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Development of the myocardial contractile system

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Summary. Recent studies regarding developmental changes in the myocardial contractile system from fetal, newborn, and adult animals are reviewed. From the data obtained so far, we conclude that in the early fetus myocardial contraction is mainly dependent on Ca which enters via the sarcolemma. Ca release from the sarcoplasmic reticulum is minimal. The role of the sarcoplasmic reticulum as a source of contractile Ca increases and the role of Ca influx across the sarcolemma in contractile system decreases with development.
Key words. Contractile system; fetus; premature myocardium; calcium; sarcoplasmic reticulum; contractile protein; sarcolemma.

Myocardial contractile function as a muscle

In the adult myocardium, a relatively small Ca influx across the sarcolemma induces the subsequent release of greater amounts of Ca from the sarcoplasmic reticulum (SR), which then activates the myofilaments[5]. Mitochondria also have a capacity to take up Ca, although it is unlikely that mitochondria regulate intracellular Ca normally. Thus the amount of Ca reaching myofilaments is dependent on the function of the subcellular organelles regulating cellular Ca concentration. Myocardial contractile function is largely dependent on the amount of Ca reaching the myofilaments and the quantity and quality of myofilaments.

Several investigators have studied postnatal changes in contractile function in the rat[3], cat[4], dog[23], and rabbit[24]. All these studies showed that the contractile force of the unit myocardium increases with development. The contractile function of the fetal heart, however, is less well known. According to the data of Sissman[30], the myocardium begins to contract at 3-somite-stage in the rat, 9-somite (about 8 days of gestation) in the rabbit, and Hamburger and Hamilton stage 10 in the chick embryo. The contractile function of these early embryonic hearts as muscles has not been studied extensively. Friedman[6] showed that contractile force in the near term fetal lamb is less than in the adult. This decreased contractile force in the premature heart was mainly attributed to the smaller amount of contractile protein per unit muscle. Developmental changes in intracellular Ca concentration have not been examined directly.

We studied developmental changes in the contractile system using the fetus at the 18th, 21st and 28th day of gestation (term: 31 days), newborn, 3–5-day-old, and adult New Zealand White rabbits[12,13]. In the rabbit, cardiovascular anatomy is established by approximately the 16th day of gestation. From the 18th day of gestation to birth, heart weight increased about 80 times (table 1). Using the isolated, arterially perfused ventricular or septal preparation[15–17,20], we measured the contractile

Table 1. Heart and body weights

Age	n	Body weight (g)	Heart weight (g)	Heart wt/body wt × 100
18-day fetus	8	1.25 ± 0.05	0.0043 ± 0.0006	0.34 ± 0.03
21-day fetus	8	2.9 ± 0.06	0.0132 ± 0.005	0.46 ± 0.03
28-day fetus	12	30 ± 0.9	0.136 ± 0.005	0.46 ± 0.01
5-day newborn	9	85 ± 5	0.34 ± 0.09	0.40 ± 0.05

Values are means ± SE. From Nakanishi et al.[12].

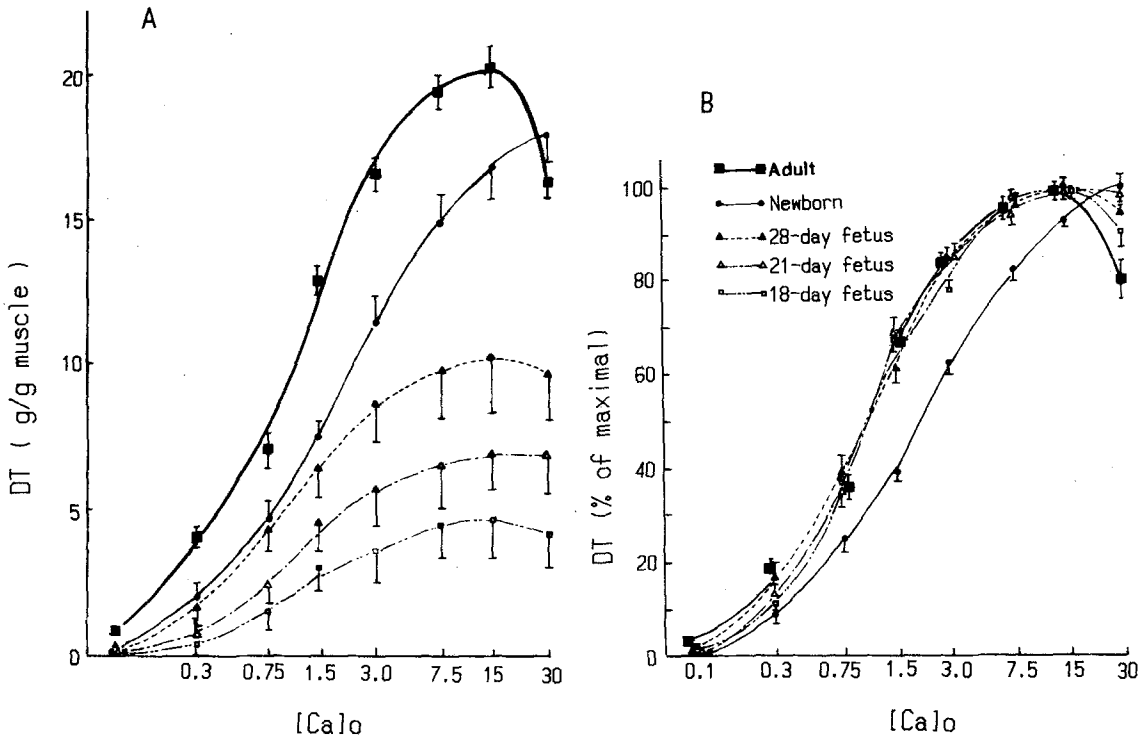

Figure 1. Effect of extracellular calcium ($[Ca]_o$) on developed tension (DT) in the isolated heart preparation of the rabbit (term of pregnancy: 31 days). DT is expressed as g/gmuscle (left panel) and as a percent of maximal values (right panel). Maximal DT observed at high $[Ca]_o$ increased with age (left panel). Dependency of DT on $[Ca]_o$ in the three fetal groups and in the adult was similar but the newborn curve shifted to the right (right panel). Data from Nakanishi et al.[12,13].

