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LIMITATIONS TO LEFT VENTRICULAR DIASTOLIC FILLING IN THE PERINATAL PERIOD

by

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ABSTRACT

The fetal heart lacks the ability to increase cardiac output in response to volume infusions and, thus, is said to lack cardiac reserve. Since this is true, it has been difficult to explain the heart's ability to increase cardiac output and stroke volume at birth. Studies conducted upon anesthetized fetal and neonatal lambs have revealed a previously unappreciated influence of the thoracic tissues upon left ventricular diastolic filling, and thus stroke volume, in the perinatal period. By measuring left ventricular minor axis dimensions (ultrasonic transducers), left ventricular end-diastolic pressure (transducer-tipped catheter), and pericardial pressure (flat, liquid-containing balloon) it has been shown that the thoracic tissues (pericardium, lungs, and rib cage) significantly limit the development of left ventricular end-diastolic transmural pressure, and thus, left ventricular preload in young lambs (1 hour to 43 days). Retraction of the lungs or of the lungs and the pericardium reduced the constraint applied to the LV and allowed left ventricular end-diastolic transmural pressure to increase at any given intracavitary pressure. Left ventricular diameters are also increased at any given end-diastolic pressure when the magnitude of constraint is reduced. The importance of the constraining influence of the thoracic tissues upon perinatal left ventricular function has also been clarified utilizing these same techniques. Prior to the initiation of pulmonary ventilation the pericardial pressure recorded in exposed fetal lambs was significantly higher ($p<0.01$) than after one hour of ventilation. The reduction in pericardial pressure was accompanied by a shift in the left ventricular pressure-diameter relationship towards larger ventricular diameters at

any given end-diastolic pressure. It was speculated that a similar reduction in constraint during natural birth could allow for an increase in ventricular volume and, through a Frank-Starling mechanism increase cardiac output. It is speculated that a reduction in constraint at birth may result from the loss of maternal tissue constraint, the change from liquid-filled lungs to air-filled lungs, and the development of a more negative pleural pressure. Finally, application of similar techniques coupled with the assessment of left ventricular stroke volume (electromagnetic flow probes) has shown that the constraint applied to the left ventricle significantly limits fetal left ventricular stroke volume. The plateau of the fetal left ventricular function curve results from the constraint imposed by the thoracic tissues on the left ventricle and not from inherent myocardial properties. When left ventricular end-diastolic pressure was maintained at 10 mmHg, eliminating the thoracic tissue constraint applied to the left ventricle resulted in a 65% increase in left ventricular stroke volume.

These studies have helped to advance the understanding of the mechanisms involved in limiting fetal left ventricular function and help to explain how cardiac output increases at birth. Since fetal left ventricular stroke volume is limited by the constraining influence of the thoracic tissues and since pericardial pressure is reduced with the initiation of pulmonary ventilation it is speculated that the increase in cardiac output observed at birth is, at least in part, dependent on a reduction in constraint. Cardiac output will increase, through the Frank-Starling mechanism, as ventricular volume increases in response to the decrease in the constraint applied to the heart.

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LIST OF ABBREVIATIONS

CCCP	Closed Chest Closed Pericardium
Dlvap	LV end-diastolic anteroposterior diameter
LA	Left Atrium
LV	Left Ventricle
OCCP	Open Chest Closed Pericardium
OCOP	Open Chest Open Pericardium
PEEP	Positive End-Expiratory Pressure
Plved	LV end-diastolic pressure
Plved(tm)	LV end-diastolic transmural pressure
Pped	Pericardial end-diastolic pressure
RA	Right atrium
RV	Right Ventricle
SO_2	Oxygen Saturation

LIST OF TERMS

Afterload	The load against which the ventricle works during contraction, best defined as instantaneous wall stress during contraction.
Cardiac Output	An indication of ventricular performance, equal to the volume of blood pumped by the ventricle per minute, calculated as the product of heart rate and stroke volume.
Contractility	Changes in contractility indicate a change in cardiac performance which are independent of changes in sarcomere length
Frank-Starling mechanism	Mechanism for changes in ventricular performance that, ultimately, are dependent on changes in sarcomere length.
Preload	The resting load of the ventricle at end-diastole which determines ventricular volume and sarcomere length; best defined as instantaneous wall stress.
Stroke Volume	An indication of ventricular performance, equal to the volume of blood ejected by the ventricle per beat.

Stroke Work	An indication of ventricular performance, equal to the (mechanical) work done by the heart during a single cardiac cycle. Stroke work can be calculated based on the area within the pressure-volume loop or as the product of stroke volume and mean systolic aortic pressure minus mean diastolic pressure.
Ventricular Function Curve	A plot of ventricular performance (stroke work, stroke volume, or [cardiac] ventricular output) plotted against a measure of preload (Plved, Plved[tm], or end-diastolic volume or dimensions).

CHAPTER 1. Introduction

The development of the cardiovascular system and its ability to satisfy the requirements of the rapidly developing fetus has been extensively studied using a variety of morphological and physiological techniques. Information has been attained from acute and chronic preparations using a variety of animal models. Even so, there remain vast areas within the realms of fetal cardiovascular physiology that are not fully understood. The developmental changes that occur in systolic and diastolic function throughout the perinatal period are two such areas.

Cardiac output depends upon four factors; preload, afterload, contractility, and heart rate (110). The first of these, preload, is of particular importance to the work which will follow. While it is impossible to consider global ventricular function without considering each of these factors, it is the author's intention to concentrate specifically on the body of knowledge which relates to the scientific work conducted as the basis of this thesis. With this in mind, it is essential to review the literature which specifically relates to the control of left ventricular function in the perinatal period.

Myocardial Cellular Development

As a starting point, a brief review of the early development of the myocardium sets the stage for understanding the continued interest in perinatal cardiac function. Early in gestation the myocardium is

comprised of immature myocytes which scarcely resemble their adult counterparts. In the immature myocyte, myofibrils are randomly oriented, peripherally located, and small in number (20, 75, 86, 109). Transverse tubule development tends to correspond to increasing cell diameter (109, 53). A primitive form of the sarcoplasmic reticulum appears early in gestation (58). It has been suggested that the sarcolemma is the site of Ca^{++} handling until quite late in gestation, at which time the sarcoplasmic reticulum becomes dominant (84). Other structures undergo developmental changes. The intercalated discs appear initially in an oblique plane, moving eventually to form a step like pattern. The nucleus, initially large, diminishes in size, and becomes elongated with development (109). The number of mitochondria increases and the amount of cellular glycogen decreases (109). The most striking developmental trend is the gradual increase in myofibril material which, in some species, continues for some time after birth (109). Although the fetal heart's cellular structures are poorly developed, the heart performs at a remarkably high level throughout gestation: in near term fetal lambs cardiac output, relative to body weight, exceeds that of the adult (450 ml/kg/min vs. 100 ml/kg/min [118]).

The fetal mammalian heart also differs from the adult heart in that the right ventricle (RV) and left ventricle (LV) function in parallel. Although after birth there can be no long term differences between LV and RV stroke volumes, this is not the case in the fetus, where roughly 65% of the combined ventricular output arises from the RV and the remaining (35%) from the LV (51, 90, 97, 98, 113, 114). RV

dominance is possible because of the unique pathways for blood flow in the fetal heart.

Fetal Circulatory Pathways

The pathway of blood through the fetal heart has been reviewed by Walsh et al. (120) and more recently Rudolph (97). Oxygenated blood from the placenta flows in the umbilical vein toward the liver and represents 45% of the total venous return. Up to 50% of umbilical venous flow bypasses the liver and goes through the ductus venosus into the inferior vena cava (97). The remainder of the umbilical blood flows through the the right and left lobes of the liver into the inferior vena cava. Blood flows through the inferior and superior vena cava to the right atrium (RA) where almost all of the inferior vena caval flow crosses the foramen ovale (a connecting pathway between the right and left atrium) into the left atrium (LA) while the superior vena caval flow passes primarily into the right ventricle (30, 92, 96, 97, 98, 120). Although superior vena caval flow represents approximately 20% of the total venous return only 2% crosses the foramen ovale (0.04% of the total venous return) (97). Early work by Dawes (26) suggested that hypoxic distress could increase the amount of superior vena caval blood which crossed the foramen ovale but work by Cohn et al. (22) did not support this.

Umbilical venous blood has a high O_2 saturation (SO_2), 80%, (14) but as it mixes with systemic venous blood in the liver and in the heart the SO_2 decreases. Remarkably little mixing of umbilical venous

blood and the systemic venous blood occurs in the inferior vena cava. In fact, in the thoracic vena cava oxygenated placental blood can be distinguished from the systemic venous blood (11, 30, 92). This lack of mixing, coupled with the preferential passage of umbilical venous blood into the LA and the limited pulmonary blood flow (8% of combined ventricular output), results in a greater SO_2 of the blood in the LV (65%) than in the RV (52%). The SO_2 of blood in the RV is lower since this blood is derived largely from hepatic venous blood, pre-ductus venosus inferior vena caval blood, and superior vena caval blood (97).

During systole LV blood is ejected into the aorta, while RV blood is ejected into the main pulmonary artery. A high pulmonary vascular resistance limits blood flow through the lungs. The ductus arteriosus allows RV stroke volume to bypass the pulmonary circulation and (in sheep) flow directly into the aorta distal to the coronary, brachiocephalic, and left subclavian arteries. LV output is distributed primarily to the arteries serving the myocardium, upper body, and head. Flow to the lower body, viscera, and placenta arises primarily from the RV (97). Since only a small fraction of LV blood actually flows into the descending aorta the SO_2 of the blood in the descending aorta is only 2%-5% above that of the RV.

The path of the blood through the circulatory system changes dramatically at birth. Pulmonary vascular resistance is reduced and, as a result, pulmonary blood flow increases. Constriction of the ductus arteriosus occurs in conjunction with the increase in arterial

PO_2. Systemic vascular resistance and systemic arterial pressure increase with the removal of the relatively low resistance pathways in the placenta (124). Cardiac output increases at this time as do LA and LV pressures. Increased LA pressure and a reduction in RA pressure leads to the closure of the foramen ovale. Since after birth aortic pressure exceeds pulmonary artery pressure, flow through the ductus arteriosus is temporarily reversed until complete closure occurs. Once these changes are complete the neonatal heart has attained the adult like form of two pumps in series.

Fetal Ventricular Performance: Preload

Cardiac function curves which relate measurements of cardiac performance including stroke volume, stroke work, and cardiac output (on the ordinate) to an index of ventricular preload (on the abscissa), have been used to express the Frank-Starling relationship (102). Force generation by cardiac muscle is dependent upon sarcomere length (110). Ventricular end-diastolic wall stress determines ventricular volume and sarcomere length and, as such, reflects ventricular preload. Approximations of ventricular wall stress (accepting errors based on wall thickness [80]) can be made using Laplace's Law which predicts that wall stress is proportional to ventricular pressure and ventricular radius of curvature, and inversely proportional to ventricular wall thickness. The pressure in this instance is the effective distending pressure i.e., ventricular end-diastolic transmural pressure.

In the intact ventricle, preload has often been quantified using ventricular end-diastolic pressure, end-diastolic volume, end-diastolic diameter, or end-diastolic transmural pressure. As we shall see, the use of end-diastolic pressure as an index of preload is an over-simplification and is inappropriate under many conditions. Although ventricular wall transmural pressure is a more appropriate index of ventricular preload, many authors continue to use intracavitary pressures as an index of preload because of the ease of measurement and, perhaps, a failure to appreciate the significance of the differences between the two measurements.

Diastolic filling will, in part, determine the amount of preload the ventricle is exposed to. Diastolic filling, itself, depends upon factors which include venous return, myocardial compliance, and any constraining influences applied to the heart by the surrounding tissues. Elastic restoring forces may also contribute to the filling of the ventricle, particularly when the heart is functioning at a high contractile level. Systolic sarcomere shortening to below resting lengths may produce restoring forces that can produce ventricular suction early in diastole and help fill the ventricle (110).

Preload: Venous Return

Cardiac output and venous return are closely interrelated. Under steady state conditions venous return must equal cardiac output. As described by Berne and Levy (14) and Levy (71), a vascular function curve relates cardiac output, and thus venous return, to central venous

pressure. Venous pressure is a consequence of cardiac output. Increases in cardiac output tend to drain the venous reservoir and decrease central venous pressure, while decreases in cardiac output increase central venous pressure. When cardiac output is zero central venous pressure equals mean circulatory pressure. Changes in the gradient between mean circulatory pressure and right atrial pressure are caused by changes in cardiac output. This contrasts with the reasoning of Guyton et al. (49) who suggest that cardiac output is determined by this pressure gradient and that cardiac output is low when right atrial pressure is high because venous return is impaired.

Vascular function curves have been recorded in exposed fetal lambs using heart-lung by-pass techniques (37, 38). In these studies the vascular function curve of the fetus was shifted upward from that of the adult. Venous return (cardiac output) was high (284 ml/min/kg in the fetus versus 115-123 ml/kg/min for the adult), as was mean circulatory pressure (15.5 mmHg in the fetus versus 7 mmHg in the adult). The high level of venous return was supported by the elevated mean circulatory pressure and a low resistance to venous return. The elevated mean circulatory pressure in the fetus results from a higher blood volume and a lower venous capacitance (37, 38, 41).

The compliant nature of the fetal liver may provide an additional mechanism for regulating venous return in the fetus (42, 97). Almost 25% of the total venous return flows through the liver (a combination of portal venous flow, hepatic arterial flow, and umbilical venous flow). Redistribution of umbilical venous flow during periods of

hypoxia, reduced umbilical blood flow, and impaired umbilical venous return can increase the fraction of umbilical blood flow which passes through the ductus venosus (19, 29, 31, 55, 56, 91).

Preload: Myocardial Compliance

Myocardial compliance has been investigated in isolated hearts and isolated heart muscle preparations from fetal and neonatal animals (36, 70, 78, 88, 94, 95). Compliance, in the true sense, is defined by the slope of a pressure volume relationship, i.e., the change in volume for given change in pressure. By convention in cardiology, if ventricular volume decreases at a given end-diastolic pressure, compliance is said to decrease and vice versa (17). Early studies by Romero et al. (94) suggest that in the fetus the RV is more compliant than the LV. Pinson et al. (88) have repeated these measurements and have confirmed that the RV is indeed more compliant than the LV. Romero et al. (94) also tried to asses changes in ventricular compliance from late gestation to adulthood. In these studies it was suggested that LV compliance is lower in the fetus than in neonate and lower in the neonate than in the adult. No differences were observed between fetal and neonatal RV compliance both of which were less compliant than in the adult. These data are difficult to assess since no indication is given as to how ventricular dimensions (used to calculate wall tension) were calculated. Errors in calculating ventricular dimensions would significantly affect the resulting data. Furthermore, they have tried to relate wall tension to changes in ventricular radius but have not obtained data for the true slack radius. These studies are often

quoted but should be repeated with accurate measurements of ventricular volumes and diameters. Until this is done these data can only be used to suggest that the fetal LV is less compliant than the neonatal and adult LV.

Studies of isolated heart muscle preparations have supported the whole heart studies (78, 36). Compared to adult muscle, fetal moderator band muscles show higher passive and lower active tensions (36). There are no apparent differences in active tension relative to age when contractile mass and cross sectional area are accounted for (36). The extent of shortening at any given level of tension is also less in fetal heart muscle than in the adult; this might explain the sensitivity of the fetal heart to elevations in afterload (28, 113, 114).

The force-velocity relationships of fetal heart muscle are shifted shifted to the left of the adult values; at any level of tension velocity is lower, with no significant differences in the extrapolated Vmax (36). However, the tension-velocity properties of the fetal muscle converge upon the adult values after correction for contractile mass. Thus, force development, extent of shortening, and velocity of shortening are reduced in the fetal heart primarily as a result of the lower fraction of contractile mass in the fetal cardiac myocyte. Intrinsic sarcomere function appears to be similar in fetal and adult heart muscle (36, 78). Recently, Nassar et al. (86) have reported that isolated cardiac cells from 3-week-old rabbits have a reduced velocity of shortening, a reduced velocity of relaxation, and a longer duration

of contraction than adult tissue as a result of differences in sarcoplasmic reticulum development.

The compliance characteristics of both the LV and the RV are also influenced by the filling conditions of the opposite ventricle (94, 88). Elevating the pressure in one ventricle significantly reduces the volume needed to attain a given pressure in the opposite ventricle (94, 88). This effect is most obvious in the fetus and more obvious in the neonate than in the adult (94).

Preload: Constraining Influences

The overall compliance of the heart is also influenced by the structures which surround it (pericardium, lungs, and chest wall). Early studies conducted on isolated whole fetal hearts (94, 70) do not refer to the state of the pericardium. Pinson et al. (88) have shown that in isolated fetal lamb hearts the pericardium limits the volume of the LV and the RV at any given pressure. Furthermore, Morton and Thornburg (82) have shown that the pericardium limits RA transmural pressure. These authors report that pericardial pressure does not significantly change over a limited range of fetal development and they dismiss the importance of pericardial pressure in influencing fetal cardiac output. These results are in conflict with the results reported in Chapters 2, 3, and 4.

Limitations of Fetal Cardiac Output

Although fetal and young lambs have a limited ability to increase stroke volume in response to volume infusions (39, 68), birth is associated with an immediate increase in both LV and RV stoke volume and thus in cardiac output (23, 67, 68). These observations would seem to be inconsistent. Heymann and Rudolph (52) reported that increasing RV end-diastolic pressure, from a level of 8-10 mmHg to in excess of 20 mmHg, only increased RV output 20%. Based on these findings it was suggested that the Frank-Starling mechanism was not important in regulating fetal cardiac output (52, 98, 100).

Several authors have used sonomicrometry to confirm that the fetal LV does indeed respond with a Frank-Starling mechanism to changes in blood volume (8, 65, 67). Significant correlations between LV stroke volume and changes in LV internal minor-axis dimensions have been observed (65, 67). A direct correlation between LV end-diastolic dimension and the extent of LV shortening has also been reported (6, 7, 8, 65, 67). LV shortening increases over a range of LV end-diastolic pressures (Plved) between 0 and 8 mmHg but further LV shortening is not observed at higher pressures. A functional Frank-Starling mechanism is also evident during periods of fetal breathing. During episodes of fetal breathing LV diameters increase and, as a result, the percentage of fractional shortening of the LV increases (67). These data suggest that under normal conditions the Frank-Starling mechanism may help regulate fetal cardiac output.

The studies using sonomicrometry have also confirmed that Plved is not an appropriate index of ventricular preload. Kirkpatrick et al. (67) stated:

> "Measurements of ventricular end-diastolic diameters appear to be a substantially more reliable indicator of resting fibre length than measurements of end-diastolic pressure."

They conclude, however, that the lack Frank-Starling response beyond a Plved of 8-10 mmHg related to an over-stretching of the myocardium. We shall see that this limitation is actually determined by the constraining influence of the surrounding tissues (see Chapters 2, 3, and 4).

Measurements of LV, RV, and combined ventricular output have also confirmed the early reports by Heymann and Rudolph (52) that the fetal heart has a limited ability to augment stroke volume and cardiac output in response to volume infusions (39, 40, 90, 113, 114). Gilbert (39, 40) reported only small increases in cardiac output in response to substantial increases in atrial pressure. Cardiac output was reduced when blood volume was reduced. Since elevations in venous return and "ventricular preload" -- as indicated by elevations in RA pressure -- did not increase biventricular cardiac output, the fetal heart was said to lack substantial cardiac reserve (39). Had a more appropriate index of ventricular preload been used to construct these cardiac function curves a different interpretation could have been made (see Chapter 4).

In separate studies, Thornburg and Morton (113, 114) provided

complete fetal LV and RV function curves over a range of mean atrial pressures using electromagnetic flow probes. Autonomic blockade, utilizing propranolol and atropine, was performed to eliminate autonomic compensations to alterations in fetal blood volume which might have complicated earlier studies (39, 40). LV and RV ventricular function curves (90, 113, 114) were similar to those described by Gilbert (39, 40). Stroke volume increased as mean atrial pressure was elevated from 1 to 3 mmHg. Stroke volume plateaued as mean atrial pressure was increased further. According to their interpretation, the ascending limb of the ventricular function curve demonstrated the functioning Frank-Starling mechanism, while the plateau was thought to represent myocardial resistance to filling and might be related to internal resistance to stretching of the sarcomeres (113). Thornburg and Morton (113, 114) have referenced mean atrial pressure to pericardial pressure which was recorded with an open-ended catheter positioned in the opened pericardium. This was said to allow for the determination of transmural pressure and, thus, would account for any influence that pericardial constraint or tamponade might have upon the curves. It has been previously reported that the open-ended catheter can significantly underestimate the true pericardial pressure in this situation (106) (see Chapter 1: Ventricular Constraint).

These studies have led to the belief that the fetal myocardium is limited, by an inherent lack of cardiac reserve, in its ability to augment stroke volume. Based on the assumption that the plateau of the ventricular function curve is not attributable to pericardial constraint, tricuspid regurgitation, or changes in afterload, it has been

suggested that the Frank-Starling mechanism, although present before birth, does not contribute to the increase in cardiac output observed at birth (113).

Fetal Ventricular Performance: Afterload

What determines the breakpoint of the ventricular function curve has not been clearly defined. Afterload is known to influence cardiac function in the adult and the fetal lamb. Afterload, the load against which the ventricle must work throughout contraction, is best defined as instantaneous wall stress during contraction. Because of the dependence of wall stress upon ventricular radius, afterload decreases during systole and can be affected by the manipulations used to generate cardiac function curves (110).

The influence that afterload has on cardiac output has been studied in chronically instrumented fetal lambs (40, 41, 90, 113, 114). Gilbert (40) measured cardiac output before and after altering ventricular afterload. Elevating aortic pressure, using methoxamine, produced a downward shift in the combined ventricular function curve. Dilatation of the peripheral vasculature, with isoproterenol, eliminated the increase in aortic pressure normally associated with volume infusions, and shifted the cardiac function curve upward. The increase in cardiac output was, however, a result of the chronotropic effect of isoproterenol since stroke volume did not increase.

Gilbert (41) has recently suggested that increases in afterload

result in only minor changes in the shape and position of the cardiac function curve. Hawkins et al. (50) disagree and argue that the increase in afterload associated with the generation of a cardiac function curve significantly limits stroke volume. Furthermore, they suggest that reductions in afterload may be an important mechanism for increasing cardiac output at birth. This is important for the RV but is unlikely to affect LV output favorably since LV afterload is known to increase at birth.

In the fetus the RV appears to be more sensitive to elevations in afterload than the LV (90, 113, 114) possibly because of geometric differences between the two ventricles. In a morphometric study of the fetal heart, Pinson et al. (88) found that the RV has a greater radius-to-wall thickness ratio. Because of this, equal elevations in intracavitary pressures would increase RV systolic wall stress more than LV wall stress, thus increasing RV afterload more than LV afterload.

Fetal Ventricular Performance: Heart Rate

Although there are, of course, transient fluctuations, heart rate progressively decreases throughout gestation. This progressive cardiac slowing results from a decrease in the intrinsic rate of the pacemaker cells (118, 119). Prior to birth a second phase of cardiac slowing is observed which is independent of the intrinsic rate and primarily dependent upon elevated parasympathetic tone. Therefore, except for the transient increase in heart rate that occurs at birth, it is

increasing parasympathetic activity that is predominantly responsible for further reductions in heart rate toward adult levels (24, 124).

At or near birth, the level of circulating catecholamines transiently increases (32, 69, 118). Elevated catecholamine concentrations and the circulating levels of thyroid hormones (18) are, in part, responsible for a transient increase in heart rate at birth and thus contribute to the increase in cardiac output.

The influence of heart rate upon cardiac output during fetal life has been investigated by several groups, each using different methods and, in many instances, generating different conclusions. During spontaneous changes in fetal heart rate LV output has been reported to be constant (67), while other studies have shown that spontaneous increases in heart rate decrease LV and RV output, end-diastolic dimensions, and LV stroke volume (3, 5, 66, 101). Variable responses in LV and RV output have also been reported when atrial pacing has been used to alter heart rate. Rudolph and Heymann (101) reported that LA pacing consistently increased RV output and had variable affects on LV output (although LV output generally decreased) while RA pacing increased both LV and RV output. Anderson et al. (3, 5) report similar studies and suggest that RA pacing does not substantially alter fetal cardiac output because of a balance between changes in heart rate and stroke volume. LA pacing did, however, consistently reduce LV output by reducing the normal right-to-left atrial pressure gradient thus reducing LV filling (101). Furthermore, there is a longer interval between atrial contraction and ventricular contraction during left atrial

pacing, particularly at elevated rates (possibly related to decreased conduction velocity) which may influence effective ventricular filling (3). No significant changes were observed in afterload during spontaneous reductions in heart rate and, as such, afterload is not thought to limit cardiac output during changes in heart rate (101).

