mention. The first problem concerns the paucity of morphometric data describing growth of the fetal lamb myocardium throughout gestation. Historically, the sheep has provided much of the physiological data, and many of the concepts of fetal cardiac function are based on studies in this species. However, quantitative ultrastructural studies have been performed in other species, notably the rat, the cat and the puppy (Hirakow & Gotoh, 1975; Legato, 1975; Sheridan, Cullen & Tynan, 1977, 1979; Anversa *et al.* 1980; Olivetti *et al.* 1980). It appears that isolated strips of myocardium from the mature fetus or newborn have a higher resting tension and a poorer contractile ability than adult tissue (Friedman, 1972; Davies, Dewar, Tynan & Ward, 1975). When the poorer mechanical performance of the perinatal tissue is corrected using an estimate of the relative amount of contractile tissue, the perinatal and adult data tend to converge (Friedman, 1972). With advancing age after birth, improvement in tension development is accompanied by growth in the relative proportion of contractile proteins in the myocyte (Sheridan *et al.* 1977, 1979). These studies support the idea that the force generating properties of the fetal and the adult sarcomere may be similar, suggesting that the greater heart mass relative to body mass in the early fetus (Fig. 13) compensates in part for the deficit in contractile material. However, morphometric data are required to test this possibility.

A second problem relates to the disordered arrangement of the myofilaments in developing myocytes. New morphometric techniques are required to quantify the development of order in myofibril structure and alignment, to complement methods which quantify the density of myofibrils. Progressive organisation and orientation of myofibrils may contribute as much to the maturation of myocyte function in the fetus as does the increasing density of myofibrils after birth (Sheridan *et al.* 1977, 1979). On the other hand, classical concepts of muscle contraction based on the regular banded appearance of mature sarcomeres may not be appropriate for developing myocardium. Whether these concepts adequately explain the mechanical properties of adult myocardium is also subject to question (Noble, 1979).

SUMMARY

The ultrastructure of the developing fetal lamb myocardium was studied in a series of animals spanning 29 days of gestation to term, and compared with newborn and adult animals. All major ultrastructural features which characterise the adult myocyte were found in early fetal life, although with considerably different degrees of development of specific features. Notably, myofibrils at 29 days of gestation are sparse and show little organisation. With advancing gestation there is an increasing number of myofibrils and the development of well defined striations. Thus, at term, the fetal tissue is not substantially different from the adult myofibril in the appearance of sarcomere structure. The observation of contractile tissue paucity and disorder in early fetal lamb myocardium is difficult to reconcile with available physiological data, which show an extraordinary pumping performance of the heart *in vivo*, and requires further investigation.

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