function of the fetal, newborn and adult rabbit heart. In order to alter intracellular Ca concentration, extracellular Ca ($[Ca]_o$ was changed. The effect of high $[Ca]_o$ on contractile force is shown in figure 1. The greatest developed tension (DT) observed in each age group at high $[Ca]_o$ increased with development (fig. 1 A). Because DT reached a plateau at high $[Ca]_o$, it is likely that the myofilaments were saturated with Ca under these conditions. Therefore, the differences in maximal DT at high $[Ca]_o$ are most likely due to the differences in the amount of contractile protein. These data are in agreement with previous studies[3,4,23,24].

The relative value of DT at various $[Ca]_o$ in each group is shown in figure 1 B. The dependency of DT on $[Ca]_o$ in

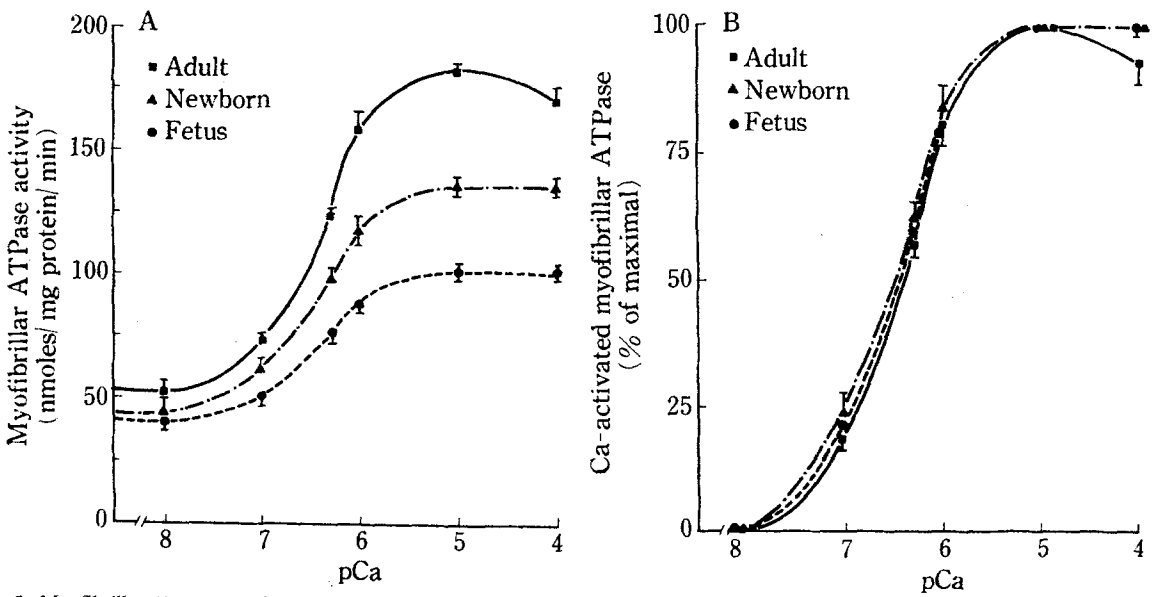

Figure 2. Myofibrillar ATPase activity as a function of pCa (−log [Ca]) in the fetal (28th the day of gestation), newborn (5-day), and adult rabbit. Myofibrillar ATPase activity expressed as nmol/mg/min increased with age (A). Ca-activated ATPase expressed as percent of the maximal value was similar in the three age groups (B). Data from Nakanishi et al.[13] with permission.

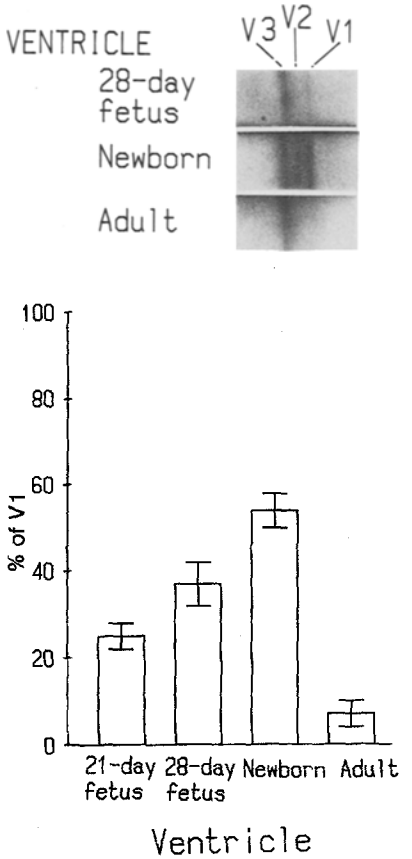

Figure 3. Myosin isozymes (V1, V2, and V3) shown by pyrophosphate polyacryramide gel electrophoresis (panel A) and proportion (%) of isozyme V1 (panel B) in the rabbit ventricle. Proportion of myosin isozyme V1 was the greatest in the newborn.

the three fetal groups and in the adults was similar but in the newborn the curve shifted to the right. Similar differences between the newborn and adult rabbit have been reported by Park et al.[24]. Since the sensitivity of myofibrillar ATPase to Ca in the fetus, newborn and adult was similar (fig. 2), it is likely that in the newborn the effect of $[Ca]_o$ on intracellular Ca concentrations is different from that in the adult and fetus. At 1.5 mM $[Ca]_o$, the relative value of DT was 63% in the fetus, 42% in the newborn, and 66% in the adult. These degrees of activation of myofibrillar ATPase were observed at pCa 6.30 in the fetus, at pCa 6.58 in the newborn, and at pCa 6.25 in the adult. This suggests that under control conditions cytosolic Ca concentration in the newborn is less than in the fetus and adult. Preliminary data supporting this hypothesis have been obtained using the intracellular Ca indicator fura-2[18]. The function of each contractile component was studied further and the data will be described below.