The variability between these studies may reflect a difference in recovery time prior to data collection. Kirkpatrick et al. (65, 67) suggest that 7 to 10 days may be needed for complete cardiac recovery. Both Rudolph and Heymann (101) and Anderson et al. (3, 5) record data after a much shorter recovery period. No indication is given as to when the majority of data was collected in the latter studies, but it might have been collected prior to the tenth day following surgery.

The response of cardiac output to pacing appears to change following birth. Increasing heart rate in young lambs (5-13 days old) does not increase cardiac output but small increases in cardiac output occur in response to increased heart rate in older lambs (15-30 days) (103). This study is complicated by the fact that ventricular pacing was used which eliminates the atrial contribution to ventricular filling

A complex interaction exists between changes in heart rate, preload, afterload, and contractility (3, 5, 66, 67). Studies in isolated heart muscle and chronically instrumented fetal lambs suggest that elevations in heart rate influence cardiac output not only through a rate change but also by changing contractility (3, 5, 6, 7, 8, 66,

67). As heart rate is increased LV end-diastolic dimensions and pressure decrease (67). Kirkpatrick et al. (67) and Anderson et al. (3, 5) have shown that, if ventricular volume is maintained, contractility increases as heart rate increases. The complex interaction between heart rate and cardiac output can also be seen in the fetal response to hypoxia. Mild hypoxia results in bradycardia and elevated systemic pressure but there is little change in cardiac output (21). Severe hypoxia also results in bradycardia and increased afterload but, in this case, cardiac output decreases.

Fetal Ventricular Performance: Contractility

Changes in cardiac contractility indicate a change in cardiac performance that is independent of preload and afterload (110). As a description of overall mechanical capacity, contractility is difficult to assess. The relation of contractility to heart rate is especially problematic, since increases in heart rate increase the value of time related parameters (e.g. dp/dt, ejection velocity indices) which sometimes are taken to indicate an increase in contractility. To eliminate this confounding variable most studies designed to detect changes in contractility involve cardiac pacing at a constant rate. Assessment of the ratio of systolic pressure and volume (elastance, Emax) has been suggested as an appropriate index of contractility (110). Other measurements of contractility, either isovolumic or ejection phase indices, have limitations (described in 110). Reliable estimations of contractile reserve have been obtained using post-

extrasystolic potentiation (ref in 110).

Assessment of fetal contractility, both in vitro and in vivo, has shown that the potential reserve for increasing contractility increases during gestation and during postnatal development as revealed by an increase in the magnitude of postextrasystolic potentiation (4). At birth there is a transient decreases in postextrasystolic potentiation in vivo, which suggests contractility has increased toward the maximal limit. This decrease in postextrasystolic potentiation was not evident in in vitro heart muscle preparations which suggests that the increase in contractility associated with birth is a result of transient hormonal changes or neural stimulation during this period (4). A decrease in the magnitude of response to beta-adrenergic challenge also suggests that contractility is increased in young lambs compared to older lambs (103, 112).

A higher level of contractility is also suggested by elevated isovolumic indices in young lambs (1-3 weeks) compared to fetal and adult values (12, 13, 93). dP/dt of new born lambs (3600-3800 mmHg/sec) exceeds that of the fetal lamb (2455-2655 mmHg/sec, [67]) and that of the adult sheep (2338-2542 mmHg/sec). These results are difficult to interpret as dP/dt depends upon ventricular volume (7) which was not controlled for in the earlier studies. Anderson et al. (8) also report that dP/dt is highest immediately after birth even after accounting for the effects of heart rate and ventricular volume. Analysis of the Pmax - interval ratio (an index of postextrasystolic potentiation in the intact animal) also suggests that contractility is

higher in the neonate than in the fetus (8).

Whether cardiac function is described in terms of stroke volume, stroke work, isovolumic contractile indices such as dP/dt, or in terms of postextrasystolic potentiation ratios such as the Pmax-interval ratio (7), the newborn lamb heart functions at a higher level than either the adult or the fetal sheep inorder to meet the added demands which occur at birth.

Autonomic Control of the Fetal and Neonatal Heart

Advancing gestational age is accompanied by the development of autonomic control mechanisms for the heart. A full review of this subject is beyond the scope of this chapter and readers are referred to recent review articles (83, 118). In brief, the development of autonomic control of the heart begins with neurotransmitter receptor formation prior to efferent nerve formation (ref in 118). Sympathetic and parasympathetic reseptor responsiveness develop at roughly the same time. Receptor functioning is followed, in order, by neuronal growth, neuronal release of neurotransmitters, and finally effective neurotransmission. Although the sequence of development of the sympathetic and parasympathetic systems are similar, the development of the sympathetic system lags behind that of the parasympathetic. This imbalance in neuronal development presents the possibility of excessive parasympathetic tone during development and is counteracted by the early appearance of beta-adrenergic receptors and circulating catecholamines (ref in 118).

Neuronal control of the heart through baroreceptors and chemoreceptors also develops during fetal and neonatal life. Large species related variations exist in the level of maturation of the baroreceptor reflex at birth; those species that are least maternally dependant have more active baroreceptor functioning (118). Whether or not baroreceptor function in utero is important is uncertain. Variability in baroreceptor function is often reported and appears independent of sleep state, fetal activity levels, and blood gas status (27, 73, 104). Baroreceptors do not appear to significantly influence the shape or magnitude of the fetal ventricular function curve (40). Chemoreceptors are active prior to birth (83), possibly being responsible for the bradycardia associated with mild fetal hypoxia (21). It is not certain if both the aortic and the carotid chemoreceptors actively control the fetal heart.

Changes At Birth

Birth is accompanied not only by the changes in the path of the blood through the cardiovascular system, but also by an increase in ventricular compliance, heart rate, and contractility. Furthermore, RV pressure decreases as pulmonary vascular resistance decreases and LV pressure increases as systemic vascular resistance increases (2, 10, 13, 33, 68, 124). A marked increase in LV and RV stroke volume and output also occurs. The increase in cardiac output is, in part, a response to the increase in metabolic demands placed upon the newborn to maintain body temperature and support rapid postnatal growth.

Comparing fetal cardiac output to that of the adult or of the neonate may, at first, appear misleading due to the parallel nature of the fetal circulation. Fetal cardiac output is often presented as combined ventricular output (400-450 ml/kg/min in the mature fetus [see 118]). The output of each ventricle increases to 400 ml/kg/min at birth and the series arrangement of the neonate/adult circulation results in systemic output equaling LV output. This value is substantially greater than the values for the adult (100 ml/kg/min in adult sheep). Combined ventricular output of the neonate would be equal to 800 ml/kg/min. Even though systemic cardiac output decrease slightly at birth, the flow available to individual tissues will increase since up to 40 to 50% of descending aortic flow in the fetus was destined for the placenta.

LV stroke volume doubles in the immediate neonatal period (68) from 1 ml/kg in the fetus to 2 ml/kg in the neonate. This level is gradually reduced over 4-6 weeks until adult levels are attained (1 ml/kg). The ability of the LV to increase its stroke volume by 100% or more reflects a major change in function which may depend, at least in part, on the Frank-Starling mechanism since LV volume increases at birth (65, 67) (see Chapter 3). Beta blockade does not abolish the increase in LV output at birth suggesting that the increase in LV stroke volume is not simply related to sympathetic stimulation (68). Stroke volume per kg body weight gradually decreases in the weeks following birth; this decrease reflects a more rapid rise in body weight than heart size, and a reduction of metabolic demands in older neonates. RV output also decreases toward adult values following birth

from early neonatal values of 290 ml/kg/min to 195 ml/kg/min after 5 weeks (123). Although cardiac output corrected for body weight decreases, actual stroke volume increases and stroke volume per kg body weight is maintained, showing that much of the decrease in cardiac output in the weeks following birth results from a reduction in heart rate.

The response of the newborn heart to volume loading also differs from that of the fetus. Newborn lambs (one week old) increase cardiac output by up to 34% in response to volume loading and six-week-old lambs by up to 58% (68). Similar interventions in fetal lambs result in limited increases in LV output (114). It has been suggested that this limitation in neonatal LV stroke volume results from a lower myocardial compliance and a greater sensitivity to afterload (94, 95). During volume loading, arterial pressure increases to a greater degree in young lambs than in older lambs or adults for similar increases in LV end-diastolic pressure (68), resulting possibly from an inability to reduce total peripheral vascular resistance during volume loading in the youngest lambs. This supports Gilbert's (40) contention that the fetal lamb (and possibly the neonatal lamb) has a limited venous capacitance due to a high blood volume and a high mean circulatory pressure.

The greater ability of older lambs to increase cardiac output in response to volume loading may relate to a shift in the resting position on the cardiac function curve. Minoura and Gilbert (79) present

data which show that 6 month old sheep normally function on the ascending limb of the cardiac function curve by maintaining a slightly lower LV end-diastolic pressure than younger lambs. This is different from adult dogs and man which function at the beginning of the plateau of the cardiac function curve (16, 87), possibly representing a species variation which requires further investigation.

Breal et al. (18) sought to explain the increase in cardiac output at birth by exploring the influence of thyroid hormones. Thyroidectomy several days before birth decreased T3 and T4 levels and, indeed, prevented the normal rise in cardiac output at birth by preventing the increase in heart rate normally associated with birth. Mean arterial pressure and O_2 consumption were also lower than normal. Thyroidectomy did not, however, prevent the increase in stroke volume associated with birth. Heart rate and cardiac output increase at birth only in the presence of normal thyroid hormone levels, possibly reflecting the importance of thyroid hormones in determining O_2 consumption and metabolic demands in the neonatal period. Stroke volume increases, even in the absence of thyroid hormones, suggesting that other mechanisms are also involved in the increase in cardiac output at birth.

Recently, several studies have utilized the technique of in utero ventilation to investigate the changes that occur in the cardiovascular system at birth (57, 81, 90, 111, 121). Attempts to isolate the effects of ventilation, oxygenation, and umbilical cord occlusion have produced conflicting results up to this point. Reller et al. (90) reported increases in heart rate and LV stroke volume and a reduction

in RV stroke volume with the initiation of in utero ventilation. Although it has been reported to mimic the process of birth, in utero ventilation with 100% oxygen did not increase either RV stroke volume or pulmonary artery flow. Iwamoto et al. (57) and Teitel et al. (111) have also reported similar studies. In utero ventilation, without altering fetal blood gas values, decreased heart rate, did not alter pulmonary or aortic pressure and did not increase cardiac output (combined ventricular output). Subsequent ventilation with oxygen did not affect cardiac output, and decreased mean descending aortic and pulmonary artery pressures. These results differ from those reported for a normal birth where cardiac output increases, heart rate increases, and systemic vascular pressures increases. It is possible that these techniques may be useful for investigating cardiovascular changes at birth, although at present they have produced conflicting results.

Ventricular Constraint

The factors which affect the ventricular pressure-volume (and diameter) relationship in the adult heart have been reviewed by Glantz and Parmley (43). Changes in the diastolic pressure volume relationship can arise without changing systolic function through changes in ventricular geometry and myocardial passive mechanical properties, and through ventricular interactions (43). It is also known that the constraining influence of the pericardium, lungs, and ribcage can substantially affect ventricular filling and thus shift the ventricular end-diastolic pressure volume relationship (35, 36, 59, 62,

63, 64, 72, 105, 106, 107, 108, 115, 116).

The magnitude of the constraint applied to the heart has been assessed using a variety of methods. Using open-ended catheters positioned in the pericardial space, Kenner and Wood (61) did not observe substantial increases in pericardial pressure when left and right atrial pressures were elevated in dogs. Using balloons and catheters, Holt et al. (54) reported that the balloon measurement of pericardial pressure exceeded that recorded with the open-ended catheter. It has since been shown that the open-ended catheter can significantly underestimate the true constraining influence of the pericardium in many instances (106). In situations where the pericardium is not watertight and when substantial volumes of fluid are not present in the pericardial space the pressure recorded with the open-ended catheter may be small while the overall constraining influences on the heart may be large. Smiseth et al. (106) demonstrated this point in adult dogs by comparing the pericardial pressure recorded with open-ended catheters and flat, liquid-containing balloons. Up to 30 ml of fluid was needed within the pericardial space in order for the catheters to measure the true constraining influence of the pericardium, while the balloon accurately recorded the magnitude of constraint regardless of the volume of fluid present in the pericardial space. Smiseth et al. (106) then demonstrated that after cutting several small slits in the pericardium, allowing fluid to escape from the pericardium, there was still substantial constraint applied to the ventricle. This constraint was reflected by the position of the ventricular pressure-diameter relationship as well as the by pressure recorded by the pericardial

balloon. The open-ended catheter registered zero pressure under these circumstances.

When the pericardium has been incised the difference between the pressure recorded by the pericardial balloon and the open-ended catheter reflects the surface contact stresses that exist between the pericardium and the heart. Surface pressures have been described by Agostoni (1) and are said to represent the deformational forces that exist between two contacting surfaces. These forces are vectorial in nature and, therefore, cannot be measured by the open-ended catheter. At end-diastole, when there is no ventricular motion, the direction of this force should be normal to the ventricular wall and can be estimated with the liquid-containing balloon (106). Not only are there forces acting on the ventricle arising from pericardial-ventricular contact, there are similar forces between the lung and the pericardium, which are then transferred to the ventricular wall and can be recorded by the pericardial balloon.

As will be mentioned in each of the following chapters, at end-diastole there exists a static equilibrium within the ventricle. The pressure within the ventricular cavity -- left ventricular end-diastolic pressure -- must be balanced by the sum of the ventricular wall transmural pressure and any additional forces applied to the ventricular wall by the surrounding tissues. If this is not the case the ventricular wall will move until a balance is achieved. We have used these assumptions to explore the effects of ventricular constraint upon the left ventricle during the perinatal period. In these studies

we used balloon transducers, similar to those described by Smiseth et al. (106), to record pericardial pressure. As mentioned previously, pericardial pressure has been assessed in fetal lambs using open-ended catheters in a limited number of studies (82, 88, 113, 114). Our results are very different from those previously described and we believe that this is because of the method used to record pericardial pressure. In each instance we have shown that our pericardial pressure measurements reflect the predicted values (as described in each of the remaining chapters).

As with any scientific work it is important to realize the limitations of the methods used. Through out this work a small pericardial balloon has been utilized to record pericardial pressures. Small is a relative term when dealing with animals of varying ages. In the fetal lambs the balloon covered roughly one half of the left ventricular free wall and was positioned on the lateral surface of the LV. Obviously the size of the LV free wall increases with advancing age and the balloon covered a smaller portion of the LV in the older neonatal lambs than in either the fetal or neonatal lambs. The balloon is constructed from compliant silastic rubber and, as mentioned in Chapter 4, had a total volume of less than 1.5 ml. It is possible that the size of the balloon many quantitatively affect the results presented in the remaining chapters. However, it is unlikely that the balloon's position or its size relative to the size of the heart will affect the qualitative results of these studies. Furthermore, the results described in Chapter 3 indicate that the balloon itself was not responsible for the shift in the pressure-diameter relationship of the fetal LV since it

was shown that larger diameters were always attained at any given end-diastolic pressure after the initiation of pulmonary ventilation, without changing the position of the balloon. At the time these studies were conducted this balloon represented state-of-the-art technology. Recently new balloons have been developed which are smaller and have a much greater frequency response. The frequency response of our balloon transducers was determined to be slightly over 15 Hz. The new balloon technology will make it possible to assess not only the diastolic components of pericardial pressure but also the systolic components.

To instrument the heart, we incised and reapproximated the pericardium. Again, the pericardial incision may have introduced quantitative differences. It is unlikely that we have significantly altered the constraint applied to the LV by the pericardium since care was taken to approximate the edges of the pericardial incision with interrupted sutures without overlapping the edges. The observations that the magnitude of constraint applied to the LV is reduced with the initiation of ventilation and that LV diameter increases at this time suggest that pure pericardial influences did not determine the pressure-diameter relationship prior to ventilation, as would be the case if the pericardium had been substantially altered. Nonetheless, alterations in the pericardium may have influenced the magnitude of the results.

Each study involved halothane anaesthetized animals. Since each animal was used as its own control and the level of anaesthesia

remained approximately constant, the qualitative changes cannot be explained by halothane. Again, the magnitude of response may vary but the overall responses should be similar.

In each study involving fetal lambs tracheal occlusion was used to prevent lung fluid loss during instrumentation. It is possible that during this time fluid accumulation occured and may have actually increased the magnitude of constraint applied to the LV. No measurements of lung fluid volume were made. No changes were observed in the baseline pericardial pressure during the the recovery period after the fetuses were instrumented, suggesting that fluid production did not have a significant impact on the observations reported herein. This uncertainty and others will be eliminated when these studies are repeated in chronically instrumented fetuses which undergo normal vaginal delivery while hemodynamic continue to be recorded.

The remaining chapters are presented in the order in which the actual studies were conducted. Our initial goal was to determine if the thoracic tissues (pericardium, lungs, and ribcage) limit ventricular diastolic filling in young lambs (Chapter 2). This study confirmed that ventricular filling in the lamb was limited by the constraining influence of the thoracic tissues and lead to the assessment of ventricular constraint in the perinatal period. Studies in Chapter 3 were designed to determine if reductions in ventricular constraint could be responsible for the increases in ventricular diameters known to occur at birth. Finally, we assessed the influence of ventricular constraint upon the shape and magnitude of the fetal left ventricular

function curve (Chapter 4). A brief global conclusion is presented in Chapter 5.

CHAPTER 2: Modulation of Left Ventricular Diastolic Filling by Thoracic Tissues in the Young Lamb

Abstract

The thoracic tissues have been seen to limit ventricular filling in adult animals and in man. The current study was designed to determine if a similar influence could be observed in young lambs in an attempt to further understand the mechanisms responsible for the dramatic changes that occur in cardiac function in the perinatal period. Left ventricular (LV) end-diastolic pressure-diameter relationships were assessed in two groups of supine halothane anesthetized lambs (Group I = one hour old, N=6, Group II = 2 to 43 days old N=8). Following sternotomy the pericardium was opened transversely along the atrioventricular sulcus and each animal instrumented to record LV anteroposterior endocardial diameters using ultrasonic transducers. A flat, liquid-containing balloon was positioned within the pericardial space over the LV free wall to assess intrapericardial pressure. The pericardium was approximated to the original volume and the chest was closed and made airtight. LV end-diastolic pressure-diameter relationships were measured first, when the chest and the pericardium were closed; secondly, when the chest was open and the lungs retracted from the heart; and finally, when both the lungs and the pericardium were retracted from the heart. The largest minor axis dimensions were recorded at any given LV end-diastolic pressure when both the lungs and the pericardium were retracted from around the heart. In Group II lambs the pressure-diameter relationship recorded

when the rib cage and the lungs were retracted (intact pericardium) maintained a position midway between that of the intact situation and that when all of the tissues were retracted from around the heart. The pressure-diameter relationship of the Group I lambs did not change significantly upon opening the chest and retraction of the lungs. The calculated transmural (i.e., LV end-diastolic pressure minus intrapericardial end-diastolic pressure) pressure-diameter relationship corresponded closely to the directly measured transmural pressure-diameter relationship (i.e., that measured when both the pericardium and lungs were retracted). These studies confirm that the thoracic tissues substantially modulate ventricular filling in young lambs and that this influence can be assessed with an intrapericardial balloon. Immediately after birth the magnitude of the constraint applied to the LV is primarily determined by the pericardium while in older lambs both the ribcage/lung combination and the pericardium constrain the LV. Pericardial pressure, as measured with the balloon transducer, is useful in calculating LV transmural pressure which is a better index of LV preload than is end-diastolic pressure.

Introduction

The thoracic tissues (pericardium, lungs and rib cage) are known to modulate left ventricular (LV) diastolic function in adult animals (35, 64, 106). The constraining influence of these tissues has been shown to significantly limit LV volume and thus limit the increase in ventricular preload that can be attained with elevations in LV end-diastolic pressure. Little attention has been given to the role that

local tissue constraint may have on LV diastolic filling in the fetal and newborn lamb. Pericardial pressure has been assessed using open-ended catheters in fetal lambs (82, 113, 114). These studies suggest that the pericardium does not significantly influence fetal cardiac function. We believe that by utilizing a flat, liquid-containing balloon, which assesses surface contact stress (106), the true magnitude of the constraining effects of the thoracic tissues can be recorded. Ventricular constraint may significantly influence LV filling and the development of ventricular preload, particularly in the fetal and early neonatal period when the fluid content of the lungs is high. We have studied the LV pressure-diameter relationships in the young lamb as a first step in determining the magnitude and significance of the constraint applied to the perinatal heart by the thoracic tissues. These studies have shown that the tissues which surround the neonatal heart limit LV diastolic filling, much as they do in the adult heart.

Methods

Left ventricular end-diastolic pressure-diameter relationships were assessed in 6 anesthetized newborn lambs (1 hour old [Group I]) and in 8 young lambs (three to 43 days old [Group II]) . The six fetal lambs which were to make up Group I were partially delivered by C-section (maternal halothane anesthesia) and instrumented while the umbilical circulation was maintained and breathing prevented by tracheal occlusion. Group II lambs were anesthetized using 1 to 1.5 % halothane (50% oxygen, balance nitrogen) and maintained in a supine

position while being ventilated (ventilatory rate and volume adjusted to maintain $PaCO_2$ between 30 and 40 mmHg, 5 cmH_2O PEEP).

In each lamb the sternum was split, the ribs retracted and a transverse incision was made in the pericardium at the level of the atrioventricular sulcus from the middle of the right atrium to the middle of the left atrium. The heart was delivered from the pericardium and endocardial ultrasonic transducers were positioned to measure mid-left ventricular anteroposterior minor-axis dimensions (Dlvap) (Sonomicrometer 120, Triton Technology Inc., San Diego, CA) (Figure 2.1).

A small (2 cm x 2 cm) flat, liquid containing, silastic rubber balloon was used to assess the intrapericardial compressive contact stress (hereafter called pericardial pressure) (106). The pericardial balloon was calibrated using a technique modified from McMahon et al. (77). The balloon was filled with degassed water and connected to a pressure transducer (Model P23ID, Gould Inc., Oxnard, CA) and amplifier (Model 13-4615-50, Gould Inc., Cleveland, Ohio) calibrated for 0-50 mmHg. The balloon was positioned in a plexiglass calibration chamber which resembled a drum; one face of the chamber was made of a silastic rubber sheet and the other of plexiglass. The balloon was positioned on the silastic sheet and a plexiglass lid was positioned over the balloon. The interior of the chamber was also connected to a pressure transducer calibrated for 0-50 mmHg. The pressure within the chamber was increased and the pressure deflections of the two transducers were recorded. The volume of water in the balloon was adjusted until equal

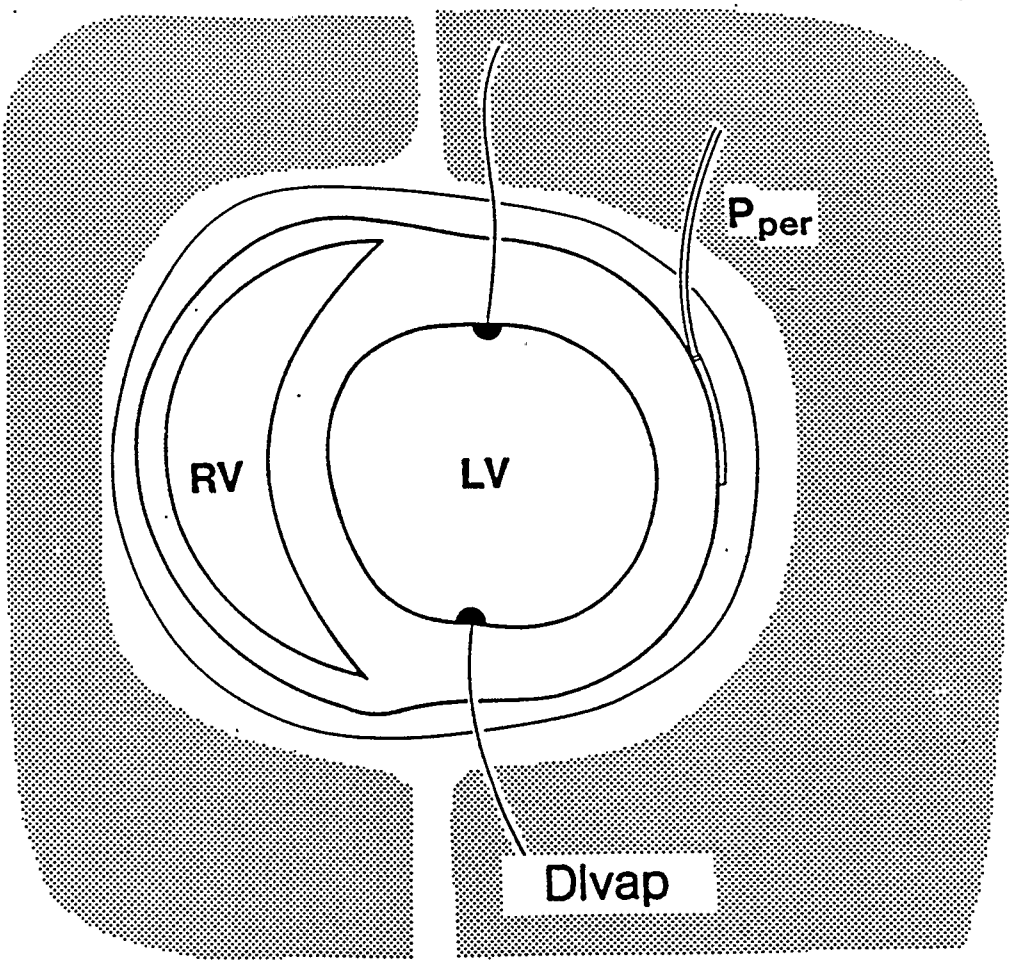


Figure 2.1: Schematic representation of the methods used to assess the influence of the the thoracic tissues upon ventricular diastolic filling (see text for detailed description). The shaded area represents the ribcage and the lungs. Left ventricular anteroposterior diameters were recorded with endocardial ultrasonic crystals, left ventricular pressure was assessed with a transducer-tipped catheter, and pericardial pressure was measured with a flat, liquid-containing balloon transducer (Pper)

deflections were registered from the two transducers over a range of pressures from 0-30 mmHg (106). The calibrated balloon was then positioned over the LV free wall within the intrapericardial space. The pericardium was approximated to its original volume with interrupted sutures. No attempt was made to seal the pericardium or make it water tight and the edges were not overlapped.

Left ventricular end-diastolic pressure (Plved) was measured using a transducer-tipped catheter (SPC-460, Millar Instruments, Houston, TX or 12CT/5F-2 Galtec Ltd., Dunvegan, Scotland) positioned in the LV via the carotid artery. LV pressures were set equal to the pressure recorded via the catheter's central lumen, or via an additional fluid-filled catheter in the LV. All pressures were referenced to the mid plane of the LV. The femoral or axillary artery was catheterized and blood was frequently sampled for analysis (IL System 1301, Instrumentation Laboratory Inc., Lexington, MA) to confirm adequate ventilation. The right jugular vein was also catheterized to allow for rapid volume infusion and withdrawal. The ECG was recorded using subcutaneous electrodes.

After instrumentation was complete, the ribcage was closed and made air tight and air evacuated with a continuous negative pressure of 2-4 cmH_2O. Each group was allowed a minimum of 15 minutes recovery before being studied. Left ventricular end-diastolic pressure diameter (Plved-Dlvap) relationships were assessed (as described below) in the Group I lambs immediately prior to the occlusion of the umbilical circulation and the beginning of mechanical ventilation (0.8%-1.4%

halothane, balance oxygen) (ventilatory rate and volume were adjusted to maintain $PaCO_2$ between 30 and 40 mmHg). After 1 hour of ventilation Group I lambs were treated in identical fashion to Group II lambs. Data were collected in both groups of lambs (Group I post ventilation) under the following 3 conditions: a) when the chest and the pericardium were closed (Closed Chest, Closed Pericardium, [CCCP]), a condition where the rib cage, lungs, and the pericardium could influence the heart; b) where the chest was widely open and the lungs were retracted away from the heart and the pericardium was closed (Open Chest, Closed Pericardium, [OCCP]), a condition where only the pericardium was in contact with the heart; and c) where the rib cage, lungs and the pericardium were retracted from the heart (Open Chest, Open Pericardium, [OCOP]). Plved was initially lowered by reducing blood volume and subsequently elevated in stepwise fashion (0-25 mmHg) with infusions of freshly collected sheep blood (and in one instance lactated Ringers solution) during each condition of study. Measurements of LV pressure, LV minor-axis dimensions, pericardial pressure, and ECG were made at each level of end-diastolic pressure only when the lungs were momentarily vented to atmospheric pressure. Dlvap-Plved relationships were reconstructed based on the data averaged from five beats during the sampling period. Data were recorded on a chart recorder (ES-1000, Gould Inc., Cleveland, Ohio) and onto FM tape for future analysis (Model 6500, Gould Inc., Cleveland, Ohio).

Curves were fitted to the mean data points collected under each of the three conditions using a cubic spline fit (SPSS Graphics Smooth Fit, SPSS Inc., Chicago IL). An analysis of variance (BMDP2V Repeated

Measures ANOVA, BMDP Statistical Software, University of California, Los Angeles, CA) was used to compare diameters interpolated from the curves over the range of end-diastolic pressures common to all of the animals studied (12.5, 15, 17.5 mmHg in Group I lambs and 5, 10, and 15 mmHg in Group II lambs). A Student-Newman-Keuls test was used to isolate differences detected by the analysis of variance with a probability (p) of less than or equal to 0.05 assumed to be statistically significant. All data are presented as means ± SD.

Assumptions

It has previously been proposed that a static equilibrium exists momentarily across the left ventricular free wall at end-diastole (115). The distending pressure of the left ventricle at end-diastole (left ventricular end-diastolic pressure [Plved]) must be balanced by the sum of the the pressure across the ventricular wall (left ventricular end-diastolic transmural pressure [Plved(tm)]) and any external forces applied by the surrounding tissues (the pericardium, lungs and chest wall) as reflected pericardial pressure at end-diastole (Pped) (Equation 1).

$$Plved = Plved(tm) + Pped \qquad \text{(Equation 1)}$$

When the rib cage, lungs, and the pericardium are held away from the heart Plved must equal Plved(tm) since the surrounding pressure is

atmospheric. Assuming that no change in ventricular distensibility occurs, subtraction of the variable contact stress exerted by the pericardium and the thoracic tissues, or by the pericardium alone, from any given Plved will produce a calculated left ventricular end-diastolic transmural pressure (Plved[tm]). This calculated pressure should equal the directly measured Plved(tm) at similar left ventricular end-diastolic diameters.

Results

Figure 2.2 displays data from a 3-day old lamb recorded when both the chest and the pericardium were closed. Volume infusions increased left ventricular end-diastolic pressure, left ventricular anteroposterior diameters, and pericardial pressure. Plved - Dlvap relationships were reconstructed from similar data recorded in each of the animals under each of the three conditions of study.

Volume infusion increased both Plved and Dlvap (Figure 2.3, Figure 2.4, Table 2.1 and Table 2.2) under CCCP conditions. In Group II lambs the Plved-Dlvap relationship was shifted upward when the rib cage/lung combination was retracted, i.e., at any end-diastolic pressure a larger end-diastolic diameter was observed as the constraining influence of the rib cage/lung combination was removed from around the heart (Figure 2.4, Table 2.2). When the ribcage/lung combination and the pericardium are retracted from around the heart of Group II lambs (OCOP), the Plved-Dlvap relationship was shifted further upward (Figure 2.4, Table 2.2), beyond that observed in either the CCCP or OCCP condition. At any given Plved the corresponding Dlvap was largest when both the lungs

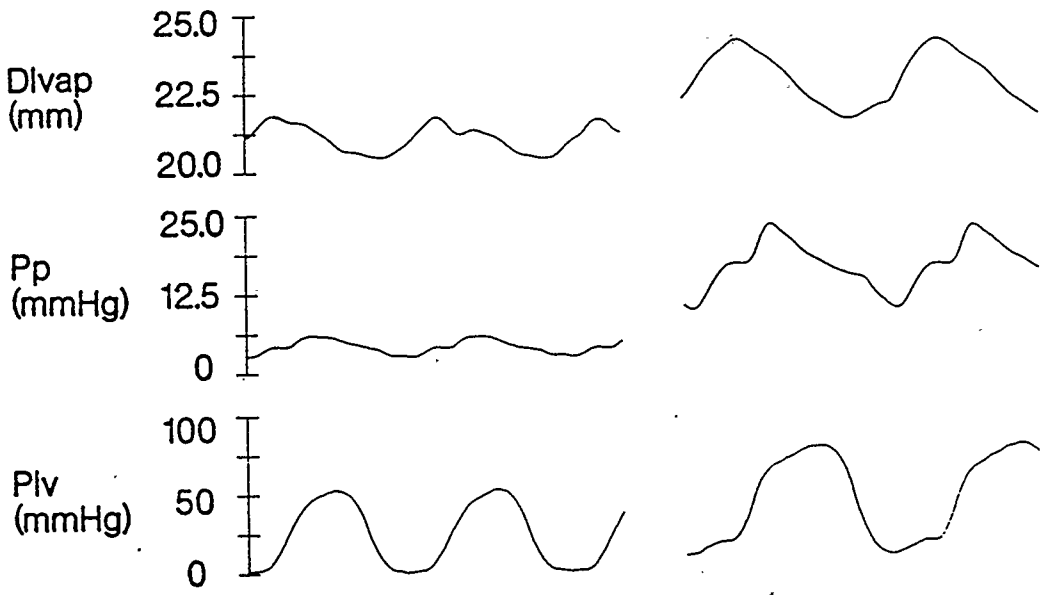
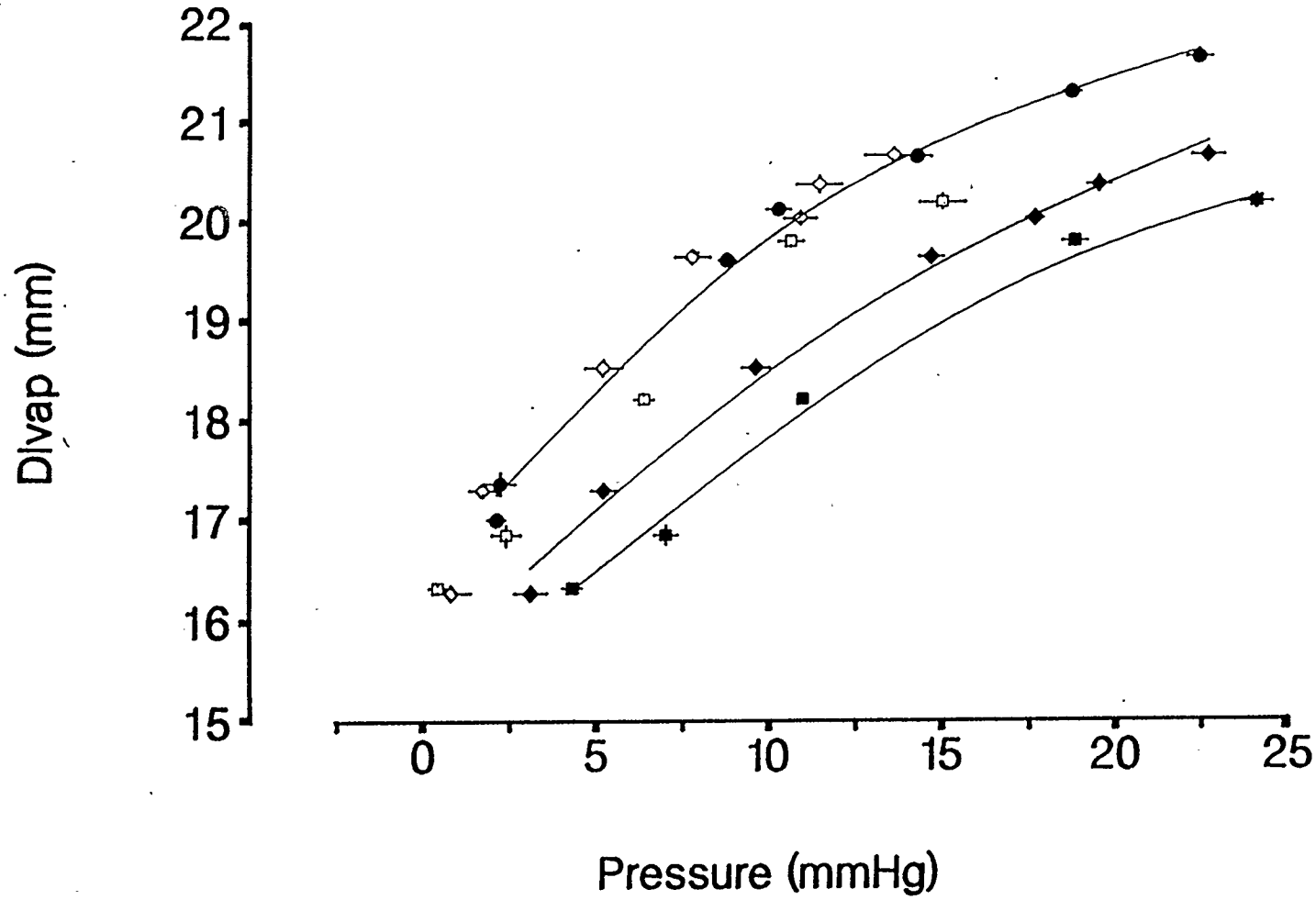


Figure 2.2: Data recorded in a 3 day old lamb under conditions where both the chest and the pericardium were closed. From top to bottom traces represent left ventricular anterior-posterior diameter (Dlvap), pericardial pressure (Pp) and left ventricular pressure (Plv). Data in the left panel were recorded prior to infusion of blood. Elevations in end-diastolic pressure were accompanied by elevations in the anteroposterior diameter and in pericardial pressure. The increase in pericardial pressure occurring in conjunction with the increase in left ventricular end-diastolic pressure limits the magnitude of the increase in left ventricular end-diastolic transmural pressure as calculated from Equation 1. Pressure-diameter relationships were constructed from similar data averaged over 5 beats recorded at end-diastolic pressures over a range of 0-25 mmHg in each of the conditions of study.

Figure 2.3: Left ventricular anteroposterior pressure-diameter relationships for 2 representative Group I lambs. Elevating Plved during conditions when both the chest and the pericardium were closed (CCCP) increased left ventricular anteroposterior diameters (Dlvap) (closed squares). Retraction of the ribecage/lung combination did not alter the pressure-diameter relationship (closed diamonds). The largest ventricular diameters were observed after retraction of both the ribcage/lung combination and the pericardium (OCOP) (closed circles). Closed stars represent the pressure-diameter relationship recorded prior to umbilical cord occlusion and ventilation. Calculated left ventricular end-diastolic transmural pressure-diameter relationships (based on equation 1) demonstrate the dependence of ventricular diameter upon transmural pressure and confirms the accuracy of the pericardial pressure recordings (open symbols as above) (Bars indicate ± SD)


Dlvap (mm)
22
21
20
19
18
17
16
15
0
5
10
15
20
25
Pressure (mmHg)
Plved
Plved-Pped
Closed chest, closed pericardium
Open chest, closed pericardium
Open chest, open pericardium

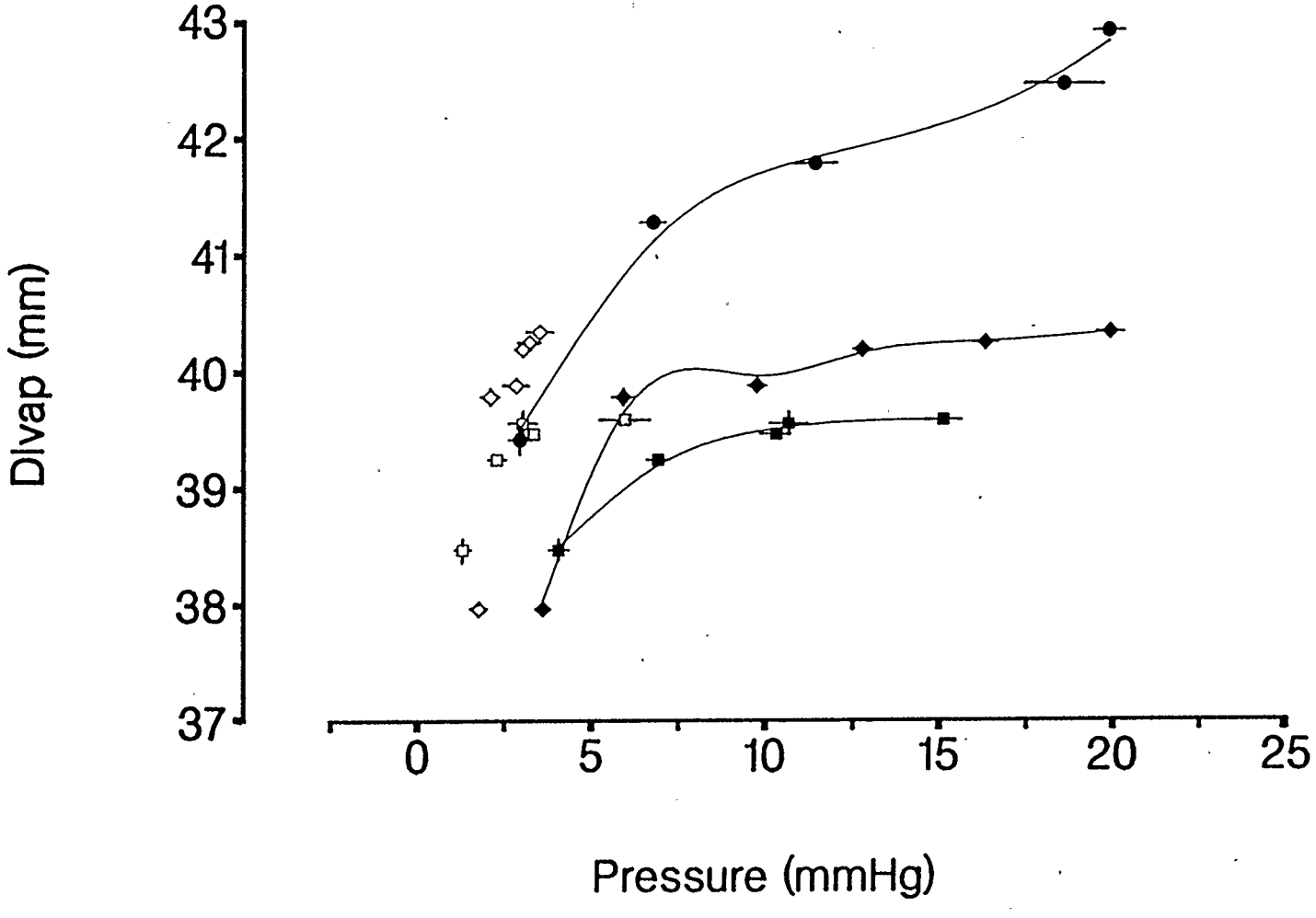
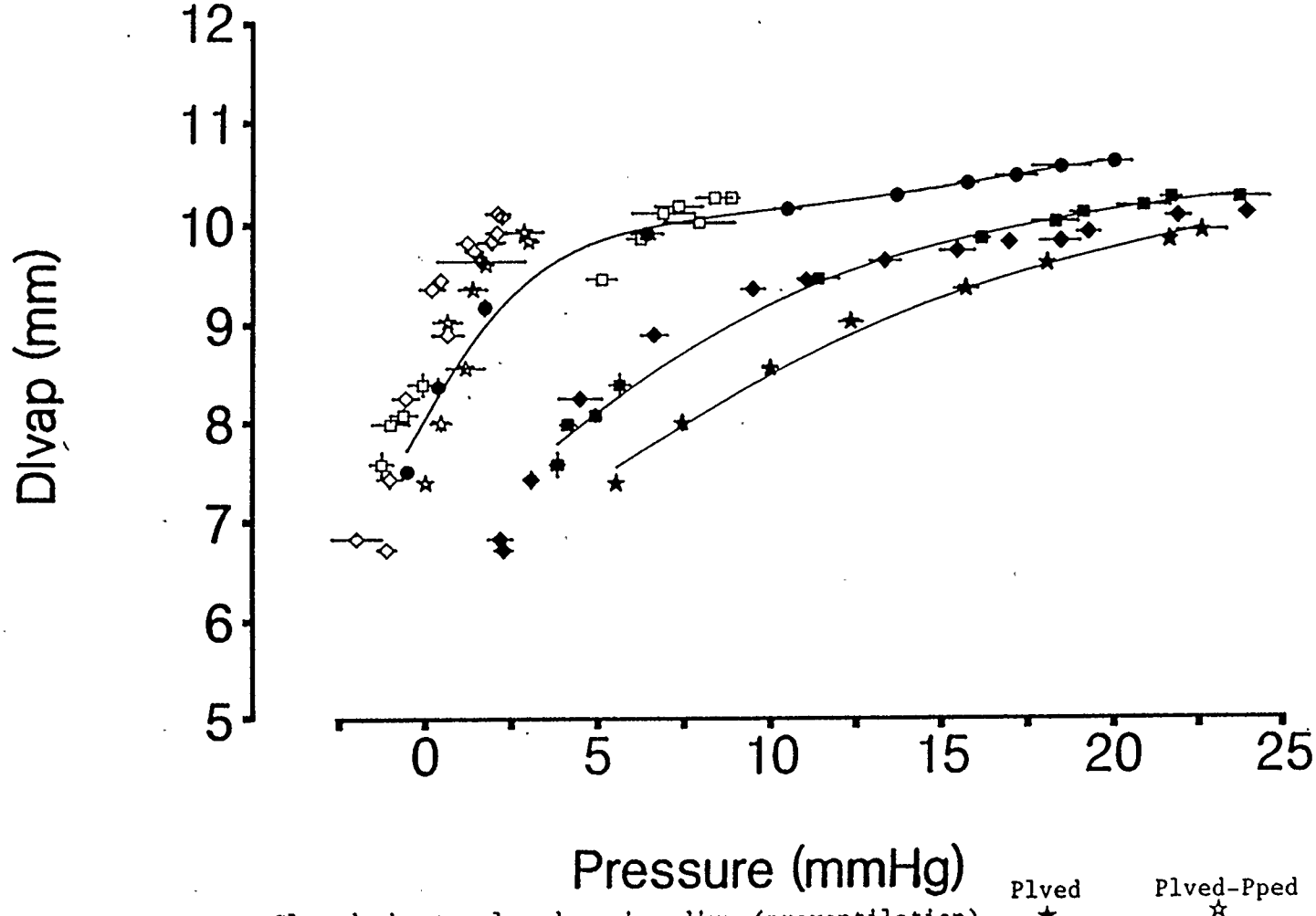

43
42
41
40
39
38
37
Dlvap (mm)
0
5
10
15
20
25
Pressure (mmHg)
Plved
Plved-Pped
Closed chest, closed pericardium
Open chest, closed pericardium
Open chest, open pericardium

Figure 2.4: Left ventricular end-diastolic pressure - anteroposterior diameter relationships in 2 representative Group II lambs. Elevating Plved during conditions when both the chest and the pericardium were closed (CCCP) increased left ventricular anteroposterior diameters (Dlvap)(closed squares). Retraction of the ribcage/lung combination shifted the pressure-diameter relationship upward (closed diamonds). The largest ventricular diameters were observed after retraction of both the ribcage/lung combination and the pericardium (OCOP, closed circles). Calculated left ventricular end-diastolic transmural pressure-diameter relationships (based upon equation 1) demonstrates the dependence of ventricular diameters upon transmural pressure and confirms the accuracy of the pericardial pressure recordings (open symbols as above) (Bars indicate ± SD).