Contractile protein

Quantity and quality of contractile protein are important factors determining myocardial contractile function. The amount of contractile protein determines contractile force and contractile protein ATPase activity mainly determines the velocity of muscle shortening[27]. We first studied the amount of myofilaments by ultrastructural methods in the fetal and newborn rabbit. Figures 4 and 5 show electron micrographs of the left ventricle and table 2 shows morphometric data. There was no significant difference in the cellular diameter in the fetus and newborn. The myofibrillar fraction in the fetus was significantly less than in the newborn (table 2). In the newborn, myofibrils were well organized (fig. 4). In the 28-day fetus, myofibrillar concentration was less than in the newborn. In the 21-day and 18-day fetuses, myofibrils were scarce and disorganized. These findings are in agreement with the data of Page and Buecker[22].

We next measured contractile protein ATPase in the fetal (28th day of gestation), newborn (3-day-old), and adult rabbits. The myofibrillar ATPase activity was determined at various Ca concentrations from 10^{-8} to 10^{-5} M[12,13]. The yield of myofibrils in the 28-day fetus (31.6 ± 0.1 mg/g muscle) was significantly less than in the newborn (42.3 ± 1.1) and the adult (45.3 ± 2.8). Maximal ATPase activity was observed at pCa 5 in all age groups and ATPase activity increased with development (fig. 2A). Figure 2B shows Ca-activated ATPase activity expressed as percent of the maximal value. There was no difference in the relative value of the Ca-ATPase activity, suggesting that the sensitivity of myofibrils to Ca does not change with development.

Recent studies suggest that isomyosin composition (V1, V2, and V3) regulates myosin ATPase[21]. Pyrophosphate polyacrylamide gel electrophoresis showed that the proportion of V1 (which has a high enzyme activity) was the greatest in the newborn rabbit and the least in the adult rabbit, in agreement with the data of Lompre[8] (fig. 3). From these data one expects to find increased ATPase activity in the newborn but the data in figure 2A show depressed ATPase activity in the fetus and newborn. Therefore, we studied ATPase activity at various conditions.

Myofibrillar preparation contains myosin, actin, tropomyosin, and troponin and its ATPase represents actomyosin ATPase in the presence of troponin and tropomyosin at low ionic strength[27]. Myosin Ca-ATPase is the ATPase of purified myosin. In the myosin preparation,

Table 2. Morphometric data

Group	Cell diameter (μm)	Organelle fractions (%) Myofibrils	Mitochondria
18-day fetus	5.3 ± 0.3	13.0 ± 1.7	7.3 ± 1.8
21-day fetus	5.5 ± 0.5	15.3 ± 1.5	7.3 ± 1.3
28-day fetus	4.9 ± 0.5	24.3 ± 2.1*	17.4 ± 0.4*
Newborn	6.8 ± 0.9	39.0 ± 2.5**	15.3 ± 1.5*

Numbers are mean ±SE of 20 measurements. The organelle fraction was determined by measuring the relative area occupied by the organelle in the cytoplasm. * = significantly ($p < 0.05$) different from the 18-day and 21-day fetal values. ** = significantly ($p < 0.05$) different from the 18-day, 21-day, and 28-day fetal values. Data from Nakanishi et al.[12].