Dlvap (mm)
12
11
10
9
8
7
6
5
0
5
10
15
20
25
Pressure (mmHg)
Plved
Plved-Pped
Closed chest, closed pericardium (preventilation)
Closed chest, closed pericardium
Open chest, closed pericardium
Open chest, open pericardium

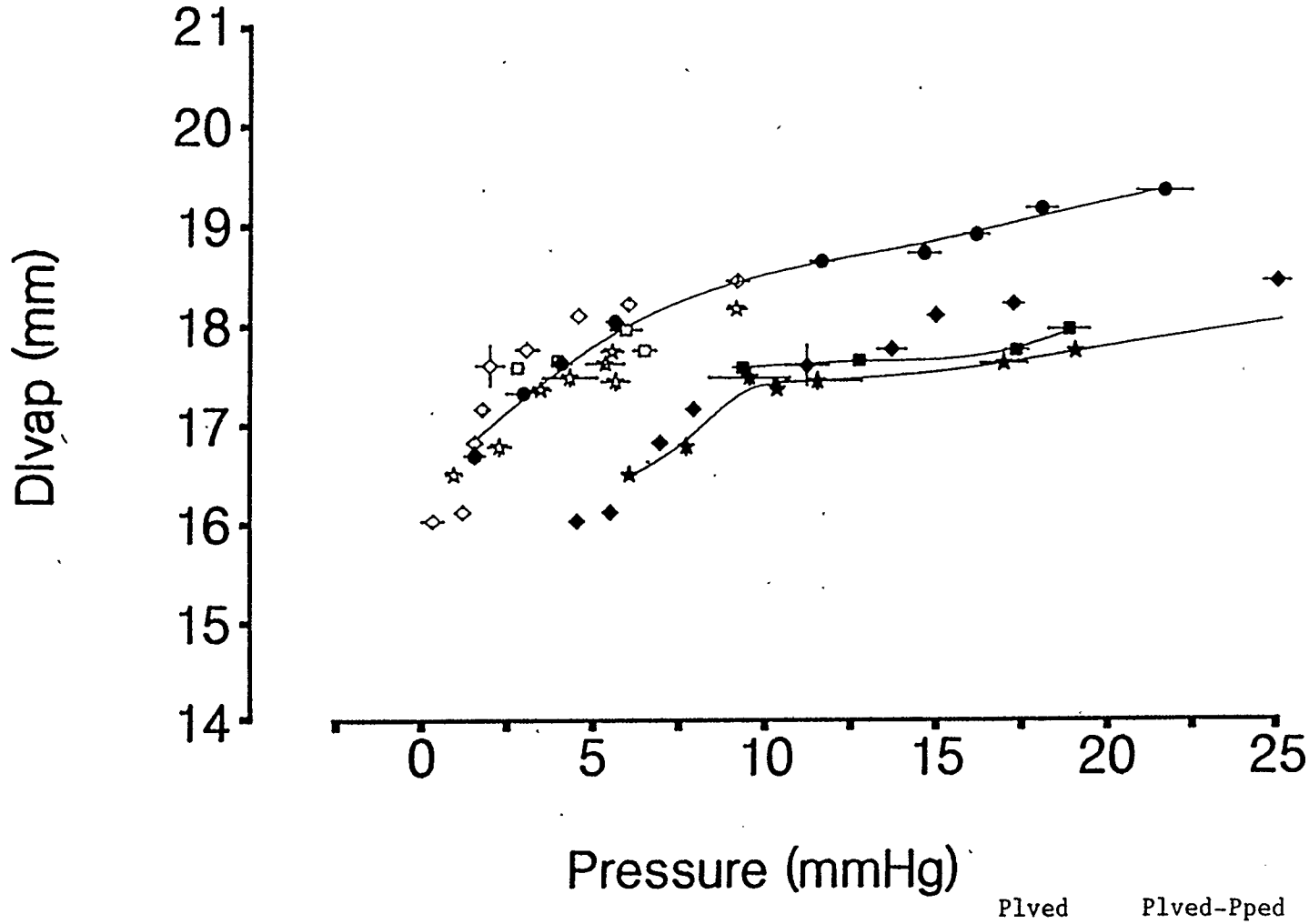

21
20
19
18
17
16
15
14
Dlvap (mm)
0
5
10
15
20
25
Pressure (mmHg)
Plved
Plved-Pped
Closed chest, closed pericardium (preventilation)
Closed chest, closed pericardium
Open chest, closed pericardium
Open chest, open pericardium

TABLE 2.1 Mean (± SD) left ventricular (LV) end-diastolic anteroposterior diameters (Dlvap) for Group I lambs (1 hour old) recorded under the three conditions of study; Closed Chest, Closed Pericardium (CCCP), Open Chest, Closed Pericardium (OCCP), and Open Chest, Open Pericardium (OCOP), at three levels of LV end-diastolic pressure (Plved).

Plved (mmHg)	CCCP	OCCP	OCOP
12.5	15.4 ± 3.6	15.4 ± 3.5	16.7* ± 3.6
15.0	15.9 ± 3.6	16.0 ± 3.5	17.2* ± 3.7
17.5	16.4 ± 3.6	16.2 ± 3.6	17.4* ± 3.8

(* significantly different from CCCP values, $p<0.01$)

TABLE 2.2 Mean (± SD) left ventricular (LV) end-diastolic anteroposterior diameters (Dlvap) for Group II lambs (2-43 days old) recorded under the three conditions of study; Closed Chest, Closed Pericardium (CCCP), Open Chest, Closed Pericardium (OCCP), and Open Chest, Open Pericardium (OCOP), at three levels of LV end-diastolic pressure (Plved).

Plved (mmHg)	**CCCP**	**OCCP**	**OCOP**
7.5	25.8 ± 12.8	26.7* ± 12.4	27.4** ± 13.0
12.5	26.4 ± 12.8	27.2* ± 12.9	28.3** ± 12.7
17.5	27.0 ± 12.7	27.7* ± 12.7	28.9** ± 12.5

(* significantly different from CCCP values, $p<0.01$)

(** significantly different from CCCP and OCCP values, $p<0.01$)

and the pericardium were retracted from the heart. In OCOP conditions Plved must equal the transmural pressure of the LV free wall since the surrounding pressure is atmospheric. A calculated Plved(tm)-Dlvap relationship was derived by subtracting Pped from Plved (Equation 1). The calculated Plved(tm)-Dlvap relationship closely approximated the directly measured Plved (tm)-Dlvap relationship (Figure 2.3 and Figure 2.4) confirming the accuracy of our measured pericardial pressure.

Group I lambs responded to a reduction in ventricular constraint in much the same way as the older lambs. At any given Plved a much larger Dlvap was recorded in the OCOP condition than in either the CCCP or the OCCP condition ($p<0.05$). Retraction of the ribcage and the lungs did not significantly influence the Plved-Dlvap relationship in the Group I lambs (Figure 2.3, Table 2.1) thus differing from the response observed in Group II lambs (Figure 2.3 and Figure 2.4). The Plved-Dlvap relationship recorded prior to umbilical cord occlusion and one hour of ventilation was below the CCCP relationship recorded one hour later. Ventilation reduced the constraint applied to the LV by the ribcage/lung combination (see Chapter 3) to such an extent that retraction of these tissues (OCCP) did not further reduce the constraint applied to the LV. After ventilation, only the pericardium limited LV filling in the Group I lambs.

Repeated volume challenges to Group II lambs in the CCCP state did not alter the Plved-Dlvap relationships observed in subsequent volume loads (Figure 2.5). Thus, the shifts observed in the Plved-Dlvap relationship between CCCP and OCCP conditions were not caused by

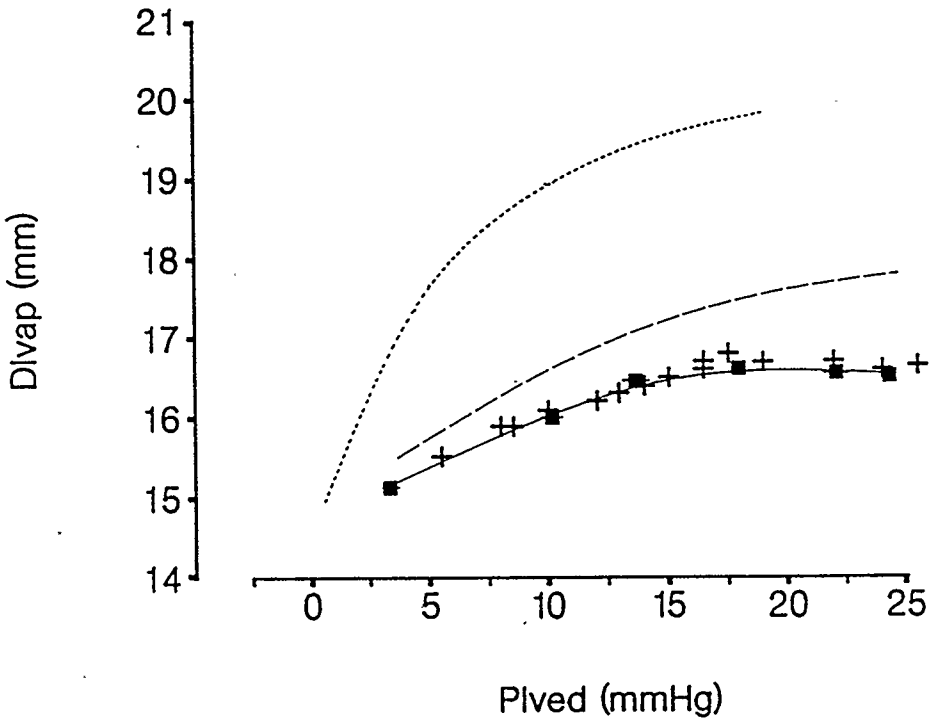


Figure 2.5: Demonstration of the response of the left ventricular end-diastolic pressure-diameter relationship in a Group II lamb to 2 sequential volume loads in a CCCP condition. The initial volume challenge produced data depicted by the closed squares while the subsequent volume challenge is depicted by the "+" symbols. Data collected during open chest, closed pericardium and open chest, open pericardium conditions are depicted by the dashed and dotted lines, respectively.

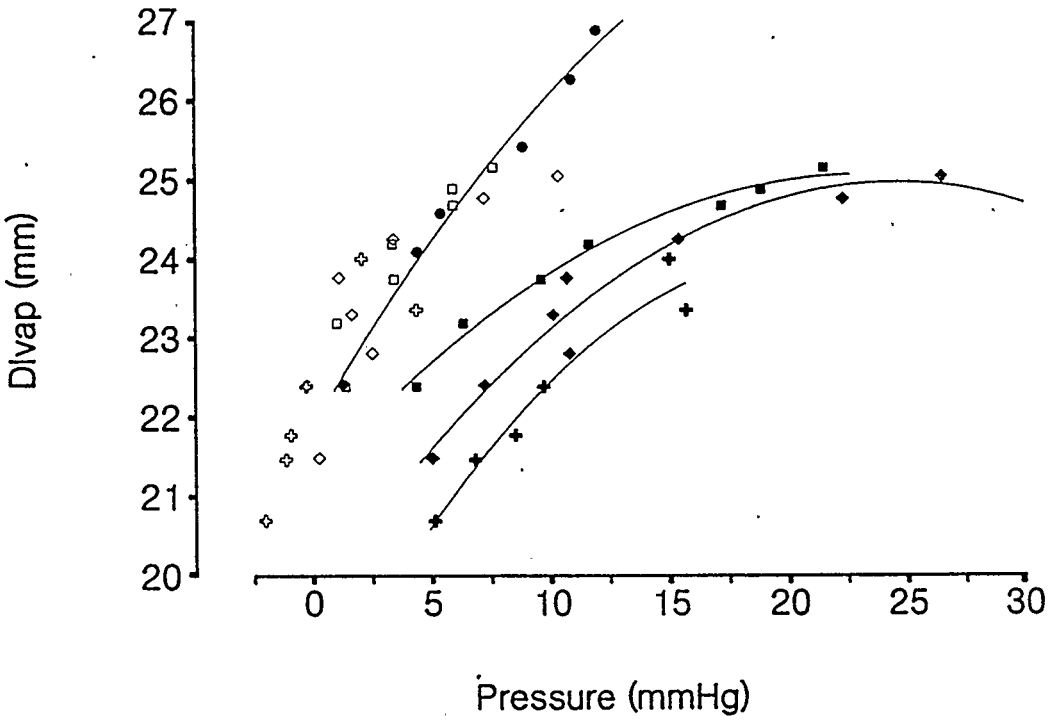


Figure 2.6: Left ventricular end-diastolic pressure-anteroposterior diameter relationship in response to elevations of positive end-expiratory pressure (PEEP). Under conditions of closed chest and closed pericardium, elevations in PEEP from 0 cmH_2O (closed squares), to 10 cmH_2O PEEP (closed diamonds) or 20 cmH_2O PEEP (closed crosses) shifted the pressure diameter relationship downward. Calculation of the left ventricular end-diastolic transmural pressure-diameter relationships at each level of PEEP (open symbols as above) closely follow the directly measured transmural pressure-diameter data as recorded with an open chest and open pericardium (closed circles).

plastic deformation of the pericardium.

Discussion

In the past an effort has been made to quantify and clarify the physiological characteristics of the fetal and neonatal heart and the transitions that occur in the cardiovascular system at birth (6, 7, 8, 39, 40, 65, 67, 97, 98, 113, 114). Not only are there functional changes in terms of the pathways that the blood follows through the heart but there are also significant increases in the heart rate, LV stroke volume, and LV minor-axis dimensions. Our present results provide insight into diastolic functioning of the LV of the neonate and suggest a new mechanism to explain the apparent lack of cardiac reserve in the fetal lamb. The shape of the fetal cardiac function curve closely resembles that of the adult (16, 39, 40, 87, 102, 113, 114). A reduction in ventricular intracavitry pressure below resting levels results in a reduction in stroke volume and cardiac output while elevation in pressure produces a minimal increase in stroke volume or cardiac output. This apparent limitation in cardiac function in fetal lambs has been previously suggested to arise from inherent myocardial properties including a reduced myocardial compliance and a functionally immature myocyte (97, 98). Our results suggest that the shape of the fetal cardiac function curve is likely to be largely influenced by the constraining forces which are applied to the heart by the surrounding tissues (see Chapter 4).

By assessing the Plved-Dlvap relationship we have demonstrated

that the thoracic tissues limit LV filling in the lamb. This observation is similar to those made previously in adult animals (106, 35, 64). When both the ribcage/lung combination and the pericardium were retracted from the heart the LV anteroposterior dimension increased significantly ($P<0.01$). In the older animals (Group II) both the ribcage/lung combination and the pericardium constrained the LV significantly. In the 1 hour old lambs (Group I) the pericardium was the major source of constraint while the ribcage/lung combination did not contribute significantly.

Retraction of the ribcage/lung combination resulted in a significant upward shift in the Plved-Dlvap relationship only in the older lambs (Group II). In general the OCCP relationship was found to be situated midway between the CCCP and OCOP data in the Group II animals. There was no shift in the Plved-Dlvap relationship following the retraction of the ribcage/lung combination in Group I (Figure 2.3, Table 2.1). This difference may relate to the transitional changes that occur in the cardiorespiratory system at birth. In another study (Chapter 4) we report that the constraint applied to the LV is reduced at birth. Based on these findings and our current results it appears that the majority of the constraint applied to the fetal LV arises from the influence of the fetal ribcage/lung combination and any additional constraint arising from the maternal tissues. The pericardial contribution must be less significant prior to birth so that the reduction in the constraint applied to the LV by the ribcage/lung combination and the maternal tissues can allow LV dimensions to increase. Alternatively, significant alterations in LV-RV interactions must occur

to allow for ventricular diameters to increase at birth. A rapid change must occur during the first several days after birth to allow the ribcage/lung combination to substantially contribute to the overall constraint applied to the LV. These adaptations may include pericardial growth, and the plastic deformation of the pericardium (creep) which has been seen to occur in adult dogs in response to prolonged distension of the pericardium (62, 72). The magnitude of the ribcage/lung contribution to overall constraint in Group II lambs appears to be larger at the higher levels of Plved than that reported for adult dogs at similar Plved (64). The magnitude of the constraint applied to the heart by the rib cage/lung contribution suggests that it may significantly influence LV systolic function. This is supported by common anecdotal observations that neonates in distress following thoracic surgery perform better hemodynamically if only the skin is closed. By leaving the sternum unapposed the internal volume of the thoracic cavity may be increased and in turn reduce the constraining influence of the ribcage/lung combination upon the heart.

Elevating end-diastolic pressure in the CCCP and OCCP conditions in both groups of lambs did not increase Dlvap to the same magnitude that was observed in the OCOP state. Since minor-axis dimensions of the LV reflect ventricular volume, the tendency for diameters to be limited by the surrounding tissues as end-diastolic pressure is elevated confirms that end-diastolic pressure does not reflect ventricular volume nor does it accurately reflect ventricular preload (43, 60). Since preload is one of the major determinants of cardiac function and since intracavitary pressures are unreliable indices of

ventricular preload, it is inappropriate to utilize intracavitary pressures when generating cardiac function curves. Preload (end-diastolic ventricular wall stress) determines muscle fibre length and thus, by the Frank-Starling mechanism, the strength of the subsequent contraction. When elevations in end-diastolic pressure are accompanied by equal increases in pericardial pressure, preload does not increase. Ventricular wall stress is dependent upon transmural pressure; therefore, transmural end-diastolic pressure is a better indicator of preload.

We have chosen to utilize the balloon transducer to record the magnitude of the constraint applied to the LV since the balloon has previously been shown to reflect the effective pericardial pressure under all conditions. Recent attempts at assessing pericardial pressure in fetal lambs have relied upon an open-ended catheter and have suggested that pericardial pressure does not influence fetal cardiac function (82, 113, 114). We believe that this is not the case and that their results reflect the methods used to assess pericardial pressure. In adult dogs it has been shown that the open-ended catheter significantly underestimates the constraining influence of the pericardium unless more than 30 ml of liquid is present (106). These same studies have shown that the balloon transducer accurately records pericardial pressures regardless of the volume of fluid in the pericardium. Furthermore, when the pericardium is not sealed or fluid tight the balloon can still be used to accurately assess pericardial pressure (contact stress). The accuracy of our assessment of pericardial pressure and of the in vitro calibration of our balloon has been confirmed

using the methods originally described by Smiseth et al. (106). By comparing the positions of the calculated Plved(tm)-Dlvap relationship to that of the directly measured transmural pressure data we have seen that the balloon reflects pericardial pressures as predicted from Equation 1.

In any preparation that requires opening and subsequently closing the pericardium there is the possibility that additional constraint has been applied to the heart. No attempt was made to seal the pericardium and the edges of the pericardial incision were not overlapped. The amount of instrumentation within the pericardial cavity was kept to a minimum and the pericardial incisions were as small as practical. It is unlikely that we have significantly increased the normal amount of constraint applied to the heart. Furthermore, alterations of the pericardium should not affect the constraint applied to the heart by the ribcage/lung combination.

It is also unlikely that plastic deformation of the pericardium in response to the initial volume load accounts for the upward shift in the Plved-Dlvap relationships in the Group II animals during OCCP conditions. Stress relaxation and or creep of the pericardium does not occur in adult dogs exposed to acute volume loading, nor is hysteresis observed in pericardial pressure-length relations (76). As the perinatal pericardium may behave differently to that of the adult we eliminated the possibility that plastic deformation of the pericardium accounted for our results by volume loading 3 of the Group II lambs two times, in sequence, during CCCP conditions. In each case the second

Plved-Dlvap relationship fell directly upon the original Plved-Dlvap relationship (Figure 2.5). In the Group I animals it is also obvious that plastic deformation has not occurred as the OCCP data did not significantly different from the CCCP data.

Preliminary observations in several Group II lambs have suggested that the neonatal LV responds to elevations in end-expiratory pressure in much the same way as do adults (34, 35, 64). Increasing PEEP sequentially from 0 to 10 and then 20 cmH_2O sequentially decreased LV anteroposterior diameters (Figure 2.6). The reductions in ventricular diameter appears to relate to reductions in ventricular transmural pressure as pericardial pressure increased. The effects of PEEP were most marked at low end-diastolic pressures as reported previously for adults (64). Even under conditions of PEEP, predictions of left ventricular end-diastolic transmural pressure can be made using Equation 1 and the pericardial pressure recorded with the pericardial balloon.

In summary, we have utilized a flat, liquid-containing balloon to assess the constraining influence that the thoracic tissue has upon the left ventricle of lambs ranging in age from 1 hour to 43 days. Left ventricular diastolic filling is indeed limited by both the rib cage/lung combination and the pericardium in lambs older than 2 days while only the pericardium has a significant constraining effect on the LV of the 1 hour old lamb. The present study does not define the influence that the surrounding tissue has upon systolic function. It has, however, clearly been shown that these structures do modulate the

diastolic filling of the left ventricle in the lamb and, as such, modulate left ventricular preload. Our studies have again confirmed that intracavitary pressures are a poor indicator of ventricular preload. As left ventricular end-diastolic pressure is elevated so, too, is the magnitude of the constraint applied to the ventricle by the surrounding tissues. The resulting change in ventricular preload and thus volume is dependent on the relative increases in intracavitary pressure and ventricular constraint. Accurate calculations of transmural pressures can be accomplished utilizing a flat, liquid-containing balloon to assess pericardial pressure. Using this technique we have accurately measured the constraint applied to the heart and confirm the importance of utilizing an index of ventricular transmural pressure when describing pressure-diameter relationships and cardiac function curves. Elevations of left ventricular end-diastolic pressure do not increase preload if a concomitant rise in the constraint placed upon the ventricle occurs (as reflected by an increase in pericardial pressure). This has been well known for the adult heart but has not received the attention it deserves in studies involving fetal and newborn animals.

CHAPTER 3: Reductions in External Left Ventricular Constraint in with the Initiation of Ventilation

Abstract

Since constraint on the left ventricle limits left ventricular diastolic filling in adults and neonates, and since this same constraint limits fetal left ventricular (LV) function, we designed a study to determine if reductions in ventricular constraint occur at birth and whether this can explain the increase in cardiac function which has been observed at birth. Six pregnant ewes (142-144 days gestation) were anesthetized (1.5%-2.0% halothane, balance O_2), ventilated and maintained supine. Each fetal lamb was partially delivered by C-section and, without interrupting the umbilical circulation, the sternum was split, and the pericardium was opened along the atrioventricular sulcus. Endocardial ultrasonic transducers were positioned to record LV anteroposterior diameters. Intrapericardial pressure was measured using a flat, liquid-containing, balloon transducer positioned over the LV free wall. The edges of the pericardium were approximated with care being taken not to reduce the original volume; the chest was closed and made air tight. LV pressure-diameter relationships were recorded under 3 conditions: State 1, in the fetus; State 2, in the neonate after interruption of the umbilical circulation and 1 hour of mechanical ventilation; and State 3, in the neonate when the chest was open and the lungs and the pericardium were retracted from the heart. A range of end-diastolic pressure was achieved by altering fetal blood volume. Comparison of the State 1 and State 2

pressure-diameter relationships revealed a significant increase ($p<0.01$) in LV diameters at any given end-diastolic pressure in State 2. Pericardial pressure was higher ($p<0.01$) at any given end-diastolic diameter in State 1 than in State 2. A close relationship was observed between the directly measured LV transmural pressure-diameter relationship (recorded when the chest and pericardium were open, State 3) and the LV transmural pressure-diameter relationship calculated on the basis of the measured pericardial pressure. The transition from State 1 to State 2 was accompanied by a reduction in the constraint applied to the heart and, therefore, larger diameters (volumes) were attained at any given distending pressure. This mechanism may account for the increase in cardiac output that is observed at birth.

Introduction

The transition from intra-uterine life to extra-uterine life is accompanied by an increase in cardiac performance as reflected in larger left and right ventricular outputs, increased heart rates, and larger left ventricular (LV) dimensions (4, 8, 65, 122). Based on a previous observation that the fetal heart lacks significant cardiac reserve (39, 40, 113, 114) it has been difficult to explain the increase in cardiac output that occurs in the immediate neonatal period. Elevations in heart rate and contractility , and hormonal changes all occur at birth but do not totally explain the increase in cardiac function.

Although it has been shown that the thoracic tissues influence the

diastolic filling of the LV in the adult dog (106, 108, 64, 35) and the newborn sheep (45), until recently little data existed to define the effect of these tissues upon cardiac function in the fetus. In a study by Morton and Thornburg (82), pericardial pressure was recorded in chronically instrumented fetal lambs using fluid-filled catheters. Although elevations in right atrial pressures beyond 3-4 mmHg were accompanied by linear increases in pericardial pressure and no concurrent data were provided to assess the influence that the elevation in pericardial pressure may have had upon ventricular function, these authors felt that the pericardium did not limit fetal left or right ventricular function (113, 114). In contrast the present study has demonstrated that the apparent lack of cardiac reserve in the fetus is not due to a lower myocardial compliance but is a result of the constraining influence of the pericardium, liquid-filled lung and unexpanded chest on the heart which limits LV transmural pressure (46, 47). In our first study of heart-lung interaction in the fetus we measured LV pressure, pericardial "pressure" (using balloon transducers which measure compressive contact stress, not just the pressure of the fluid in the pericardium), and aortic flow (SV). In contrast to other studies which showed that little or no increase in LV stroke volume was generated by increasing fetal blood volume beyond control levels (39, 40, 113, 114) we have demonstrated a mechanism which explains the capacity of the fetal LV to increase its stroke volume at birth (46, 47). By reducing the constraint applied to the LV of the fetal lamb, by retracting the thoracic tissues from around the heart, we observed significant increases (65%) in LV stroke volume, beyond the maximal levels observed under control conditions.

Prior to this investigation there have been no studies which explore the role of constraint from the pericardium and the thoracic tissues upon diastolic filling during the transition from fetus to neonate. The present studies were designed to assess the influence that the tissues which surround the heart (i.e., the pericardium, and the lung-chest wall combination) have upon fetal LV diastolic filling and to record the transitional changes that occur in intrapericardial pressure with the initiation of ventilation. Our results indicate that the thoracic tissues significantly restrict diastolic filling of the fetal LV. A reduction in constraint on the ventricle within 1 hour of the onset of ventilation permits the increase in ventricular diameters seen at birth (8, 65) and may account for a portion of the increase in cardiac output at birth.

Methods

Six pregnant ewes (mixed western breeds) (142-144 days gestation) were anesthetized with sodium thiopental (1 mg/kg) and then ventilated with oxygen and 1.5%-2.5% halothane. A mid-line laparotomy was performed and the fetal head delivered through a hysterotomy into a saline-filled rubber glove to prevent the fetus from breathing air. Each fetus was tracheostomized with an occluded tracheal tube. The upper body was delivered and allowed to lie in a supine position on the ewe's abdomen while care was taken to maintain the umbilical circulation. The sternum was split and the pericardium incised transversely from the the right atrium to the middle of the left atrium along the atrioventricular sulcus. The heart was delivered from the pericardium

and ultrasonic transducers were positioned on the endocardium to assess mid-LV anteroposterior minor axis dimensions (Dlvap) (Sonomicrometer 120, Triton Technology Inc., San Diego, CA) (Figure 3.1). Following each experiment the lambs were killed with an injection of saturated KCl and the position of the ultrasonic crystals was verified.

A small (2 cm x 2 cm), liquid-containing, silastic rubber balloon was used to measure intra-pericardial end-diastolic pressure (Pped) (106). The intra-pericardial balloon was connected to a calibrated (0-50 mmHg) transducer (Model P23ID , Gould Inc., Oxnard CA) and amplifier (Model 13-4615-50, Gould Inc., Cleveland Ohio). The balloons were calibrated using the technique of McMahon et al. (77) before being positioned on the LV free wall. Calibration of the balloon was confirmed at the end of each experiment. LV pressure was recorded using a transducer-tipped catheter (SPC-460, Millar Instruments, Houston, TX) positioned in the LV via the carotid artery. LV pressures were set equal to the fluid pressure recorded from the catheter's central lumen using a calibrated transducer (0-100 mmHg). All pressures were referenced to the mid-plane of the LV. The heart was returned to the pericardium and the incision loosely approximated with interrupted sutures, and the edges were not overlapped, to maintain the original pericardial volume. No effort was made to seal the pericardium since the pericardial balloons have previously been shown to reflect intra-pericardial pressure (contact stress) accurately under these conditions (106). The chest was closed, made air tight, and evacuated of air with constantly applied negative pressure (2-4 cmH_2O).

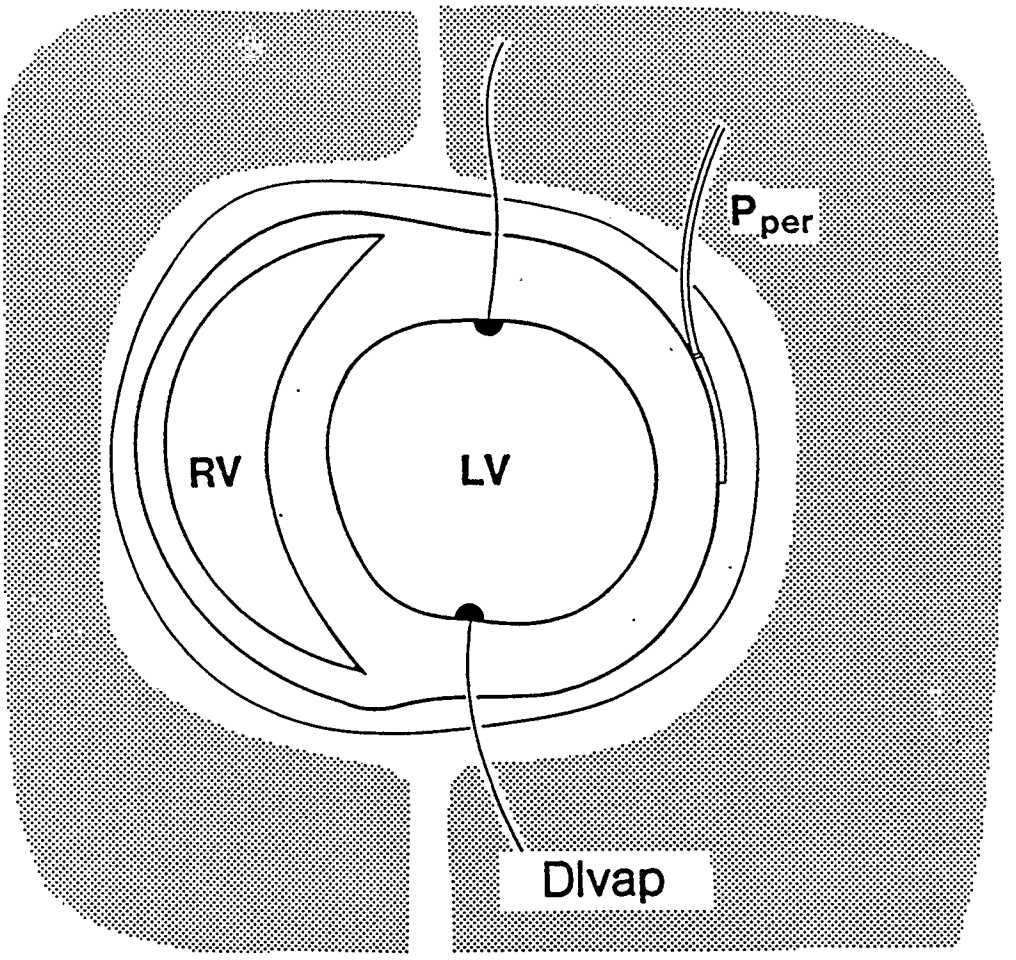


Figure 3.1: Schematic diagram of methodology. Intra-pericardial pressure (Pp) was assessed with a fluid containing balloon positioned over the left ventricular (LV) freewall. Mid LV endocardial anteroposterior dimensions (Dlvap) were measured using ultrasonic transducers. LV pressure was recorded using a transducer-tipped catheter positioned in the LV via the carotid artery. LV end-diastolic pressure - diameter relationships were recorded over a range of LV end-diastolic pressures by altering fetal blood volume under the three conditions of study: State 1, closed chest and closed pericardium in the fetus; State 2, closed chest and closed pericardium after interruption of the umbilical circulation and the beginning of mechanical ventilation in the newborn; and State 3, open chest and open pericardium in the newborn.

A catheter was positioned in the axillary artery to allow blood sampling for blood gas analysis throughout the study (IL System 1301, Instrumentation Laboratory Inc, Lexington, MA) and the left jugular vein was catheterized to allow access for volume infusions. The ECG was recorded. Body temperature (esophageal) was maintained with a heating lamp. The fetal lambs were allowed to recover for 15 to 30 minutes after the completion of instrumentation. All data were recorded on chart paper (ES 1000, Gould Inc, Cleveland, Ohio) and onto FM tape for further analysis (Model 6500, Gould Inc, Cleveland, Ohio).

LV end-diastolic pressure-diameter relationships were recorded over a range of left ventricular end-diastolic pressures (Plved) by rapidly removing and subsequently reinfusing fetal blood and additional maternal blood to attain a maximum Plved of 20-25 mmHg under 3 distinct conditions. Before ventilation was begun pressure-diameter curves were generated while the umbilical circulation was maintained, the lungs were free of air, and the chest and pericardium closed (State 1). Plved was then returned to a normal level, the umbilical circulation interrupted, and ventilation begun (initially volume was 15 ml/kg and rate was 40/min; oxygen and 0.8%-1.5% halothane). Ventilatory rate and volume were adjusted to maintain arterial $PaCO_2$ between 30 and 40 mmHg.

At 5 minute intervals the trachea was momentarily opened to the atmosphere, and data collected without altering fetal blood volume. A complete LV end-diastolic pressure-diameter relationship was assessed by volume loading the neonate (with a closed chest and closed pericardium [State 2]) at the end of 1 hour of ventilation. Only data

collected during the brief periods when the trachea was open to atmospheric pressure were analyzed. A final pressure-diameter curve was generated after the neonatal chest had been opened, the lungs retracted and the pericardium widely incised (open chest, open pericardium [State 3]). During State 3, the unconstrained pericardial balloon was used as a siphon to register any change in the midplane of the LV.

Assuming that a static equilibrium is achieved at end-diastole (106), left ventricular end-diastolic pressure must be balanced by the sum of the left ventricular wall transmural pressure and any additional forces (as measured by the intra-pericardial balloon) applied to the heart by the surrounding tissues. When the rib cage, lungs, and the pericardium are widely retracted from the heart the surrounding pressure is atmospheric and LV end-diastolic transmural pressure is directly measured in that the pressure within the ventricle is then equal to the pressure across the ventricular wall. Left ventricular end-diastolic transmural pressures (Plved[tm]) were calculated from data collected in the State 1 and State 2 by subtracting intra-pericardial end-diastolic pressure (Pped) from left ventricular end-diastolic pressure (Plved) (Equation 1)

$$Plved(tm) = Plved - Pped \qquad \text{Equation 1}$$

Curves were fitted to the mean of five consecutive end-diastolic data points collected at each sampling interval under each condition using a cubic spline fit (SPSS Graphics Smooth Fit(10), SPSS Inc, Chicago, IL) and an analysis of variance (BMDP2V Repeated Measures

ANOVA, BMDP Statistical Software, University of California, Los Angeles, CA) was used to compare the diameters (converted to a percent of the State 1 value attained at a Plved of 12.5 mmHg) interpolated from the curves over a range of Plved (12.5, 15, and 17.5 mmHg) common to all of the animals and all of the conditions. A Student-Newman-Keuls test was used to isolate differences detected by the analysis of variance. By transposing Equation 1, it is apparent that the difference between Plved when the pericardium is intact and that measured at the same diameter after the removal of the pericardium (i.e., the directly measured Plved[tm]) should predict Pped. Accordingly, a paired t-test was used to compare these differences (at the maximal diameter achieved in the fetus) in the State 1 data and the State 2 data to determine if there was any difference in the constraint applied to the LV by the thoracic tissues in those 2 conditions. A probability (p) of less than or equal to 0.01 was assumed to be statistically significant. All data are represented as mean ± SD.

Results

The blood gas data are presented in Table 3.1. Compared to previous studies of exposed, anesthetized fetal lambs the initially reduced PaO_2, elevated $PaCO_2$ and reduced pH reflect the extensive preparation required for this study. Following the initiation of ventilation, rate and volume were optimized to maintain a $PaCO_2$ between 30 and 40 mmHg.

TABLE 3.1: Blood gas data, gestational age and body weight of the six lambs utilized in the study. Blood gas data were recorded under conditions of closed chest and closed pericardium in the fetus (State 1) and the newborn (State 2) as well as under conditions of open chest and open pericardium in the newborn (State 3) (± SD)

	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	pH	Gestational age (days)	body wt. (kg)
Fetus State 1	59 ± 14	18 ± 4	7.18 ± 0.11	142 ± 1.1	4.56 ± 0.98
Newborn State 2	31 ± 5.4	96 ± 23	7.38 ± 0.08		
Newborn State 3	32 ± 6.9	162 ± 67	7.35 ± 0.09		

A complete LV end-diastolic pressure-diameter (Plved-Dlvap) relationship for 1 of the fetal animals is depicted in Figure 3.2A. In State 1 volume infusions produced substantial increases in Dlvap in most animals until a Plved of 10 to 13 mmHg was attained, beyond which smaller increases in Dlvap were observed as Plved was increased. Following 1 hour of ventilation the State 2 Plved - Dlvap relationship was shifted upward. End-diastolic diameters were larger at each Plved in State 2 than in State 1 (Figure 3.2A). As such, the State 2 heart achieved a given Dlvap at a lower Plved than did the State 1 heart.

The directly measured LV end-diastolic transmural pressure-diameter (Plved[tm]-Dlvap) relation (State 3) was shifted upward beyond that of both the fetus and neonate (Figure 3.2B). The predicted pericardial pressure observed at any Dlvap in the State 1 animal exceeded that recorded in the same animal following ventilation (Figure 3.2B, horizontal arrows, Table 3.2). The calculated Plved(tm)-Dlvap relationships in both State 1 and State 2 closely reflected the directly measured transmural pressure diameter data (State 3) (Figure 3.2C) and confirm that the pericardial balloon accurately recorded the intra-pericardial pressure in both State 1 and State 2.

Figure 3.3 represents the Plved - Dlvap relationships observed in each of the animals studied. The Plved - Dlvap relationship of the State 2 animals was shifted significantly upward ($p<0.01$) at a Plved of 12.5, 15, and 17.5 mmHg when compared to the State 1 curves (Table 3.3). In one instance the State 1 Plved - Dlvap relationship appeared as a straight line and it was not until an Plved of 10 mmHg was

attained that the neonatal relationship was positioned above that of State 1. In every instance the largest LV diameters at any given Plved were recorded in the State 3 condition. Calculated Plved(tm) - Dlvap relationships approximated the directly measured transmural pressure-diameter relationships confirming that the pericardial balloon accurately recorded Pped.

The relationship between Dlvap and pericardial pressure is depicted in Figure 3.4 for a typical animal in State 1 and State 2. At any given Dlvap the Pped recorded in State 1 was greater than that recorded in State 2. This was true in each of the animals when Plved was equal to or greater than 10 mmHg; in 3 of the 6 animals it was also true in the lower range of Plved (see Figure 3.3).

Recordings were made every 5 minutes following the initiation of ventilation (without altering Plved) to determine the time course of the changes in LV constraint. In all but 1 experiment the Plved - Dlvap relationship was shifted upward beyond the State 1 curve within 10 minutes of the initiation of ventilation. The interval required to approximate the State 2 pressure-dimension curve was, however, variable: in 2 animals the transition was completed in 5 minutes or less, 2 other animals required 10 to 20 minutes and the remaining 2 required 50 minutes.

Discussion

A series of adaptations of the cardiovascular and respiratory

Figure 3.2: Left ventricular (LV) end-diastolic pressure-anteroposterior diameter relationships in a representative lamb. Elevations of LV end-diastolic pressure (Plved) resulted in increases in LV anteroposterior diameter (Dlvap) in State 1 (Figure 3.2A, closed squares). One hour after ventilation (State 2) the Dlvap was larger at any given Plved (Figure 3.2A closed diamonds). Retraction of the lungs and pericardium from around the neonatal heart (State 3) resulted in the largest Dlvap at any given Plved (Figure 3.2B, closed circles). Predicted pericardial pressures (Figure 3.2B, arrows) for the fetus (State 1) at any diameter were greater than that of the newborn (State 2) at the same diameter. Comparisons of the pressure diameter relationship obtained with an open chest and open pericardium and the calculated transmural pressure diameter data for both State 1 (open squares) and State 2 (open triangles) confirm that the intra-pericardial balloon accurately reflects intra-pericardial pressure (Figure 3.2C). (Vertical and horizontal bars equal ± SD)

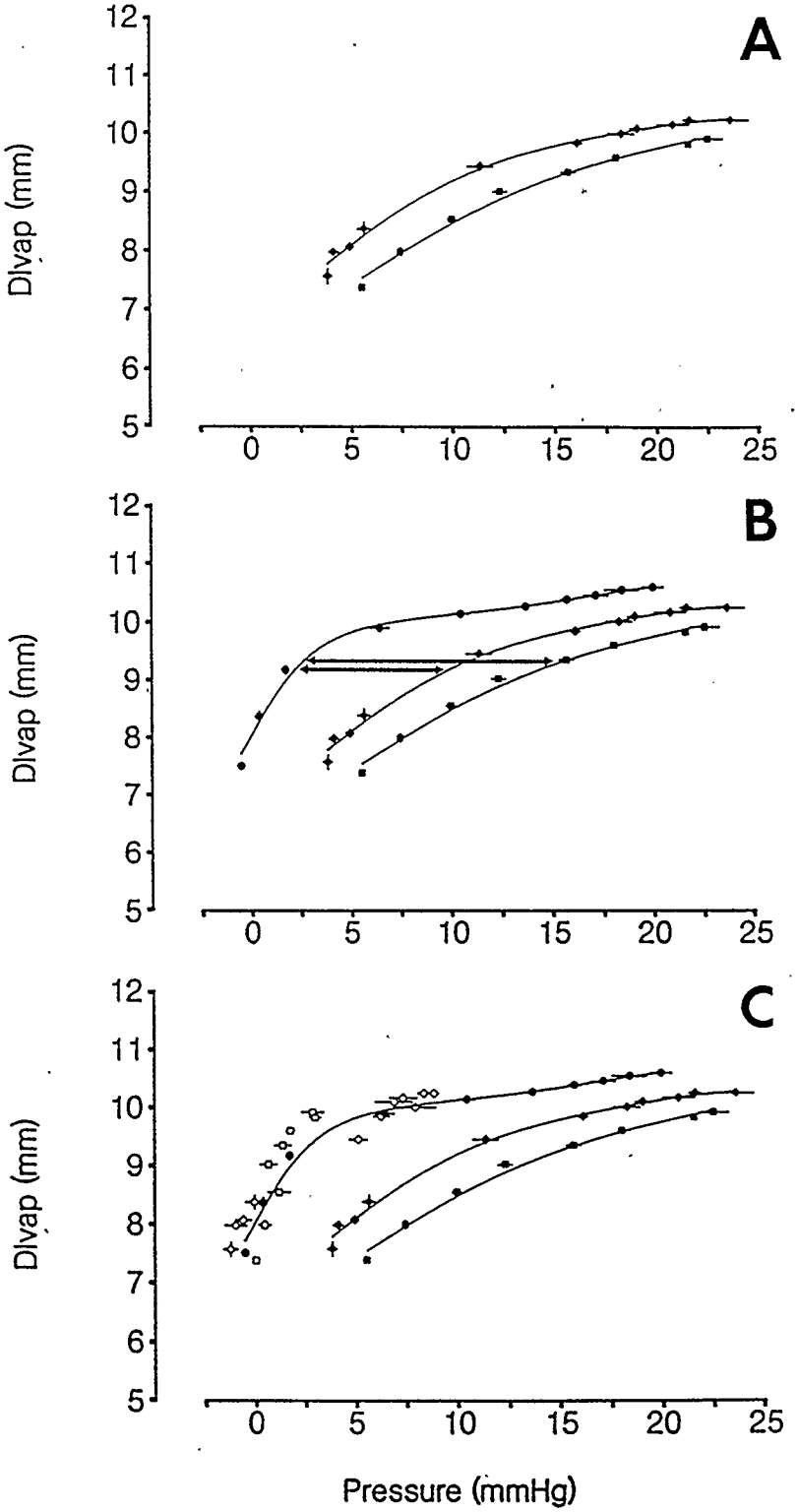
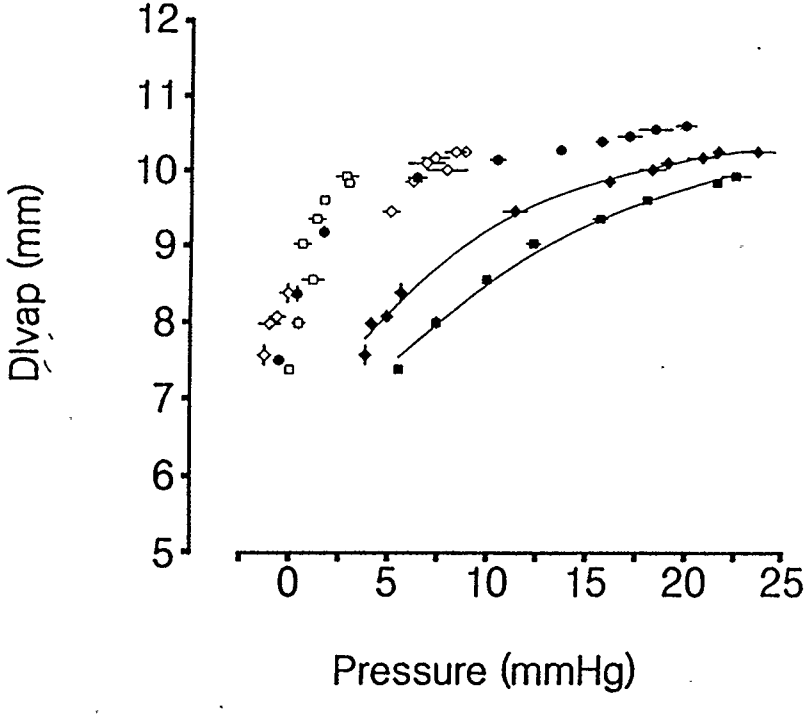

A
B
C
Dlvap (mm)
12
11
10
9
8
7
6
5
0
5
10
15
20
25
Pressure (mmHg)
Plved
Plved-Pped
State 1
State 2
State 3

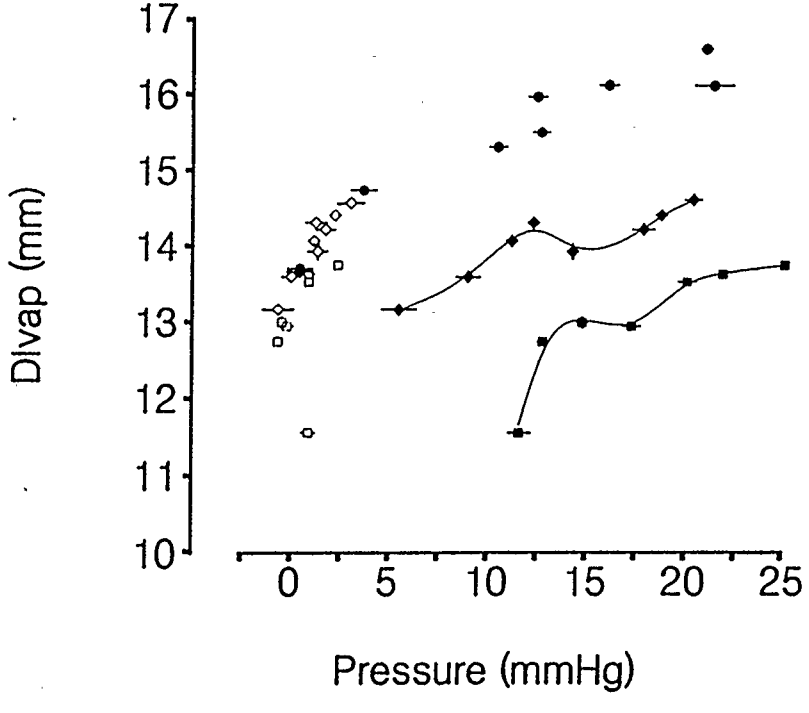
TABLE 3.2: The mean (± SD) predicted end-diastolic pericardial pressures (Pped) under conditions of a closed chest and closed pericardium in the fetus (State 1) and the neonate (State 2). Pericardial pressure for each condition was assessed at the maximal left ventricular end-diastolic diameter recorded in the State 1.