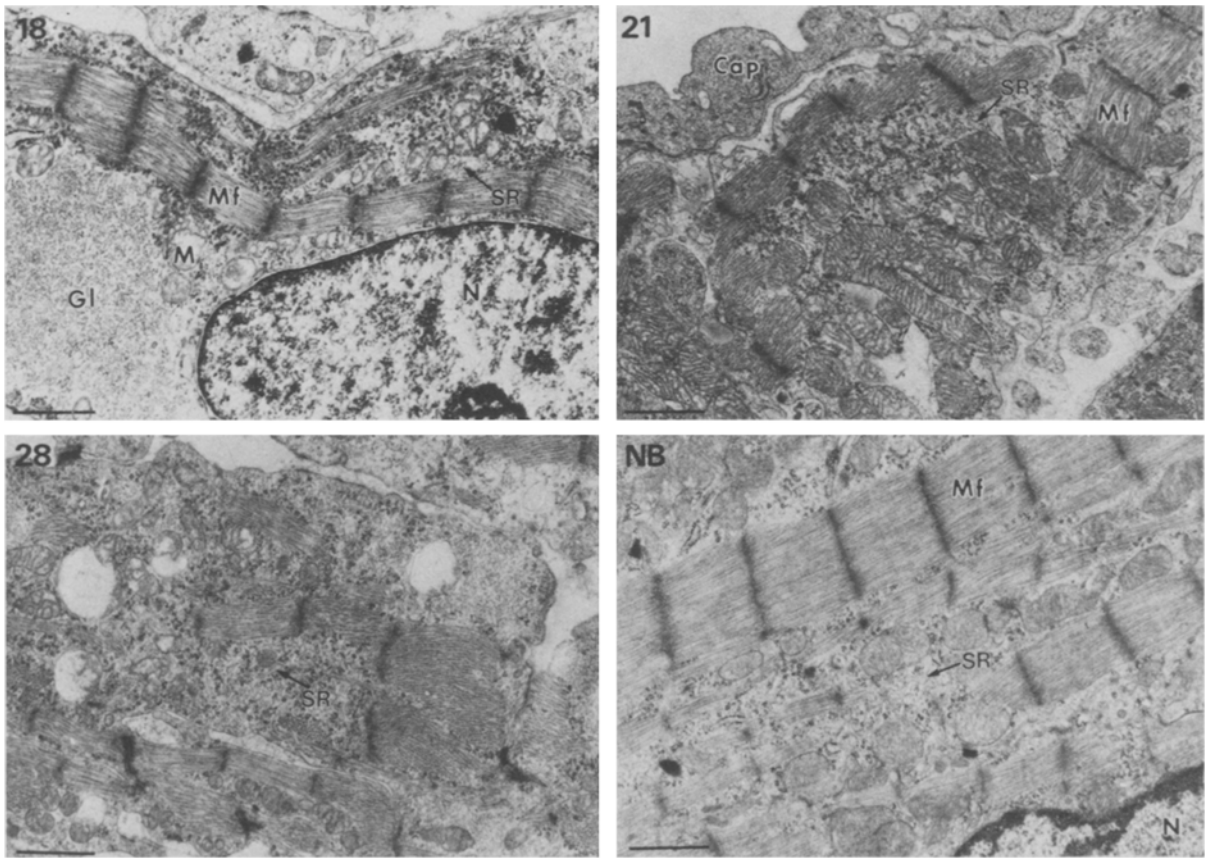

Figure 4. Low magnification of electron micrographs of the left ventricle. From the 18-day fetus to the newborn, the amount of myofibrils increased with development. 18, 18-day fetus; 21, 21-day fetus; 28, 28-day fetus; NB, newborn; Gl, glycogen granules; Mf, myofibrils; M, mitochondria; N, nucleus; SR, sarcoplasmic reticulum. Calibration bars are 1 μm. From Nakanishi et al.[12] with permission.

Ca-ATPase activity in the newborn was the greatest, followed by the fetal and adult values (fig. 6). The higher Ca-ATPase activity in the newborn is most likely due to the increased V1 myosin isozyme. To determine the mechanisms for the lower myofibrillar ATPase activity at low ionic strength in the premature heart, we added actin and troponin-tropomyosin complex, which had been isolated from adult rabbit skeletal muscle, to the myosin preparation. As shown in figure 7, actin-activated myosin ATPase was the greatest in the newborn. Therefore, it is unlikely that actin-activation is less in myofibrils of the fetus and newborn. In the presence of actin, tropomyosin, and troponin, however, myosin ATPase activity was greatest in the adult and least in the fetus (fig. 7). These data suggest that the low myofibrillar (actomyosin) ATPase activity in the premature heart may be due to the age-related difference in the interaction of myosin with troponin-tropomyosin.

In summary, myofibrillar (actomyosin) ATPase is low in the fetus and newborn rabbit. Myosin Ca-ATPase is dependent on the myosin isozyme and the ATPase activity is low in the fetus, higher in the newborn, and again lower in the adult. The physiological significance of the developmental changes in the myofibrillar and myosin ATPase activity remains unclear. Since Saeki et al.[26] showed that alteration of the ATPase activity changes isometric contractile force, the low myofibrillar ATPase in the fetus and newborn is in part responsible for the decreased contractile force. The relationship between the ATPase activity and the isotonic contraction parameter has not been examined in the developing myocardium but several studies indicate that muscle shortening velocity is lower in the premature heart[3, 32]. The lower myofibrillar ATPase activity may be in part responsible for this.

Sarcoplasmic reticulum

The regulatory mechanisms of intracellular calcium concentration are complex but the sarcoplasmic reticulum has an important effect on $[Ca]_i$. We attempted to elucidate developmental changes in the function of the sarcoplasmic reticulum using the isolated heart preparation. Previous studies suggested that the inotropy of paired electrical stimulation (PES) results from Ca release from intracellular sites including the sarcoplasmic reticulum and T-tubular system[11]. Therefore, to determine developmental changes in Ca release from the sarcoplasmic reticulum, the effect of PES was studied. There was no significant inotropic effect of PES in the 18- and 21-day fetus. This suggests that the sarcoplasmic reticulum may