	FETUS	**NEONATE**
Predicted	18.8	10.2 *
Pped (mmHg)	± 4.0	± 1.8

(* indicates significantly different from previous value, paired t-test, $p<0.01$)

Figure 3.3: Left ventricular (LV) end-diastolic pressure-diameter relationships for the animal shown in Figure 3.2 (see Figure 3.2C) and the remaining 5 animals. Similar relationships were observed as described in Figure 3.2. The directly measured transmural pressure-diameter relationship obtained with the lungs and the pericardium retracted (closed circles) in each case revealed the largest LV anteroposterior diameters at any given LV end-diastolic pressure. The calculated transmural pressure diameter relationships closely approximated the directly measured transmural pressure diameter data (open squares = fetus [State 1], open diamonds = neonate [State 2]). (Abbreviations as in Figure 3.2)

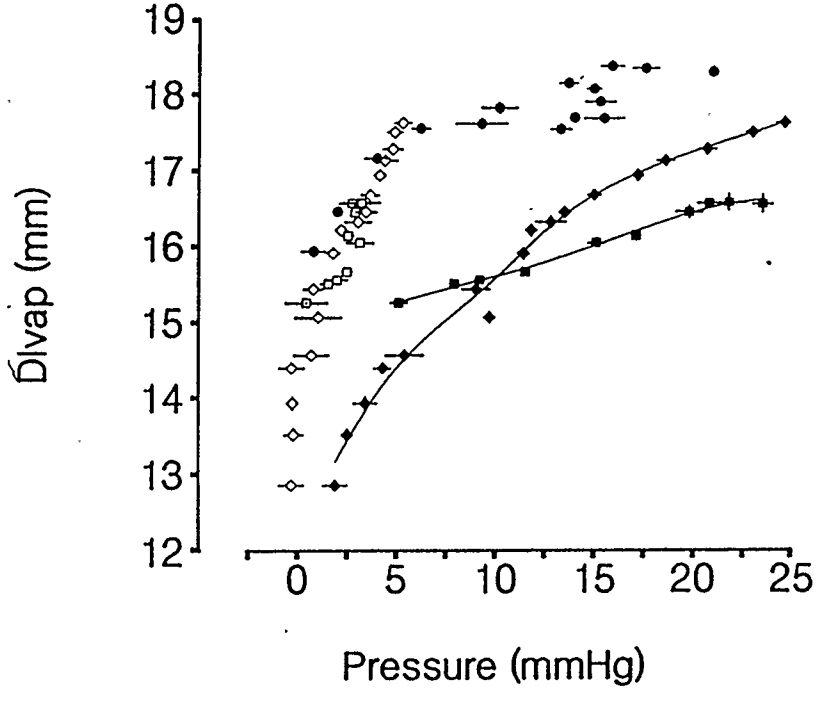


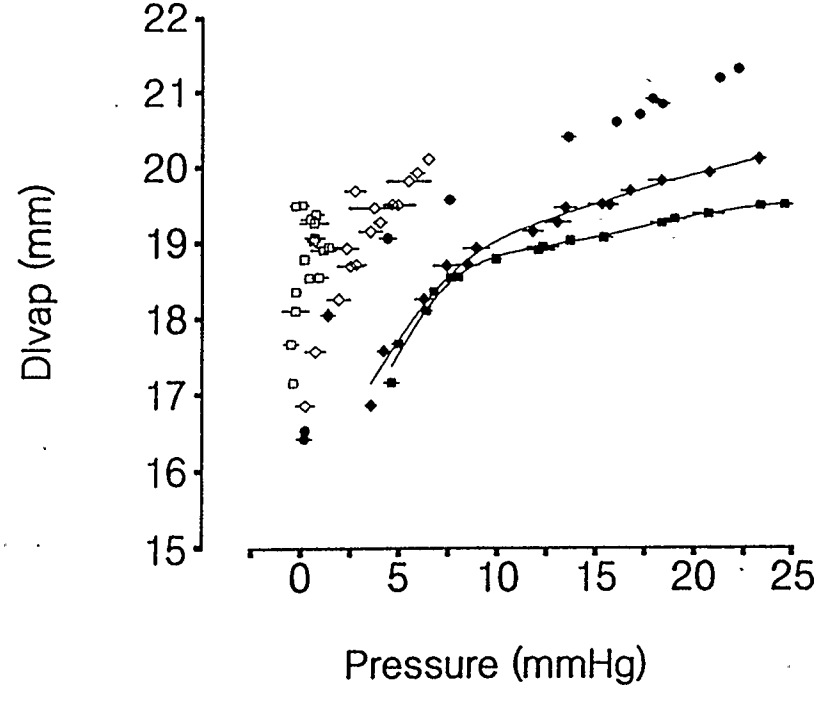


State 1 Closed chest, closed pericardium
State 2 Closed chest, closed pericardium
State 3 Open chest, open pericardium

Plved ■ ◆ ●

Plved-Pped □ ◇

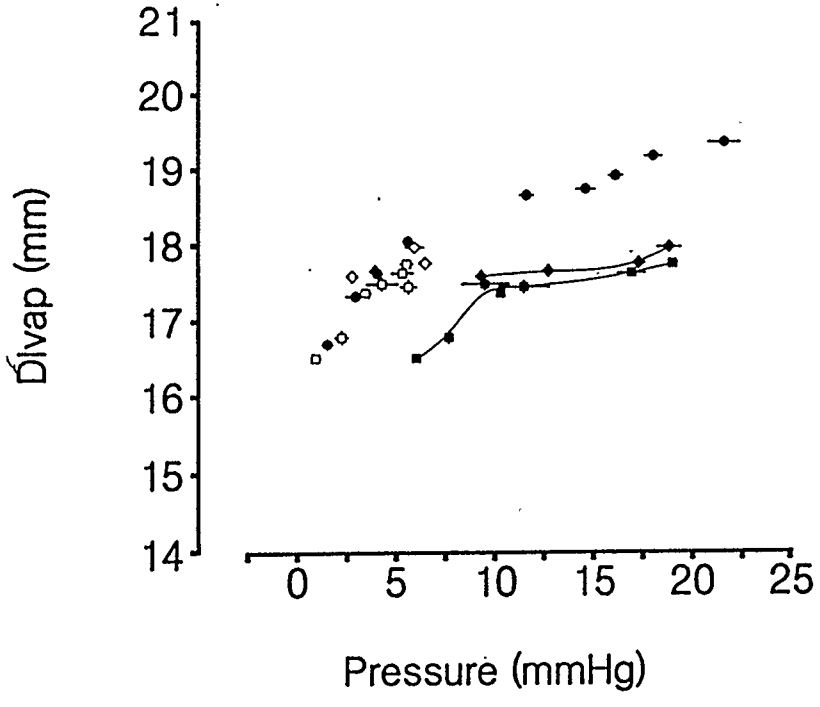


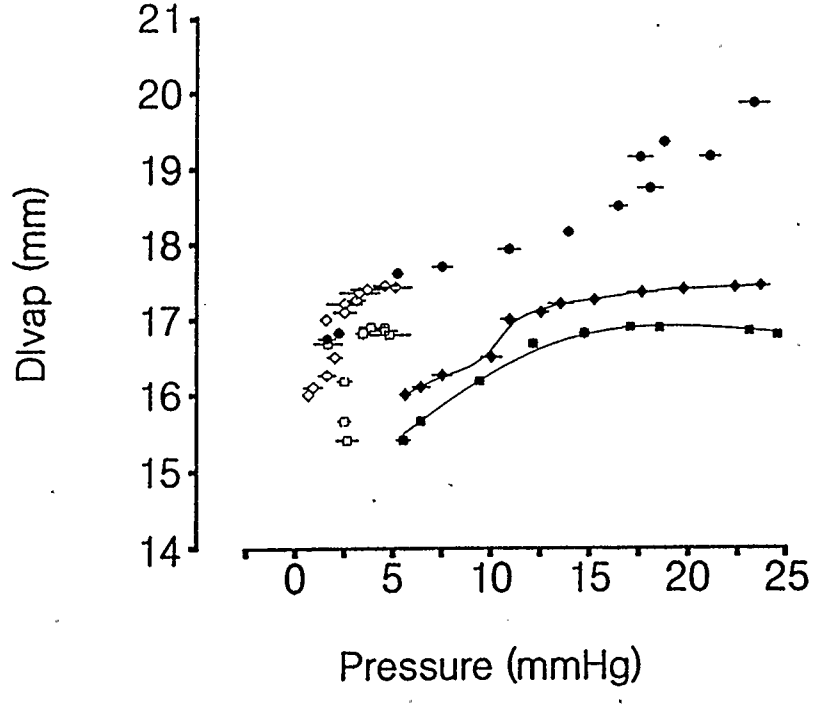


State 1 Closed chest, closed pericardium
State 2 Closed chest, closed pericardium
State 3 Open chest, open pericardium

Plved ■ ◆ ●

Plved-Pped □ ◇





State 1 Closed chest, closed pericardium
State 2 Closed chest, closed pericardium
State 3 Open chest, open pericardium

Plved Plved-Pped

TABLE 3.3: Mean (± SD) left ventricular (LV) end-diastolic anteroposterior diameters (Dlvap) (as a percent of the diameter recorded in State 1 at a Plved of 12.5 mmHg) recorded under the 3 conditions of study; Closed chest, Closed Pericardium (State 1), Closed Chest, Closed Pericardium (State 2), and Open Chest, Open Pericardium (State 3), at 3 levels of LV end-diastolic pressure (Plved).

Plved (mmHg)	State 1 fetus	State 2 newborn	State 3 newborn
12.5	100.0	105.0* ± 4.6	112.1** ± 7.6
15.0	102.0 ± 1.8	105.1* ± 4.2	113.7** ± 6.9
17.5	103.1 ± 2.4	107.4* ± 4.8	116.9** ± 9.4

(* indicates significantly different from State 1 values, $p<0.01$)

(* indicates significantly different from State 1 and State 2 values, $p<0.01$)

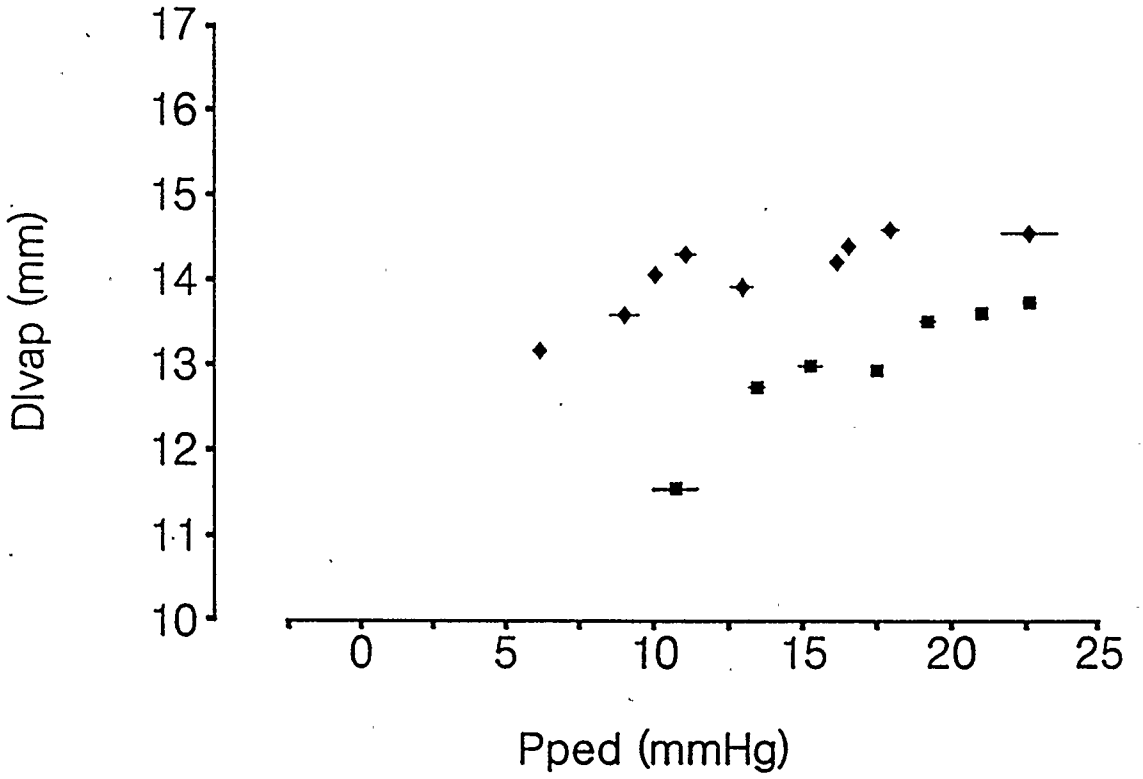


Figure 3.4: Pericardial end-diastolic pressure recorded in a representative fetus (State 1) with a closed chest and closed pericardium at any given end-diastolic diameter (Dlvap) (closed squares) was greater than that observed in the neonate (State 2) 1 hour after the initiation of ventilation (closed diamonds). (Abbreviations as in Figure 3.2).

systems must occur during the transition from the fetus to neonate. Much is known about these transitions, although several aspects have eluded explanation. It is thought that the fetal heart is functioning near its maximal level prior to birth. Nonetheless, at birth significant changes occur in the pathways of the blood through the heart, and there is an increase in cardiac output (4, 97, 98). How the fetal heart can increase its level of function to produce the increases observed at birth is not fully understood. Elevations in heart rate and contractility, reductions in pulmonary arterial pressure, and increases in ventricular diameters and volume are thought to contribute (4, 65, 97, 98). How ventricular volume increases has not been explained. The present studies have shown that the constraining influence of the thoracic tissues upon the LV is reduced at birth. Reducing Pped would allow the end-diastolic diameter of the LV to increase at any given Plved. This, in turn, would effectively increase LV preload and should lead to an increase in stroke volume through a Frank-Starling mechanism.

Previous studies have observed that the diameters of the LV are increased at birth (4, 8, 65, 122). Our studies confirm that the anteroposterior diameter of the LV does increase and confirms a previous report of 1 fetal/neonatal lamb which suggested that these changes can occur immediately after birth (65). Our study shows that, in some instances, these changes occurred very quickly after birth. Within 1 hour after the first breath the Plved - Dlvap relationship had significantly shifted. Increases in end-diastolic diameter were accomplished through a reduction in the constraining influence of the

thoracic tissues as reflected by a reduction in pericardial pressure. Because of the position of our pericardial pressure sensor, the present studies do not clearly define the source of this reduced constraint. Wladimiroff et al. (122) noted a 9% increase in LV diameters following the birth of human infants. Our results show a mean increase of 5.0% at a Plved of 12.5 mmHg. Although this change is less than that observed by Wladimiroff et al. (122) it must be remembered that a significant reduction in constraint may already have occurred as a result of delivering the fetal chest from the uterus.

Plastic deformation of the pericardium during the course of our experiment might have, in part, explained the reduced constraint; however, previous studies in this laboratory (Chapter 2) have shown that a single volume challenge to young lambs did not significantly change the pressure-diameter relationship of the LV to subsequent volume loads. Repeated volume loads to fetal lambs have not been reported to result in shifts in fetal cardiac function curves (113, 114). These studies tend to argue against a stretch in the pericardium as the underlying mechanism for the reduction in intra-pericardial pressure at birth.

We believe that the liquid-filled lungs of the fetal animal may offer more of a constraining influence on the fetal heart than the air-filled lungs of the neonate. Similarly, the positive pleural pressure (relative to atmospheric pressure) in the fetus (117) would be expected to limit LV filling. While in utero, the constraining influences on the heart would also include forces applied to the fetus by the

amniotic fluid and the maternal tissues. At birth these forces would also be reduced. This concept is supported by the past work of Kirkpatrick et al. (65) who noted cyclic changes in LV diameters related to changes in intra-uterine pressure during maternal movements. Furthermore, in one lamb in which LV anteroposterior dimensions were recorded during vaginal delivery, LV diameters increased immediately after delivery of the chest, and increased further with the onset of ventilation. Thus, it is likely that our results underestimate the true reduction in Pped which occurs during a normal delivery and subsequently on initiation of respiration because, in our study, the initial observation was made only after the maternal constraining influences were removed.

Other authors have attempted to measure the constraining influence of the pericardium (113, 114, 82, 88) in chronically instrumented fetal lambs. In these experiments the authors failed to note any major contribution of the pericardium to the fetal cardiac function. They reported, however, that the pericardium significantly influences the pressure-volume relationship of the left and right ventricles in the isolated fetal lamb heart (88) and limits the transmural filling pressure of the right atrium (82). The failure to record any effect of pericardial pressure upon fetal ventricular function arises from methodological problems. They have relied upon fluid-filled catheters to record pericardial pressure in their in vivo studies. Underestimation of pericardial pressure by fluid-filled catheters has previously been reported when the pericardium was not sealed and did not contain significant amounts of fluid in it (106). More recently,

we (46, 47) have shown that the fetal thoracic tissues significantly limit the development of LV transmural pressure and, as such, limit LV stroke volume. We believe that the differences between these studies is simply reflected in the methods of instrumentation. As was clearly shown by Smiseth's "fish net" experiments, a fluid-filled catheter underestimates the effective pericardial pressure whenever the volume of fluid is small within the pericardial space (as must be the case when the pericardium has not been sealed). We have made no attempt to seal the pericardium in our studies. Furthermore, we have confirmed our measurements using the same rational described in the original work of Smiseth et al. (106). By comparing the transmural pressure-diameter relationship calculated according to equation 1 to the directly measured transmural pressure-diameter relationship, we were able to see how accurately the balloon transducer measured Pped. In each case our measured pericardial pressure closely reflected the predicted pericardial pressure.

To avoid exaggerating the normal pericardial constraint no attempt was made to seal the pericardium and the amount of instrumentation was minimized. Pericardial incisions were as small as possible and the incisions were made transversely to avoid altering the characteristics of the pericardial sac overlying the ventricle. However, even the possibility of some unrecognized pericardial alteration does not in any way alter our most important finding -- the constraint applied to the fetal LV was reduced after 1 hour of ventilation as reflected by both the predicted and the recorded Pped.

The reduction in constraint may, in part, be due to the fact that intrathoracic pressure is more negative in the neonate than the fetus. The rapid time course involved for changes in ventricular diameters observed in several of our experiments and in those of past reports (65) suggest that a rapid mechanical change may be involved. Expansion of the rib cage with the initiation of ventilation could increase intrathoracic volume and allow ventricular filling to increase. Recently Maloney et al. (74) have reported results from x-ray transmission studies of lung fluid clearance at birth which suggest that significant increases in thoracic volume may occur in the early neonatal period .

The fluid-filled lungs of the fetus may provide a hydrostatic pressure gradient to the heart. A further reduction in ventricular constraint may occur during the transition from liquid-filled lungs to air-filled lungs following birth. The time required to fully clear fluid from the lungs remains uncertain, beginning in some studies within 30 min of birth (48) and as late as 1 to 3 hours after birth in others (15, 25) and continuing for up to 24 hours. Therefore, it is possible that further decreases in pericardial pressure and increases in LV diameter may occur beyond the first hour after birth.

The present results support the previous contention that elevations of Plved do not accurately reflect elevations in LV volume if Pped increases concomitantly (60). Thus intracavitary pressures do not reflect elevations of fetal LV preload and should not be used to assess cardiac function. An inherently lower myocardial compliance (94) is

not, in itself, responsible for the limitations in fetal cardiac performance since the development of transmural pressure is limited in the perinatal LV by the surrounding tissues. In essence reduced ventricular compliance is the mechanism for the limitation in Dlvap that was observed in State 1, but it is the constraining influences of the surrounding tissues which determines the effective compliance of the LV. Larger LV diameters in the immediate neonatal period (State 2) suggest that the maximal dimensions of the ventricle, as determined by myocardial compliance alone, had not been attained in State 1 and had been, in fact, limited by the surrounding tissues.

It is unlikely that the compliance of the ventricle was increased in the neonatal period because of an improvement of blood gas status. The time course for the changes in several of the fetuses studied was shorter than that required for blood gas improvement. In the adult heart, hypoxia has been shown to reduce LV compliance while global ischemia and its associated acidosis has been shown to increase LV distensibility (28, 29). The reduced pH initially observed in State 1 would be expected to mask any changes in compliance induced by the slightly reduced PaO_2. Furthermore, the upward shift in the Plved - Dlvap relationship following one hour of ventilation can be accounted for by magnitude of the reductions in Pped. Analysis of the calculated Plved(tm) - Dlvap relationships also suggest that myocardial compliance had not significantly changed between State 1 and State 3. Had ventricular wall compliance been substantially reduced in State 1 the calculated Plved(tm) - Dlvap relationship would have been shifted to the right of the directly measured Plved(tm) - Dlvap curve.

We have assessed only the anteroposterior LV diameter in this study. It is known that significant changes occur in ventricular diameters at birth as represented by an increase in LV diameters in the transverse plane and a decrease in right ventricular septal to free wall diameter (122). Work by Wladimiroff et al. (122) and Rein et al. (89) show that the fetal and neonatal LV maintains a round shape in the transverse plane during diastole and that LV septal to free wall diameters are similar to anteroposterior diameters. It is only during systole that the septal configuration is deformed, showing flattening during the first 4 to 5 days after birth (88). If LV minor axis dimensions in the transverse plane are equal at end-diastole, a crude estimate of the LV volume can be obtained using the equation for the volume of one half of a sphere. The increases in Dlvap attained at a Plved of 15 mmHg in State 2 could reflect a 12% ($\pm$ 7.9% SD) increase in LV diastolic volume when compared to State 1 data at the same Plved.

Recently attention has been payed to the role that changes in afterload may play in the developmental changes in cardiac function (40, 113, 114, 90, 50). At birth there is a substantial reduction in pulmonary vascular resistance. The LV, on the other hand, is exposed to an increase in afterload as the low-resistance, parallel placental circulation is lost (4, 123). As such, a reduction in LV afterload is not the mechanism responsible for the increase in LV stroke volume observed at birth. To increase LV output in the face of increasing afterload requires adaptations which include the increase in ventricular volume and the associated shift in position on the Frank-Starling curve. This shift in the ventricular diastolic pressure-

diameter relationship appears to arise from substantial reductions in pericardial pressure.

In summary, the present studies have revealed a new mechanism to explain the increase in cardiac output at birth. Birth is accompanied by a reduction in ventricular constraint and, thereby, an increase in transmural end-diastolic pressure and an increase in left ventricular minor axis diameter within 1 hour of the initiation of ventilation. The reduction of the constraint applied to the heart by the surrounding tissues (i.e., maternal tissues, amniotic pressure, and fetal thoracic tissues) and the resulting increase in left ventricular end-diastolic volume can, through a Frank-Starling mechanism, account, at least in part, for the rise left ventricular output which occurs at birth.

CHAPTER 4: Effects of External Constraint on the Fetal Left Ventricular Function Curve

Abstract

Fetal left ventricular (LV) function was studied in six halothane-anesthetized fetal lambs (142-144 days gestation) to determine the influence that the pericardium, lungs and rib cage have upon the shape and magnitude of the fetal LV function curve. The pericardium was incised transversely along the atrio-ventricular sulcus and an electromagnetic flow probe was positioned on the ascending aorta to measure LV stroke volume. Intrapericardial end diastolic pressure (Pped) was measured with a liquid-containing balloon positioned over the LV. LV pressure was measured with a transducer-tipped catheter. The pericardium was approximated to the original volume and the chest closed. LV function curves were generated by changing fetal blood volume. When stroke volume was plotted as a function of LV end-diastolic pressure (Plved), an LV function curve was generated which displayed an ascending limb at lower pressures followed by a plateau in which smaller increases in stroke volume were generated regardless of the end-diastolic pressure developed. When LV end-diastolic transmural pressure (Plved minus Pped) was plotted against stroke volume, the plateau was absent. LV function curves generated while the rib cage, lungs and pericardium were widely retracted revealed much larger stroke volumes at any given Plved ($p<0.01$). These data show that constraint of the heart by the thoracic tissues significantly influences the shape and magnitude of the fetal LV function curve. The plateau of this

curve arises because elevations in Plved are counteracted by increases in Pped; thus, transmural pressure does not increase in proportion to the increase in Plved. The fetal myocardium has an intrinsic ability to increase its performance as Plved is elevated but is limited in the intact animal by the surrounding tissues.

Introduction

Cardiac function curves, relating a measurement of systolic performance on the ordinate and an index of ventricular preload on the abscissa, have shown that the fetus does not increase its cardiac output with increases in atrial pressure beyond control levels (39, 40, 113, 114). Nevertheless, the fetal heart has a functioning Frank-Starling mechanism (67) and it responds to reductions in blood volume by reducing its output (39, 40, 113, 114). Thus, the fetal heart functions at or near the beginning of the plateau of its ventricular function curve and has been described as lacking reserve (98). As yet, the factors which determine the inflection point and plateau phase of the fetal cardiac function curve have not been established. It is possible that the immature structure of the fetal myocyte limits contractile function or that the relatively less compliant fetal heart does not respond with sarcomere lengthening when distending pressure is elevated (67, 68, 94, 97). Since the thoracic tissues (rib cage, lungs, and pericardium) limit left ventricular (LV) end-diastolic diameters in the newborn as they do in adult animals (106, 45) it is conceivable that the shape of the fetal LV function curve may be substantially affected by these structures. By limiting the diameter of

the LV, and thus ventricular volume, the thoracic tissues may limit cardiac output by controlling the position that the heart maintains on the Frank-Starling curve. Elevations in end-diastolic pressure do not effect elevations in ventricular preload (i.e., LV end diastolic transmural pressure) if the increase in end-diastolic pressure is accompanied by an equal increase in pericardial pressure, reflecting an increase in constraint placed upon the heart by the surrounding tissues. It has been suggested that the pericardium is not important in determining the inflection point of the cardiac function curve of the fetal lamb (82, 113, 114). However, these investigators measured Pped using a fluid-filled catheter, which may seriously underestimate pericardial pressure (106). By determining intra-pericardial pressure utilizing a flat liquid-containing balloon (106) we have constructed LV function curves with LV end-diastolic transmural pressure as the index of preload. These data reveal that external ventricular constraint, arising from the tissues which surround the heart, accounts for the plateau of the fetal LV function curve and limits fetal LV stroke volume.

Methods

Seven pregnant ewes (Marino-Border Leicester cross, 140-144 days gestation) were anesthetized (sodium thiopental 1 mg/kg) and ventilated (oxygen and 1.0%-2.0% halothane) in a supine position. A mid-line laparotomy was performed and the head of the fetus was delivered from the uterus into a saline-filled bag to prevent the initiation of air breathing prior to the trachea being ligated to maintain lung liquid

volume. The anterior body of the fetus was delivered and placed in a supine position upon the ewe's abdomen so as not to compromise the umbilical circulation.

The sternum was split and the ribs retracted. At the level of the atrioventricular sulcus a transverse incision was made in the pericardium from the middle of the right atrium across to the left atrium. A 1 cm incision, perpendicular to the transverse incision, was made along the pulmonary artery to allow the cable of an electromagnetic flow probe to leave the pericardium. The ascending aorta was dissected free of the pulmonary trunk and a flow probe (Micron Instruments Inc. Los Angeles) was positioned on the aorta to measure LV stroke volume (less the coronary flow). Flow probes were calibrated in vitro using the methods of Gordon et al. (44) after comparisons of in vitro and in vivo calibrations revealed only a 2% difference, well within the error of the instrument (4%). The output of the flow meter (Micron RC1000) was connected to a Hewlett-Packard (HP) medium gain amplifier (HP 8802A, Hewlett-Packard, Palo Alto, CA) to measure ascending aortic flow and to an integrator (HP 8815A) to measure LV stroke volume.

Intrapericardial pressure (Pp) (compressive contact stress) was measured using a small (2 cm x 2cm internal dimensions), liquid-containing, silastic rubber balloon (106) connected to an pressure transducer (HP model 1280) and carrier amplifier (HP 8805B). The pericardial balloon was filled with gas-free liquid to prevent bubble formation and calibrated (77) prior to being secured within the intrapericardial space over the LV free wall.

LV pressure was measured using a transducer-tipped catheter with a central lumen (SPC-460, Millar Instruments, Huston, TX) connected to a carrier amplifier (HP 8805B). The catheter was positioned in the LV through the LV free wall. LV pressures were set equal to the fluid pressure measured via the catheter's central lumen. In turn, this pressure was referenced to the middle of the LV. The pericardium was approximated to its original volume and closed with interrupted sutures. No attempt was made to seal the pericardium since the pressure of the fluid within the pericardial cavity is unrelated to pericardial constraint (except in the presence of effusion or tamponade [106]). The chest was closed and air was removed from the thorax by suction. Instrumentation was completed by implanting ECG electrodes and by catheterization of the left carotid artery for arterial blood sampling and the jugular vein for fluid infusion.

Each fetus was allowed to recover for 15 to 30 minutes after instrumentation. Body temperature (esophageal) was maintained throughout the experiment using a heating lamp. Propranolol (1.0 mg/kg) and atropine (0.2 mg/kg) were administered to block autonomic compensations during generation of the LV function curves (113). Supplementary doses of atropine were given at 15 minute intervals.

Measurements of left ventricular pressure (Plv), high-gain Plv (for measurement of left ventricular end-diastolic pressure [Plved]), intra-pericardial end-diastolic pressure (Pped), aortic flow, LV stroke volume, and ECG were made while Plved was varied by rapidly withdrawing and subsequently reinfusing fetal blood. Maternal blood was used as

necessary to elevate Plved to 25 mmHg. Alterations in Plved were made under two conditions: a) with a closed chest and closed pericardium (CCCP), a condition where the pericardium, lungs and the rib cage could influence the heart; and b) with an open chest and open pericardium (OCOP) when the rib cage and lungs were widely retracted from around the heart and the pericardium was widely incised.

Measurements were recorded on 8 channel magnetic tape and thermal chart recorders. LV function curves were generated from up to 300 beats collected over the range of Plved attained with hemorrhage and volume infusions. Both Plved and left ventricular end-diastolic transmural pressure (Plved[tm]) were used as indices of preload when constructing LV function curves.

Plved(tm) was calculated by subtracting Pped from Plved. As discussed by Smiseth et al. (106), assuming that a static equilibrium exists in the free wall of the LV at end-diastole:

$$Plved = Plved(tm) + Pped \quad \text{(Equation 1)}.$$

Where Plved equals left ventricular end-diastolic pressure, Plved(tm) equals left ventricular end-diastolic transmural pressure and Pped equals intra-pericardial end-diastolic pressure. When the thoracic tissues are held away from the heart (OCOP condition) Plved(tm) equals Plved since the surrounding pressure is atmospheric.

Curves were fitted to the data points using a spline fit (SPSS Graphics Smooth Fit, SPSS Inc. Chicago, IL) and an analysis of variance (BMDP2V Repeated Measures ANOVA, BMDP Statistical Software, University of California, Los Angeles, CA) was used to compare LV stroke volumes interpolated from the curves at a Plved of 10, 15, and 20 mmHg as well as at the visually determined breakpoint. LV stroke volumes were also compared between the CCCP and OCOP conditions at equal peak ventricular systolic pressures (using peak LV systolic pressure as an index of afterload) over the range of pressures common to all animals (55 - 60 mmHg) using the analysis of variance. A Students-Newman-Keuls test was used to isolate differences detected by the analysis of variance. A probability (p) of less than or equal to 0.05 was assumed to be statistically significant.

Results

Table 4.1 lists the mean control data for the fetal animals studied. Mean values for P_aCO_2, pH and P_aO_2 recorded with a CCCP reflected near normal values. A slight deterioration in blood gases (but not pH) data was evident following the initial phase of the study.

Figure 4.1 illustrates measurements in a 142 day fetus. Under CCCP conditions, volume infusions increased LV stroke volume from 0.34 ml/kg to 0.48 ml/kg. In the same animal shortly after the rib cage and lungs were retracted and the pericardium widely incised (OCOP), volume infusions increased LV stroke volume from 0.33 ml/kg to 0.87 ml/kg at comparable values of Plved. Thus the maximal LV stroke volume was 70%

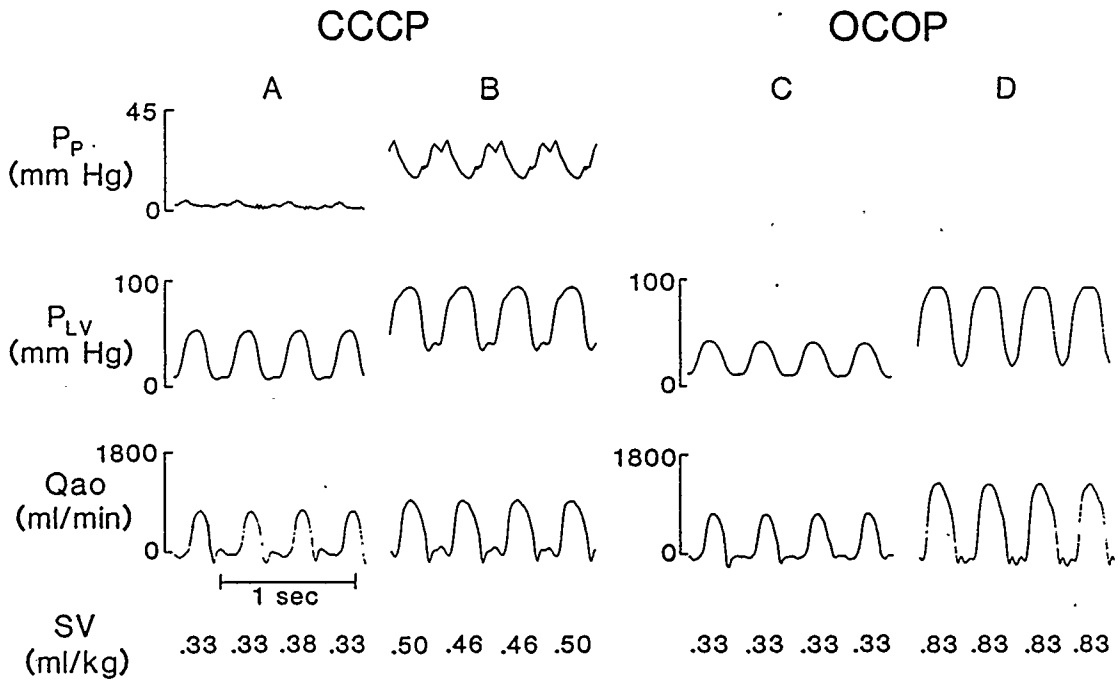


Figure 4.1: Representative measurements obtained from a fetal lamb (142 days). From top to bottom the traces represent intrapericardial pressure (Pp), left ventricular pressure (Plv), and aortic flow (Qao). Panels A and B were recorded before and after the blood infusion when the chest and pericardium were closed (CCCP). Panels C and D were recorded at similar left ventricular end-diastolic pressures (Plved) (as in panels A and B) after the chest and pericardium were widely retracted (OCOP). In the CCCP state elevating Plved from 8mmHg to 34mmHg increased LV stroke volume from 0.34 ml/kg to 0.52 ml/kg and this was accompanied by an increase in intrapericardial end diastolic pressure (Pped) from 2 mmHg to 24 mmHg. In the OCOP state an increase in Plved from 7 mmHg to 28 mmHg and increased LV stroke volume from 0.33 ml/kg to 0.87 ml/kg. Elevations in Plved during CCCP conditions resulted in a dramatic increase in Pped (panel B) which limited the increase in left ventricular wall transmural pressure. This was responsible for a smaller increase in LV stroke volume with CCCP than with OCOP, for a similar increase in Plved.

TABLE 4.1: Mean control data for the fetal lambs used in this study (mean ± SD of the mean) under conditions of Closed Chest Closed Pericardium (CCCP) and Open Chest Open Pericardium (OCOP).

	CCCP	OCOP
P_aCO_2 (mmHg)	45 ±11	54 ±15
P_aO_2 (mmHg)	20 ±4	17 ±4
pH	7.24 ±0.05	7.22 ±0.09

BODY WEIGHT (kg) = 4.5 ± 0.4

GESTATIONAL AGE (days) = 142 ± 1.3

greater after the thoracic tissue constraint was eliminated.

Figure 4.2 shows the LV function curves generated in one of the 7 fetal lambs studied. The LV function curve generated in the CCCP state with Plved as the index of preload (Figure 4.2A) resembled those of previous studies (39, 40, 113, 114). After an initial withdrawal of fetal blood, elevation in Plved produced by transfusion increased LV stroke volume. Subsequently a plateau developed in which successive increases in Plved were accompanied by smaller or no further increases in stroke volume. An LV function curve was also generated in the same animal after the surrounding tissues were retracted from the heart (Figure 4.2A), again utilizing Plved as the index of preload (in this condition, Plved is equivalent to Plved(tm) because surrounding pressure is atmospheric). In this instance much larger stroke volumes were generated at any Plved. The stroke volumes generated in the OCOP state were found to be significantly greater than those at similar Plved in the CCCP state ($p<0.01$)

LV function curves in the CCCP state were also generated utilizing the calculated Plved(tm) as the index of preload (Figure 4.2B). In this case a plateau in the LV function curve was not observed and the generated curve fell closely upon the directly measured transmural pressure - stroke volume curve (Figure 4.2C).

Figure 4.3 presents the LV function curves generated for the remaining 6 animals studied (a directly measured Plved(tm) - stroke volume curve was not obtained in one fetus which deteriorated rapidly due to maternal hypotension). In each case a plateau of the LV

Figure 4.2: Left ventricular function curves for a 142 day fetal lamb utilizing Plved as the index of preload (panel A) when both the chest and the pericardium were closed (CCCP) (closed circles). Retraction of the thoracic tissues from around the heart allowed for larger stroke volumes at any given Plved (OCOP, Panel A) (open circles). When Plved(tm) (Plved minus Pped) (+) was used to construct the cardiac function curve from the data in Panel A, the plateau phase was absent in the resulting curve (Panel B) and closely approximated the cardiac function curve derived when the lungs and the pericardium were retracted from the heart (open circles) (Panel C). Arrow indicates the Plved utilized as the break point of the ventricular function curve.

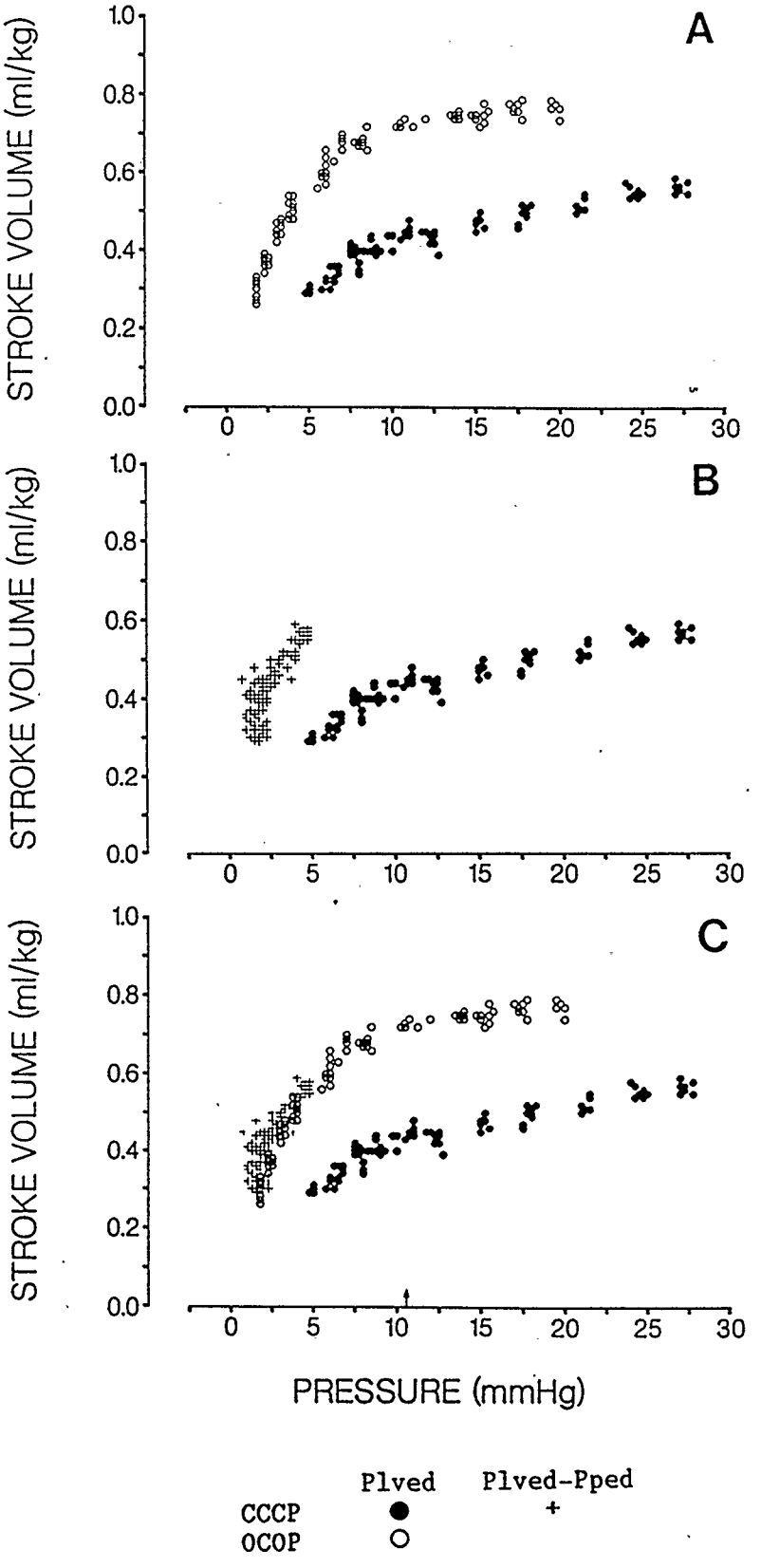

A
B
C
STROKE VOLUME (ml/kg)
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
PRESSURE (mmHg)
Plved
Plved-Pped
CCCP
OCOP

TABLE 4.2: Comparison of left ventricular stroke volume (SV) under conditions of closed chest closed pericardium (CCCP) and open chest open pericardium (OCOP). (mean values ± SD of the mean)

	CCCP		OCOP	
	Plved[¶] (mmHg)	SV[Π] (ml/kg)	Plved[¶] (mmHg)	SV[Π] (ml/kg)
Breakpoint of LV function curve.	9.7 ± 1.58	0.15 ± 0.06	9.7 ± 1.58	0.63* ± 0.10
Plved[¶] 10 mmHg		0.38 ± 0.15		0.63* ± 0.09
Plved[¶] 15 mmHg		0.41 ± 0.06		0.69* ± 0.09
Plved[¶] 20 mmHg		0.41 ± 0.06		0.63* ± 0.10
Plvs[#] 60 mmHg		0.39 ± 0.06		0.52* ± 0.09

* = statistically greater than CCCP ($p<0.01$)

Π = Left ventricular stroke volume

¶ = Left ventricular end-diastolic pressure

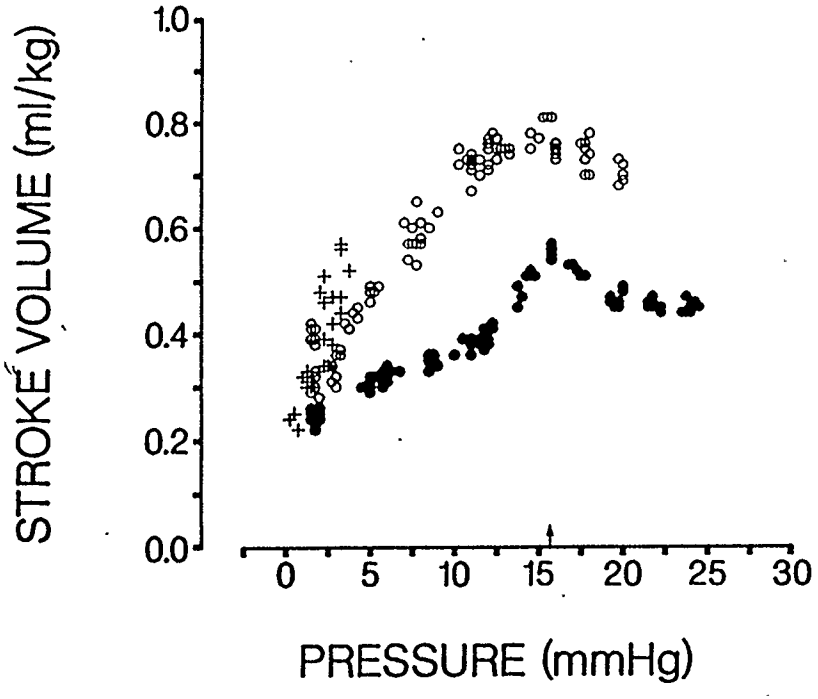
= Left ventricular peak systolic pressure

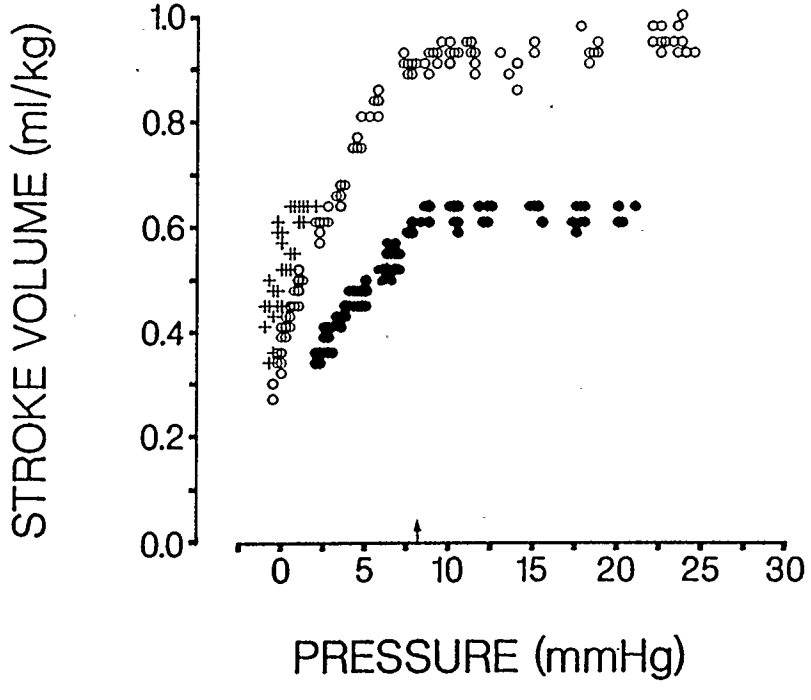
function curve was present when Plved was used as the index of preload. When the calculated Plved(tm) was used as the index of preload the plateau was absent and the LV function curves corresponded almost identically to those generated when the rib cage, lungs, and pericardium were widely retracted (OCOP).

Figure 4.4 illustrates the relationship between Pped and Plved and between the calculated Plved(tm) and Plved in one typical fetus under CCCP conditions. Elevations in Plved were accompanied by almost one-to-one increases in Pped while calculated Plved(tm) increased only slightly over the range of Plved. The average slope of the calculated Plved(tm) vs. Plved relationship (0.13 $\pm$ 0.036 SD) was substantially less than that observed in the Pped vs. Plved relationship (0.87 $\pm$ 0.036 SD).

To assess the possibility that a reduction in LV afterload may have accounted for the increase in LV stroke volumes observed when the thoracic tissues were retracted, LV stroke volume was plotted against peak LV systolic pressure (Plvs) in both states (Figure 4.5). It is obvious that, at any value of Plvs, the OCOP stroke volume points lay above those recorded with CCCP. This difference widened as Plvs increased. LV stroke volumes were found to be significantly greater ($p<0.01$) in the OCOP state than in the CCCP state (Table 4.2) at equivalent LV afterloads over the range of Plvs common to all animals.

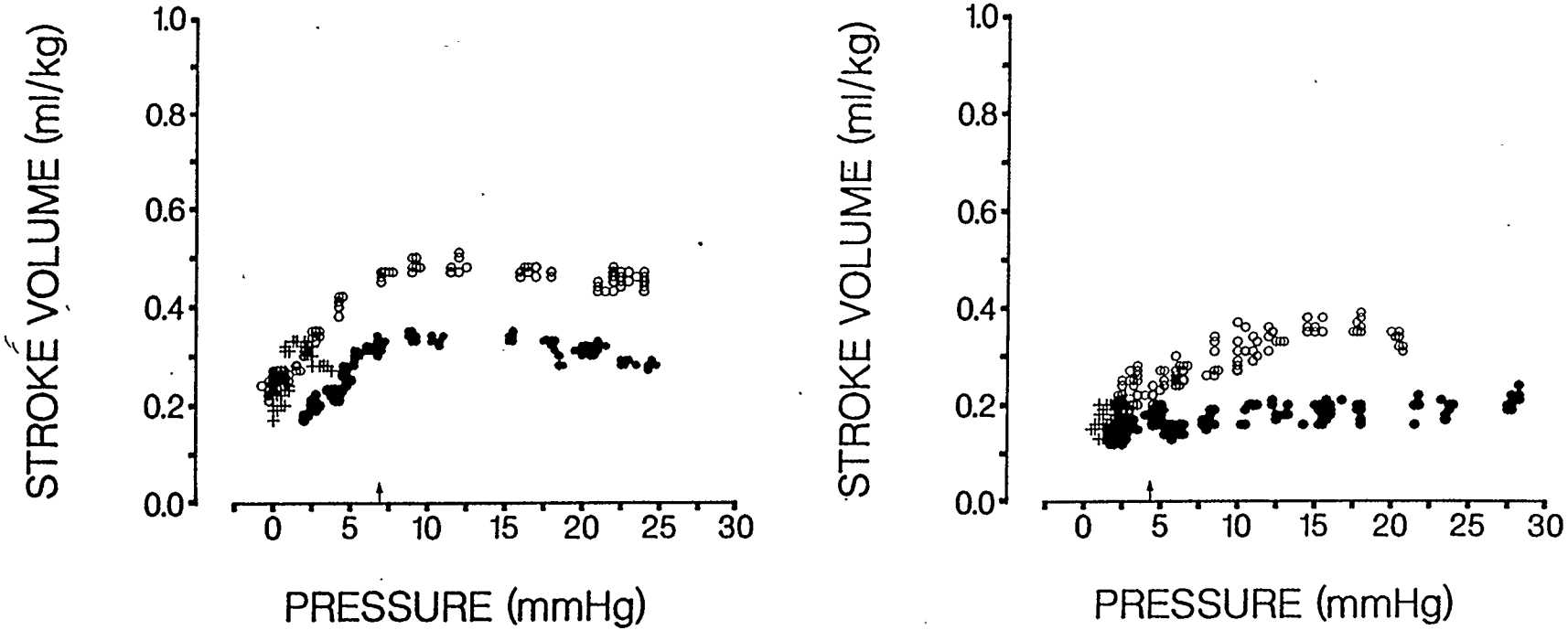
Figure 4.3: Left ventricular function curves for the other 6 fetuses studied using Plved as the index of preload when the chest and pericardium were closed (Plved, CCCP) (closed circles) and when the chest and pericardium were open (Plved, OCOP) (open circles). Retraction of the thoracic tissues during OCOP allowed the left ventricle to generate larger stroke volumes than when both the chest and the pericardium were closed at the same Plved. By subtracting Pped from Plved during conditions of CCCP, left ventricular transmural pressure (Plved[tm]) was derived which, when used as the index of preload, resulted in a LV function curve (+) which closely approximated the OCOP data. For clarity, only 20% of the Plved(tm) data points (as determined by random sampling) are depicted so as not to obscure the OCOP data. Arrows indicate the Plved utilized in each animal as the break point of the ventricular function curve.