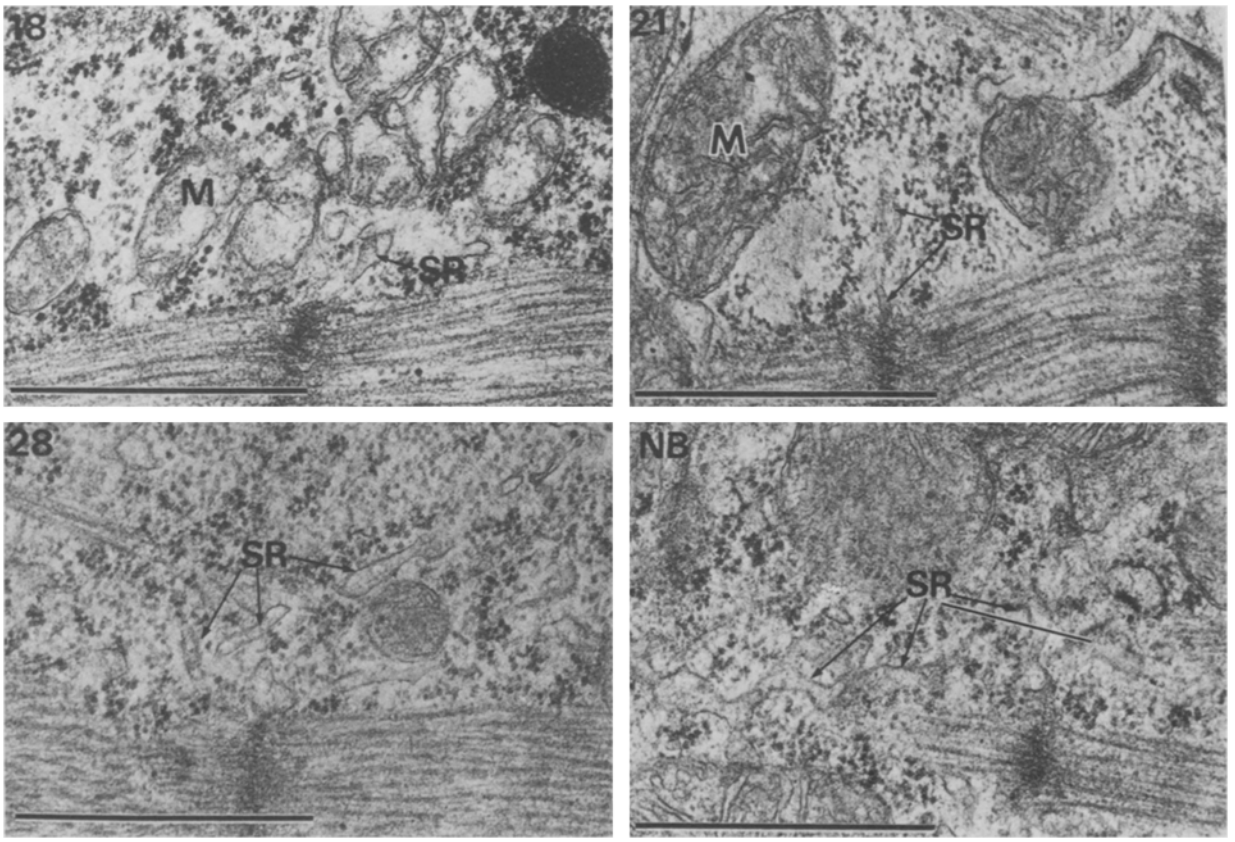

Figure 5. Medium magnification electron micrographs of the left ventricle. Loose network of the sarcoplasmic reticulum is observed in the newborn. In the 18- and 21-day fetuses, the sarcoplasmic reticulum was very scarce. Calibration bars are 1 μm. From Nakanishi et al.[12] with permission.

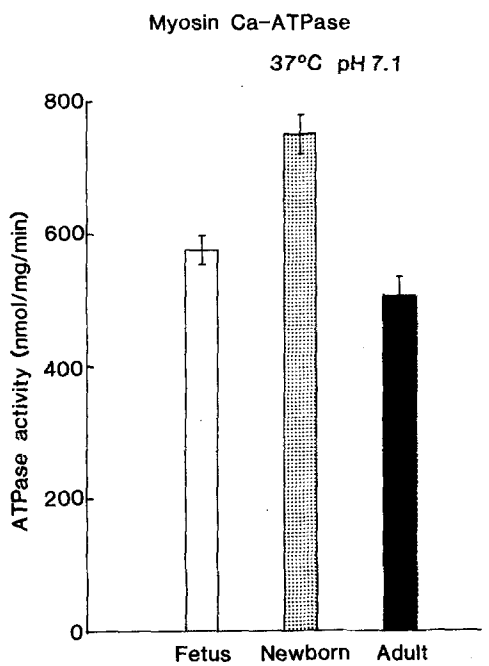

Figure 6. Myosin Ca-ATPase activity in the myosin preparation obtained from rabbit ventricle. Ca concentration = 10 mM. Other experimental conditions, see Nakanishi et al.[14]. Myosin Ca-ATPase activity was the greatest in the newborn.

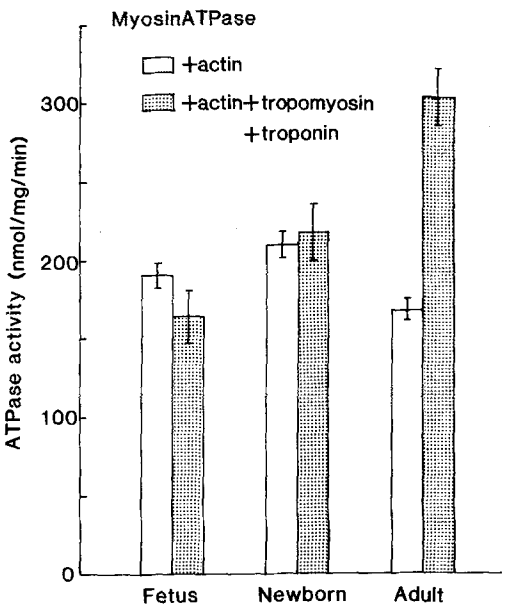

Figure 7. Actin-activated myosin ATPase and actin-activated myosin ATPase in the presence of tropomyosin-troponin complex. Although actin-activated myosin ATPase was the greatest in the newborn, actin-activated myosin ATPase activity in the presence of troponin-tropomyosin complex was the greatest in the adult, followed by the newborn and fetus (28-day). Data from Nakanishi et al.[14].

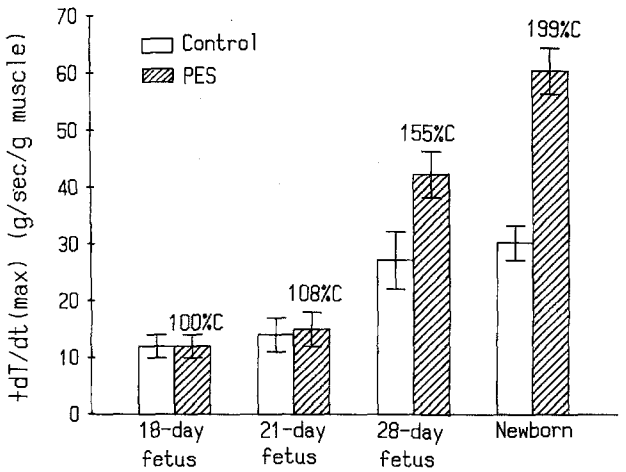

Figure 8. Effect of paired electrical stimulation (PES) on the rate of tension development (+dT/dt). There was no significant inotropic effect in the 18- and the 21-day fetuses. From Nakanishi et al.[12] with permission.

not be functioning significantly at these stages. Significant inotropy was observed in the 28-day fetus but it was less than in the newborn (fig. 8). The inotropic effect of PES in the adult was also greater than in the newborn[28]. These data suggest that the function of the sarcoplasmic reticulum increases with development.

In order to further study the function of the sarcoplasmic reticulum in the premature myocardium, ryanodine was used. Ryanodine selectively inhibits Ca uptake and release from sarcoplasmic reticulum[10, 31]. In the newborn, ryanodine (10^{-5} M) significantly increased time to peak tension (TPT) and decreased the rate of tension development (fig. 9). TPT was not altered significantly by this drug in the 18- and 21-day fetuses. A negative inotropic effect was observed in all age groups, but it was minimal in the 18- and 21-day fetuses. Changes in +dT/dt (max) in the 28-day fetus was greater than in the 18- and 21-day fetus but less than in the newborn (fig. 10). Changes in those variables in the adult were greater than in the newborn. The data obtained using ryanodine are in agreement with the PES data and suggest that the role of the sarcoplasmic reticulum in the myocardial contractile system increases with development.

Fabiato and Fabiato[5] showed that Ca-induced Ca release was not observed in the 2-day-prepartum rat fetus and the threshold of Ca to induce Ca release from sarcoplasmic reticulum is higher in the 2-day-old newborn than in the adult rat. This study also suggests that Ca release from the sarcoplasmic reticulum increases with development.

We evaluated the development of sarcoplasmic reticulum in the rabbit heart by ultrastructural methods. In the newborn, loose networks of sarcoplasmic reticulum were observed (fig. 5). In the 28-day fetus, sarcoplasmic reticulum was less than in the newborn. In the 18- and 21-day fetuses, intracellular organelles were packed more loosely; sarcoplasmic reticulum was very scarce and observed only occasionally (fig. 5). Since Ca may be released from dyadic junctions between sarcoplasmic reticulum and plasma membrane, Page and Buecker[22] measured surface density of the dyads in the rabbit heart and they showed that surface density of dyadic junctions between sarcoplasmic reticulum and external plasma membrane increases during embryonic life until it reaches the adult value one day after birth. After T-tubules develop at about 10 days after birth, dyads between the T-system and the sarcoplasmic reticulum also appear and their surface density remains constant.

We also examined the function of the sarcoplasmic reticulum biochemically[13]. The yield of sarcoplasmic reticulum in the fetus (28th day of gestation) is less than in the newborn and adult rabbits (table 3). Within all age groups studied, Ca-ATPase activity, Ca uptake, and Ca binding to sarcoplasmic reticulum were similar (table 3). In contrast, Nayler and Fassold[19] showed that the yield of sarcoplasmic reticulum in the fetus, newborn and

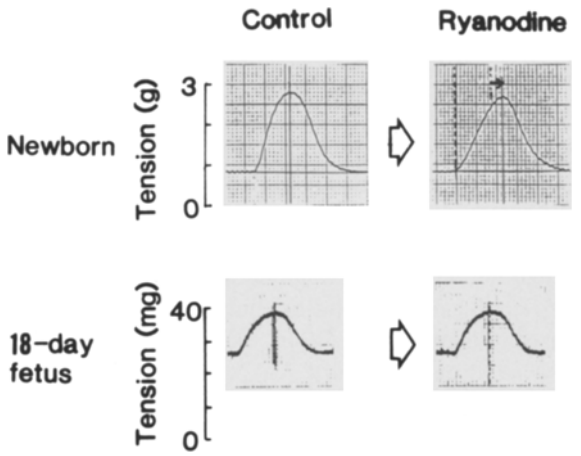

Figure 9. Typical experiments that show effect of ryanodine. After ryanodine infusion, time to peak tension (TPT) increased and rate of tension rise (+dT/dt) decreased in the newborn (indicated by the solid arrow), but not in the 18-day fetus. From Nakanishi et al.[12] with permission.

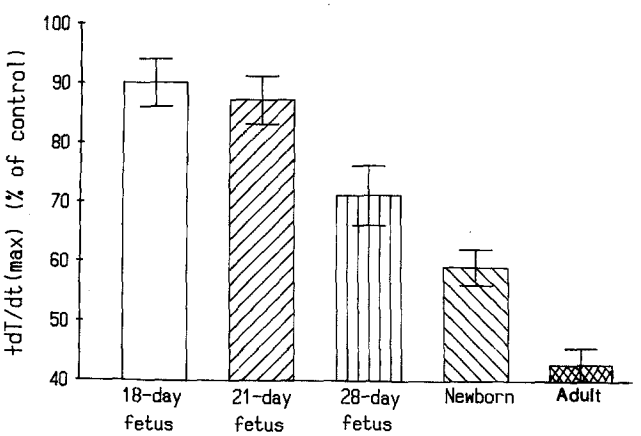

Figure 10. Effect of ryanodine on the time to peak tension and +dT/dt (max). The effect of this drug in the 18- and 21-day fetuses was minimal. The ryanodine effect increased with age. From Nakanishi et al.[12] with permission.

Table 3. Yield, Ca ATPase, and Ca uptake by sarcoplasmic reticulum

Group	SR yield (mg/g muscle)	Ca-ATPase (nmol/mg protein/min)	Ca uptake (nmol/mg protein/min) pCa 6.3	pCa 6	pCa 5	Ca uptake, capacity (μmol/mg protein/30 min) pCa 4
Fetus	0.15 ± 0.06	152 ± 21	144 ± 16	179 ± 13	226 ± 19	3.95 ± 0.28
Newborn	0.49 ± 0.10	172 ± 13	150 ± 15	214 ± 26	242 ± 14	4.13 ± 0.46
Adult	0.82 ± 0.08	157 ± 13	102 ± 13	138 ± 16	196 ± 24	4.26 ± 0.33

Values are means ± SE of 5 assays. Ca-activated ATPase was calculated as ATPase activity at pCa 5 minus ATPase activity at pCa less than 8 at 27 °C. Ca uptake was determined in the presence of ATP and oxalate at 27 °C (data from Nakanishi et al.[13] with permission).

adult rabbit was similar but Ca uptake by sarcoplasmic reticulum in the fetus was less than in the adult. This discrepancy may be due to the different methodology utilized, but both studies indicate that Ca uptake by sarcoplasmic reticulum in the unit amount of muscle is depressed in the premature heart. The data obtained in sheep is more consistent: both Mahony et al.[9] and Pegg et al.[25] showed decreased Ca-ATPase activity and Ca uptake in the fetus (100–145th day of gestation, term being 145 days) compared to the adult data. In the chick, Boland and Martonosi[2] showed that Ca-ATPase and Ca uptake in the 10–14-day embryo is less than in the 10–40-day-old chick.