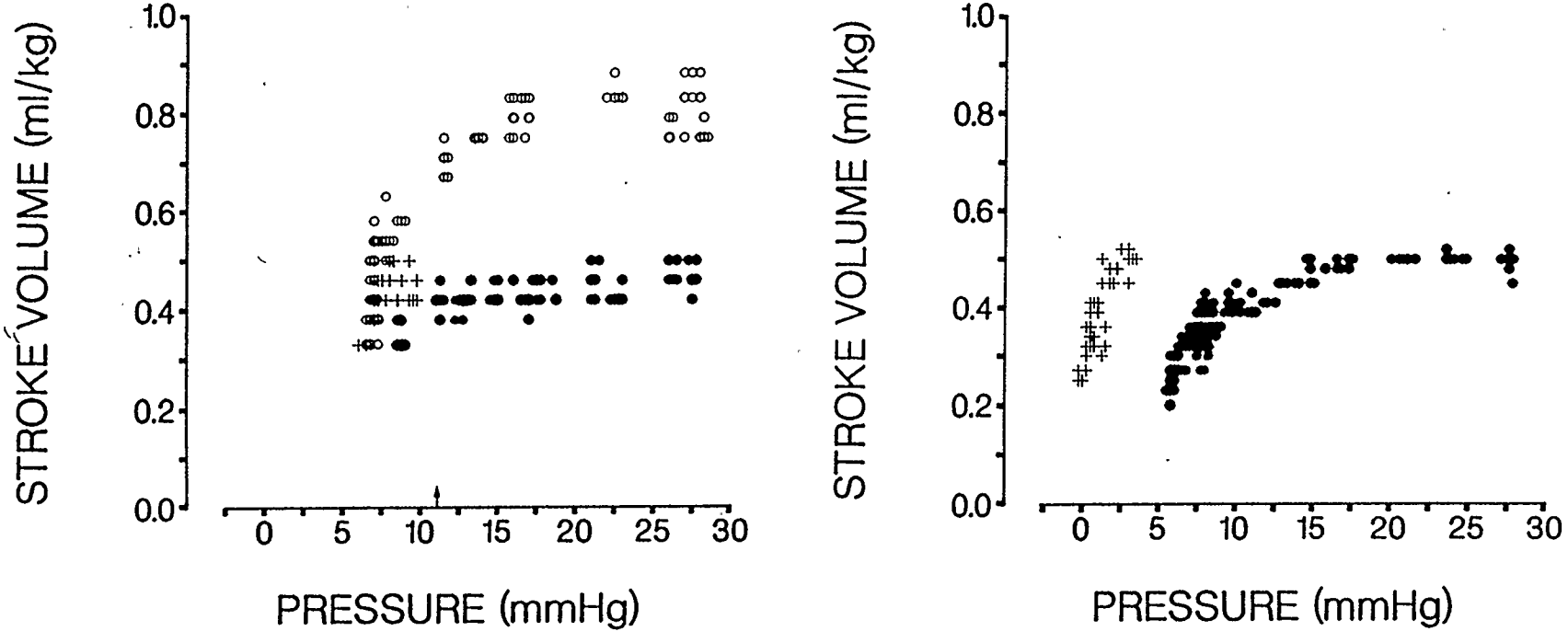
Closed chest, closed pericardium
Open chest, open pericardium

Plved ● ○
Plved-Pped +



Closed chest, closed pericardium
Open chest, open pericardium

Plved ● ○ Plved-Pped +



Closed chest, closed pericardium
Open chest, open pericardium

Plved ● ○
Plved-Pped +

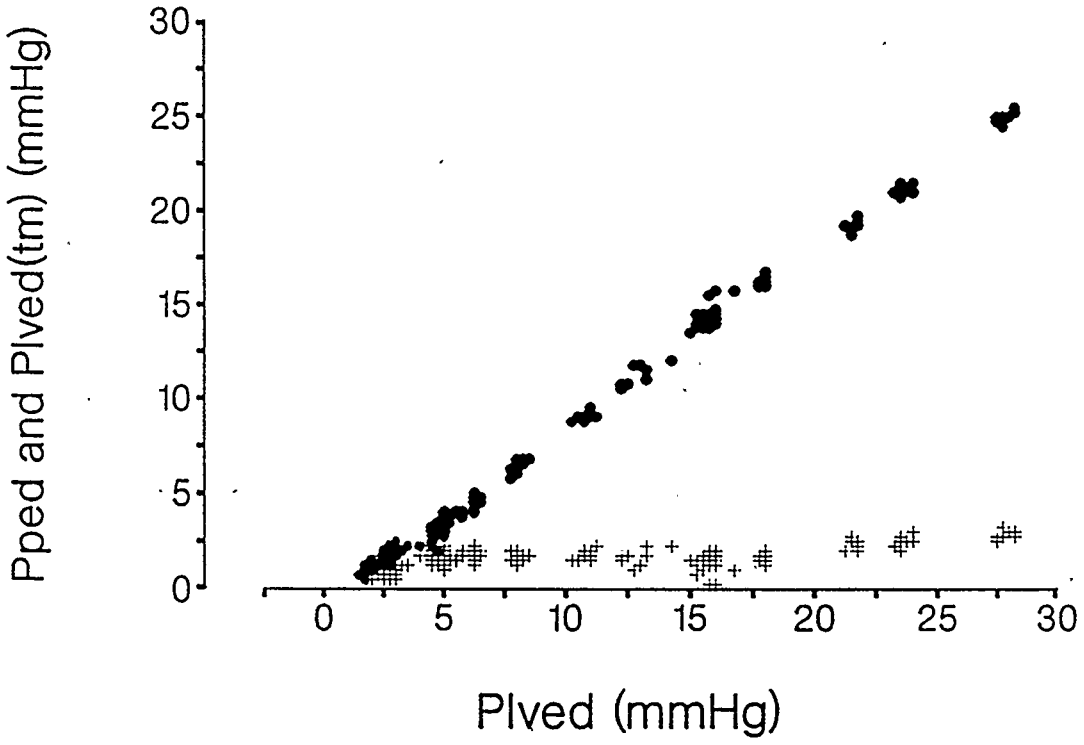


Figure 4.4: When the chest and the pericardium were closed (CCCP) elevations in left ventricular end-diastolic pressure (Plved) were accompanied by almost equal increases in intrapericardial end diastolic pressure (Pped) (closed circles). Thus, left ventricular end-diastolic transmural pressure Plved(tm) (+) increased only minimally. Data from each of the other animals revealed similar relationships.

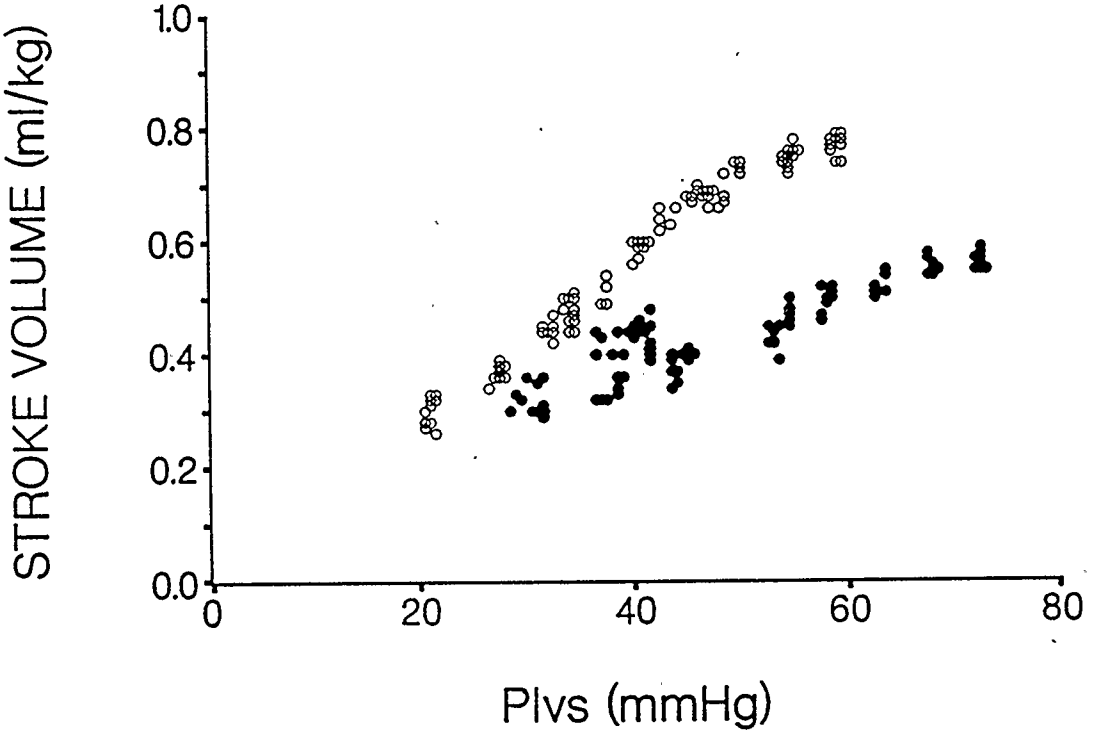


Figure 4.5: The relationship between LV stroke volume and LV systolic pressure, an index of LV afterload, is depicted for one representative fetus for both the CCCP (closed circles) state and the OCOP state (open circles). LV stroke volume in the OCOP condition was equal to or greater than that in the CCCP condition at equivalent afterloads.

Discussion

Contrary to past studies (39, 40, 82, 113, 114) we have shown that the fetal left ventricle has the inherent ability to increase systolic function beyond control levels, but is limited in doing so by external ventricular constraint. The plateau phase of the fetal left ventricular function curve arises from elevations in Pped occurring as ventricular pressures are elevated and thus reflects a limitation in ventricular preload as assessed by Plved(tm). When an appropriate index of LV preload, such as Plved(tm), is used in constructing the ventricular function curve a plateau phase is not observed and an LV function curve with a new shape is observed.

Previous analysis of the fetal cardiac function curve in terms of shape and magnitude have suffered from two major limitations. First, most of these studies have utilized atrial or ventricular intracavitary pressures as the index of preload instead of a more appropriate index of ventricular preload such as Plved(tm). Secondly, attempts to address the question of how the pericardium influences the fetal cardiac function curve (82, 113, 114) have relied on a fluid-filled catheter to record the intrapericardial pressures in fetal lambs. In a pioneering study Katz (60) predicted that if the dimensions of the heart were limited by the pericardium changes in Plved became meaningless in terms of changes in ventricular volume. By utilizing a measurement of Plved(tm) we have avoided the inherent problems of utilizing Plved as an index of preload (43) and have revealed a fetal LV function curve which is much different in shape than previously reported. The

"normal" plateau of the fetal cardiac function curve, which has been used to suggest that the fetal myocardium has only a limited ability to increase its performance when ventricular preload is raised (39), was not present when Plved(tm), a more appropriate index of ventricular preload, was utilized. Elevations in Plved do not always indicate elevations in ventricular preload, for example, when elevations in Plved are accompanied by similar elevations in Pped. In these circumstances, LV preload will not change since Plved(tm) has not changed. This clearly occurred in the present studies (Figure 4.4) since large increases in Pped occurred as Plved increased in the CCCP state. Since elevations in Plved(tm) were minimal LV preload did not change substantially.

The present studies show for the first time that the fetal myocardium does have a significant intrinsic reserve in terms of the Frank-Starling relationship beyond the breakpoint of the CCCP LV function curve. This is clearly evident when the surrounding tissues have been retracted. In these circumstances Plved is equal to Plved(tm) since the surrounding pressure is atmospheric and, as Plved is increased, LV output continues to increase beyond the previous limit imposed by the surrounding tissues. These data suggest that in the normal state stroke volume is largely limited by the tissues which surround it, rather than by any inherent limitation on myocardial function. While recognizing that the fetal cardiovascular system may have additional limitations imposed by structural immaturity, the present data emphasizes that the previously recognized limitation in systolic function is largely determined by the constraining influence of the surrounding

tissues. Moreover, the plateau of the fetal cardiac function curve is conceptually erroneous in that it is found only as a result of assuming that atrial or ventricular pressures reflect preload. When Plved(tm) is used as the preload index the plateau is absent; however, it is obviously true that fetal stroke volume is limited by an inability to develop a greater Plved(tm) with elevations in Plved as increases in Pped closely match increases in Plved (Figure 4.4).

We have utilized a smaller version of the liquid-containing pericardial balloon described by Smiseth et al. (106) to assess the influence of the pericardium and surrounding tissues upon fetal LV function. In contrast to the fluid-filled catheter (or even a micromanometer situated to measure the pressure of the liquid within the pericardial space), this device is capable of measuring the constraint imposed by the normally near empty pericardium or the unsealed pericardium. Also, the balloon transducer reliably measures the pressure of the liquid when the amount of liquid is ample ($\geq$ 30-40 ml in the adult dog). The repeated correlation between the LV function curves plotted with the calculated and directly measured Plved(tm) (Figures 4.2 and 4.3) supports the accuracy of the measurement of pericardial pressure in our present study. Our previous studies utilizing ultrasonic crystals and the balloon measurements of pericardial pressure also substantiate the accuracy of the pericardial balloon (45).

Recently, Morton and Thornburg (82) have also reported data to

show that pericardial pressure does significantly limit cardiac transmural filling pressures but state that the limitation of cardiac transmural pressure does not influence the fetal cardiac function curve. We believe that the conflict between our results and those of Thornburg and Morton (82, 113, 114) arise primarily from differences in methodology. In the experiments in which they have recorded limitations in transmural filling pressures by elevations in pericardial pressure they have not measured stroke volumes. Furthermore, we believe that the fluid-filled catheters used in their studies to record pericardial pressure significantly underestimates the true pericardial pressure. The volume of fluid needed in the fetal pericardium to allow a fluid-filled catheter to accurately record pericardial pressure is unknown although initial work of Thornburg and Morton (82) would suggest that as little as 2 ml is required in a sealed pericardium. Although it is possible that the most recent work of Thornburg may have recorded pericardial pressure accurately using a fluid-filled catheter, it is highly unlikely that the fluid-filled catheter used to record pericardial pressure in their earlier studies would have reflected true pericardial pressure since in those studies the pericardium was not sealed. It has been clearly shown in Smiseth's "fish net" experiments (see figure 6 in reference 10) that the pressure measured by a fluid-filled catheter significantly underestimates true pericardial pressure when the pericardium is not sealed. Our data suggest that the past studies, (113, 114) which have relied on fluid-filled catheters to record pericardial pressure, may well have underestimated the magnitude and, consequently, the significance of pericardial pressure. The underestimation of pericardial pressure in the early studies would

explain the observed plateau in the fetal ventricular function curves and account for the inappropriate conclusion that limitations in transmural filling pressures, arising from elevations in pericardial pressure, do not effect the fetal ventricular function curve. Our present data clearly show that elevations of Plved are accompanied by almost equal increases in Pped (Figure 4.4). Plved(tm), as determined by subtracting Pped from Plved, is limited by this increase in Pped. This being true, Plved(tm) more closely predicted the changes in LV stroke volume than did Plved. Therefore, elevations in Pped largely account for the plateau of the fetal LV function curve.

Afterload is also known to influence fetal ventricular stroke volume (40, 90, 113, 114). We verified that a reduction in ventricular afterload was not responsible for the larger LV stroke volumes attained in the OCOP state by observing the relationship between stroke volume and peak LV systolic pressure over the range of Plved attained during volume infusion. LV stroke volume recorded at equal peak LV systolic pressure, although not necessarily at a similar Plved, (LV systolic pressures between 55 and 60 mmHg) was found to be significantly greater in the OCOP state than in the CCCP state (Table 4.2 and Figure 4.5). This confirms that the increases in stroke volume observed in the OCOP state were not primarily a result of a reduction in afterload. We believe that the plateau phase of the LV function curves generated in the CCCP state using Plved as preload is not fundamentally determined by increases in afterload. This is not to say that elevations in ventricular afterload during volume infusions do not alter the ventricular function curve, but that the primary determinant of the

plateau of the fetal LV function curve are the previously unappreciated elevations in Pped in the CCCP state. In the OCOP state increases in Plved(tm) are not limited by tissue constraint and in this condition elevations in ventricular afterload may well have limited the maximal stroke volume generated.

Our studies were conducted on acutely instrumented, anesthetized, exposed fetal lambs which were nearly normal according to measurements of blood gases and acid-base status, but which did deteriorate slightly (in terms of blood gas status) before the OCOP phase of the study. Nevertheless, this apparent deterioration would be expected to impair rather than enhance cardiac function (22, 85). The influence of the anesthesia may be reflected in the smaller stroke volumes of our animals as compared to the more recent work of Reller et al. (90). However, the present data suggest that, by not closing the pericardium, previous investigators may have actually recorded greater stroke volumes than those which would normally be seen. In a preliminary observation loosely closing the pericardial incision during preparation of the fetus substantially reduced LV stroke volume (from 0.75 ml/kg to 0.33 ml/kg at a Plved of 8 mmHg). Consequently, throughout this study care was taken not compromise the pericardial volume; thus instrumentation was kept to a minimum (total balloon volume was less than 1.5 ml as measured by displacement) and the edges of the pericardium were not overlapped.

Previous studies have explained the breakpoint of the fetal cardiac function curve as a result of the reduced compliance of the fetal

heart (94, 97, 98). In a real sense, "reduced compliance" is the essence of the explanation that we propose. The confusion derives from the previous lack of appreciation for the phenomenon of pericardial constraint. It was almost universally assumed that pericardial "pressure" was negligible or perhaps negative and equal to intrathoracic pressure. Previous investigators therefore assumed that changes in left ventricular compliance were due to changes in the structural properties of the myocardium and this is what is meant by "reduced compliance". In this paper we have shown that the alterations in the ventricular function curve can be explained by a shift in the diastolic pressure-volume relationship (as demonstrated elsewhere, [45]) and that the increase in Pped in the CCCP state explains that shift.

The results of the present study suggests a mechanism for the increase in cardiac output that occurs at birth. A reduction in ventricular constraint at birth would shift the ventricular function curve upward resulting in greater stroke volumes at any given Plved. In this study the retraction of the thoracic tissues resulted in an increase in LV stroke volume (more than 65% at a Plved of 10 mmHg). Coupled with an increase in inotropic and chronotropic stimulation, this could account for a large portion of the approximately 100% increase in LV output that occurs at birth. Reduction in constraint applied by the surrounding tissues at birth may be effected by the transition from fetal fluid-filled lungs to air-filled lungs and the associated establishment of negative intrathoracic pressure in the

neonate. In addition, the constraint imposed by the amniotic fluid and that of the maternal tissues would be removed at birth.

In summary, the present studies reveal that the fetal lamb heart is significantly influenced by the tissues which surround it. The plateau of the fetal left ventricular function curve is largely a result of the limitations placed upon ventricular filling by the rib cage, lungs and pericardium. In-vivo, the maternal tissues and amniotic fluid may also impose an additional constraint to the fetal left ventricle. When an appropriate index of left ventricular preload (such as left ventricular end diastolic transmural pressure) is utilized in constructing the left ventricular function curve, a plateau is not developed and left ventricular stroke volume is limited by the inability to increase left ventricular end diastolic transmural pressure. On opening the chest and pericardium it is always possible for stroke volume to increase above that observed with a closed chest and closed pericardium; thus, it is clear that the observed plateau is not due to any inherent myocardial limitation but rather to the fact that the increase in true preload is severely limited by the pericardium and the surrounding tissues. In these circumstances elevations of intracavitary pressures do not reflect changes in ventricular preload and as such cannot be expected to increase left ventricular stroke volume.

CHAPTER 5. Conclusions

As described in the introduction, the fetal cardiovascular system must undergo significant change at birth. Included in the changes are an increase in cardiac output, increase in LV and RV stroke volume and an increase in LV systolic and diastolic dimensions. The studies presented here have provided a new explanation for at least a portion of these changes. By using a liquid-containing balloon to measure pericardial pressure it was possible to assess how thoracic tissues influence LV diastolic filling. The first of the three projects (Chapter 2) has shown that the thoracic tissues significantly limit diastolic filling. This study has also confirmed past studies which suggest that LV end-diastolic pressure does not reflect LV preload.

The results from the first set of studies suggested that the constraint applied to the LV may also influence diastolic filling, and thus systolic function, in the fetus. By recording the changes that occur in the magnitude of constraint applied to LV at birth, the second study (Chapter 3) provided a new explanation for the increase in cardiac output at birth. Reductions in the constraint applied to the LV were shown to occur with ventilation. This allowed for greater ventricular diameters at any given end-diastolic pressure. By decreasing pericardial pressure LV end-diastolic transmural pressure can increase and LV preload is increased. This should augment cardiac output through a Frank-Starling mechanism.

The last predictiion was proven true in that the fetal LV has a

substantial ability to increase its stroke volume beyond the normal maximal values if pericardial pressure is reduced. The final study looked at fetal LV function and the influence that thoracic tissue constraint may have upon both the magnitude and the shape of the ventricular function curve. As predicted, the fetal LV was shown to have a substantial reserve which was normally masked by the constraint applied to the LV. Stroke volume could increase well beyond the normal limit when the thoracic tissues were retracted and ventricular constraint reduced. This study also provided insight into the cause of the plateau of the fetal cardiac function curve. Again, elevations in end-diastolic pressures were accompanied by substantial increases in pericardial pressure, the result being that LV end-diastolic transmural pressure was limited. When transmural pressure is used as the index of preload the LV function curve has a much different shape and lacks a plateau. Thus, the plateau phase represents a limitation of transmural pressure and thus ventricular preload and not necessarily an inherent myocardial property. Other authors (88, 113, 114) have attempted to explore the role of pericardial pressure on influencing fetal cardiac function but have underestimated the magnitude of the constraint by utilizing fluid-filled catheters to record pericardial pressure.

Although some of the observations recorded in the present studies have been previously reported i.e., increases in ventricular diameters at birth (65) and limitation of transmural pressures by the pericardium (81), it has not been until now that the overall significance of thoracic tissue constraint upon perinatal LV function has been demonstrated. Furthermore, these studies have explained two important

observation in the area of fetal cardiac physiology. The shape and magnitude of the fetal cardiac function curve has been clarified by utilizing a more appropriate index of preload. These studies have also explained how ventricular diameters can increase at birth and suggest that at least a portion of the increase in cardiac function observed at birth is related to the Frank-Starling mechanism. Finally these studies have emphasized that left ventricular end-diastolic pressure is not a good index of preload, a fact well recognized in adult cardiovascular literature but often ignored in fetal physiology.

In summary, reducing the constraint applied to the left ventricle of the exposed fetal lamb has been shown to increase stroke volume at any given end-diastolic pressure. It has also been shown that the constraint applied to the left ventricle decreases with the initiation of pulmonary ventilation and that this decrease in constraint is accompanied by an increase in ventricular dimensions. Therefore, it is predicted that a portion of the increase in cardiac output at birth occurs through a change in the heart's position on its Frank-Starling curve as a direct result of the reduction in constraint and the increase in ventricular volume.

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