Sarcolemma

Developmental changes in Ca flux across the sarcolemma have not been studied directly. We examined the role of the sarcolemma in the contractile system in the fetal, newborn, and adult rabbit heart using lanthanum (La^{3+}). La^{3+} displaces Ca bound to the sarcolemma and blocks sarcolemmal Ca influx, but it does not permeate the plasma membrane[1]. When the muscle was perfused with a solution containing La^{3+}, this caused a rapid decrease in DT and +dT/dt(max) and the mechanical function reached a new steady state within 30 min. La^{3+} caused a severe decline in +dT/dt(max) in the 18- and 21-day fetuses (fig. 11). The La-induced decrease in +dT/dt(max) in the 28-day fetuses was greater than in the newborn but less than in the 18- and 21-day fetuses.

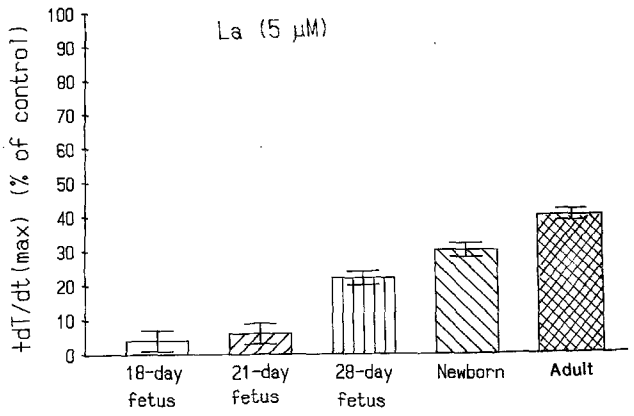

Figure 11. Effect of lanthanum on +dT/dt(max). The negative inotropic effect of La in the 18- and 21-day fetuses was significantly greater than in the 28-day fetus and in newborn. The effect was the least in the adult. From Nakanishi et al.[12] with permission.

The decrease in +dT/dt(max) in the adults was less than in the newborn. These data are in agreement with the study of Seguchi et al.[29] who showed that the negative inotropic effect of verapamil and diltiazem in the newborn rabbit heart was greater than in the adult. These data suggest that in the 18- and 21-day fetal hearts most contractile Ca enters via the sarcolemma and the role of Ca influx in the contractile system decreases with development.

Mitochondria

We measured mitochondrial Ca uptake in the rabbit heart[13]. Mitochondrial Ca uptake occurred at pCa less than 6 (Ca > 1 μM) in all age groups (fig. 12). Since under

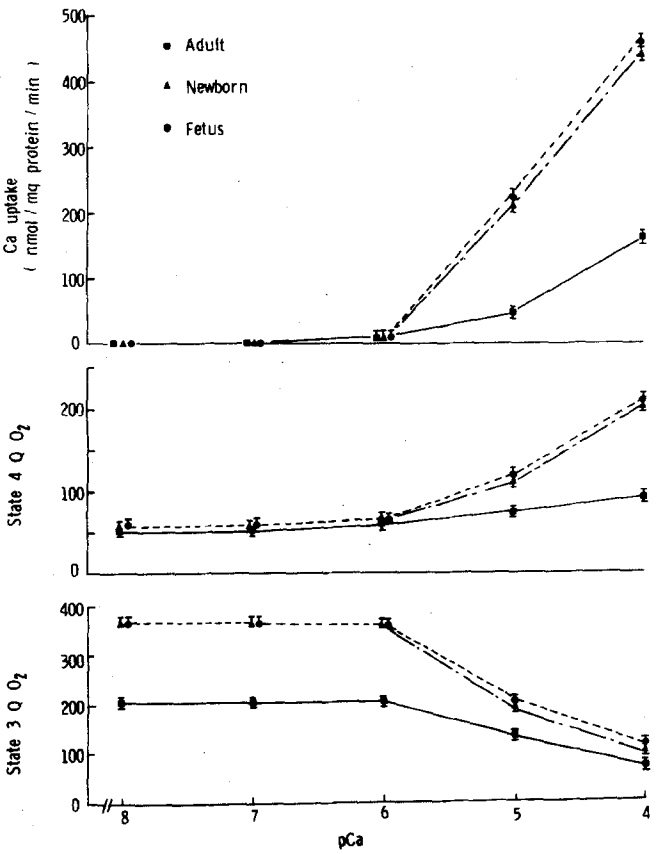

Figure 12. Mitochondrial Ca uptake and respiratory function measured in the mitochondrial preparation isolated from fetal (28-day), newborn (5-day), and adult rabbit ventricle. pCa = – log Ca. Significant Ca uptake was observed at pCa less than 6, suggesting that mitochondria may not regulate $[Ca]_i$ under normal conditions. Mitochondrial respiratory function in the newborn and fetus was greater than in the adult. From Nakanishi et al.[13] with permission.

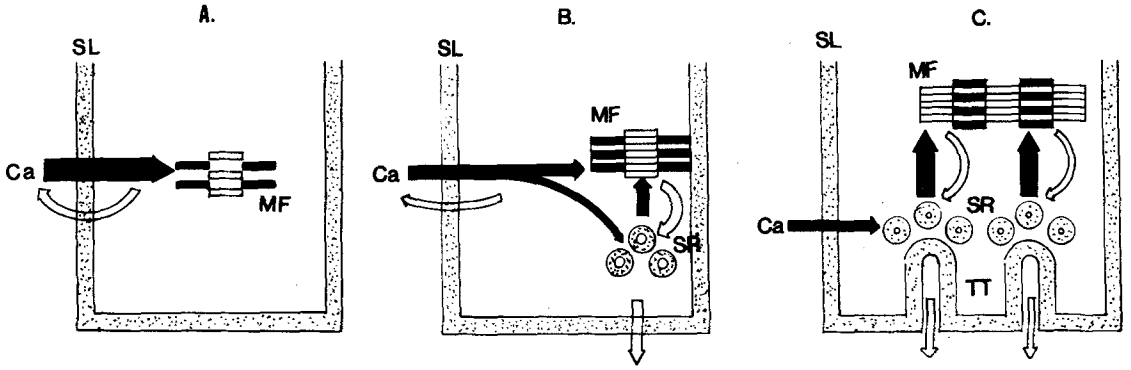

Figure 13. Schematic diagrams showing developmental changes in the excitation-contraction coupling. The arrows represent Ca ion movements. In the early fetus (panel A), Ca entering the cell across the sarcolemma (SL) directly reaches the myofilaments (MF). In the newborn (panel B), Ca influx across the sarcolemma induces Ca release from the sarcoplasmic reticulum (SR), which then activates the myofilaments. In the adult (panel C), the amount of Ca released from SR is more important.

control conditions intracellular Ca concentrations are less than 1 μM, mitochondria may not normally play a role in regulating intracellular Ca. When intracellular Ca concentrations increase, however, mitochondria may take up Ca and act as a Ca buffer.

Summary of the contractile system

The difference between the premature and mature myocardium is shown schematically in figure 13. We conclude that the contractile system develops from the early fetal type (panel A) to the newborn type (panel B), and then to the adult type (panel C); Ca release from SR increases and sarcolemmal Ca influx decreases with development. In the rabbit the $[Ca]_o$-tension curve shifts to the right in the newborn and that in the fetus and adult in similar, suggesting that $[Ca]_i$ is lower in the newborn. The precise reasons for this remain unclear but the intracellular Ca concentration is dependent on the relative capability of the Ca releasing system and the Ca sequestering system. The Ca sequestering system may be underdeveloped in the fetus and for this reason intracellular Ca concentration in the fetus may be higher than in the newborn. Since the Ca releasing system is well developed in the adult, $[Ca]_i$ may be relatively high. It is not clear whether similar age-related changes in $[Ca]_i$ exist in other species. As stated above, since $[Ca]_i$ is dependent on the relative function of Ca release and Ca sequestration, developmental changes in $[Ca]_i$ should be examined in each species separately.

Acknowledgment. This study was supported by Research Grants 59480241, 59570411, 61770706, and 62570440 from the Japanese Ministry of Education, Science, and Culture and by a grant-in-aid from the Japan Research Promotion Society for Cardiovascular Diseases.

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0014-4754/88/11-120936-09$1.50 + 0.20/0

Role of extracardiac factors in heart development

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Summary. Many factors extrinsic to the developing heart play important roles in determining its final form. The neural crest has been shown to provide ectomesenchyme to the pharyngeal apparatus and outflow tract, as well as the postganglionic innervation of the heart. Ablation of the neural crest providing ectomesenchyme to the outflow tract results in various cardiac malformations. These malformations have in common either outflow and/or inflow tract malalignment. Although the reason for this malalignment is not understood, it is thought that hemodynamic parameters during early cardiac morphogenesis may be disrupted causing cardiac dysmorphogenesis. The most likely area for this alteration to occur is in the pharyngeal apparatus which houses the aortic arch arteries. Various possibilities are discussed. The innervation of the heart by neural crest-derived autonomic neurons and nodose placode-derived sensory neurons is outlined, and the interactions between the two progenitive sites is discussed.
Key words. Cardiac morphogenesis; neural crest; nodose placodes; chick embryos; heart malformations.

Much of the information and most of the cellular precursors needed for cardiac morphogenesis are contained in the primitive endothelial tube from which the structure of the fully formed heart is derived. However, many factors extrinsic to the developing heart play important roles in determining its final form. These factors include vascular resistance, seeding of the outflow tract with extracardially derived ectomesenchyme, and innervation. Circulating factors such as polypeptides and hormones which are derived from endothelium and a variety of other sources probably play some role in differentiation and growth of the heart although almost nothing is known about the influences of circulating factors on the early period of cardiac morphogenesis.

The neural crest has been shown to be of great importance in cardiac morphogenesis because of direct involvement of neural crest-derived ectomesenchyme in outflow tract septation, and also because the neural crest is important for maintenance of the aortic arch arteries which provide the major conduits for blood leaving the developing heart[4, 16]. The neural crest[24, 25] and nodose placodes[8, 48] provide the innervation of the heart and there is increasing evidence that innervation plays a role in maturation of signalling mechanisms in the myocardial membrane[9] and myocardial growth[7, 41]. Hence, the neural crest plays a multifaceted role in development of the heart (fig. 1).

Neural crest

The neural crest arises from the neural folds which develop from the lateralmost part of the neural plate[15, 30, 49]. As the neural plate closes to form the neural tube, neural crest cells are released from the neural folds[46]. The neural crest cells extend processes and actively migrate away from the vicinity of the neural folds. The neural crest is divided into two regions based on the potential for formation of ectomesenchyme[15, 30, 49]. Cranial neural crest (fig. 2) extends from the mid-diencephalon to the caudal limit of somite 5[34]. Neural crest cells derived from this cranial region have the prospective potency to differentiate into mesenchyme which has been called ectomesenchyme because of its unique origin from the ectoderm. Ectomesenchyme derived from cranial neural crest provides a variety of mesenchymal derivatives that are important in development of the face, pharyngeal appara-