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IN UTERO RIGHT VENTRICULAR OUTPUT IN THE FETAL LAMB: THE EFFECT OF HEART RATE

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SUMMARY

1. The effect of heart rate on right ventricular output was examined in six lambs during a period extending from 126 to 139 days of gestation. The fetuses had been surgically instrumented at least four days previously with a main pulmonary artery flow probe, right ventricular dimension transducers and left and right atrial pacing electrodes.

2. During spontaneous variations in heart rate, rate was correlated positively with right ventricular output ($P < 0{\cdot}0001$) and end-diastolic dimension ($P < 0{\cdot}0001$) among the lambs considered as a group, but no significant effect of rate on stroke volume was found. When individual responses were examined, output increased significantly with rate in sixteen out of seventeen observations.

3. With left atrial pacing, heart rate did not affect output. With right atrial pacing, rate correlated negatively with output ($P < 0{\cdot}0001$). With pacing from either site, rate correlated negatively with end-diastolic dimension ($P < 0{\cdot}0001$) and stroke volume ($P < 0{\cdot}0001$).

4. The introduction of a longer paced interval during each pacing rate inhibited the rate-related decrease in dimension and allowed the ventricle to fill to the same end-diastolic dimension. The systole following these longer intervals had a greater stroke volume than did the preceding systoles with smaller end-diastolic dimension. The faster the preceding paced rate, the greater was the increase in stroke volume ($P < 0{\cdot}001$).

5. Right ventricular dimensions and volumes were measured *in vitro*, and the relationship was found to be linear using regression analysis.

6. This study demonstrates that experimentally induced variations in heart rate produce changes in end-diastolic volume and contractility which prominently affect right ventricular stroke volume. As a consequence, rate has, over a broad range, either no significant effect on output or a negative one. With spontaneous variations in rate, additional changes in contractility and venous return occur which affect stroke volume and end-diastolic volume and enhance right ventricular output. These relationships are similar to those in the adult heart, and demonstrate the absence of a maturational change in the effects of rate on ventricular function from the fetus to the adult.

INTRODUCTION

Heart rate is thought to modulate fetal right ventricular output (Rudolph & Heymann, 1976). In the adult, in contrast, rate does not significantly alter cardiac output (Ross, Linhart & Braunwald, 1965; Sugimoto, Sagawa & Guyton, 1966; Cowley & Guyton, 1971). Such an apparent maturational change in the control of right ventricular output could result from changes in the intrinsic characteristics of the right ventricle, or from developmental changes in other properties of the cardiovascular system, e.g. the effects of birth on the right and left ventricular contributions to cardiac output (Dawes, 1968; Rudolph & Heymann, 1970).

The increase in both fetal right and left ventricular output with an increase in heart rate (Rudolph & Heymann, 1976) suggests that a change in intrinsic myocardial properties occurs with development. That such changes are intrinsic to the myocardium is suggested by the similarity of the effect of heart rate on both ventricles despite the marked differences in the work of each ventricle. However, the basis of this suggestion is not supported by other studies which have found that fetal left ventricular output, like that of the adult, is not enhanced by rate (Kirkpatrick, Pitlick, Naliboff & Friedman, 1976; Pitlick, Kirkpatrick & Friedman, 1976; Anderson, Glick, Killam & Mainwaring, 1986).

To test whether fetal right ventricular output is dependent on rate and, if so, to determine the basis of the relationship, this study uses techniques which allow right ventricular filling and stroke volume to be monitored. A new fetal application of sonomicrometry allows the measurement of right ventricular end-diastolic dimension, while a main pulmonary artery electromagnetic flow transducer, as used by Rudolph & Heymann (1976), measures right ventricular stroke volume. The use of these techniques in combination is similar to their use in a fetal study of the relationship between rate and left ventricular output (Anderson *et al.* 1986), which found that monitoring changes in end-diastolic volume and stroke volume was very useful in understanding how rate affects fetal left ventricular output.

This study of the chronically instrumented *in utero* lamb demonstrates that the dynamic interaction of pacing interval, right ventricular filling, and contractility has a marked effect on fetal stroke volume. As a consequence of this interaction, right ventricular output is not affected by rate during left atrial pacing, and is negatively affected by rate during right atrial pacing. When rate varies spontaneously, the stimulus which alters rate is associated with additional changes in venous return, contractility, and pulmonary artery pressure which modulate stroke volume and so affect output. These findings demonstrate that fetal right ventricular output is modulated by the same mechanisms which control cardiac output in the adult heart (Ross *et al.* 1965; Sugimoto *et al.* 1966; Cowley & Guyton, 1971; MacGregor, Covell, Mahler, Dilley & Ross, 1974; Mahler, Yoran & Ross, 1974) and left ventricular output in the fetal heart (Kirkpatrick, Naliboff, Pitlick & Friedman, 1975; Kirkpatrick *et al.* 1976; Gilbert, 1980; Anderson *et al.* 1986). The presence *in utero* of mechanisms which give rise to these relationships allows their use by the neonatal heart, and demonstrates that they are not acquired with maturation from fetal to adult life.

METHODS

The preparation

Six fetal lambs from age-dated pregnancies underwent surgery *in utero* at 125±2 days of gestation (mean ± standard deviation (S.D.)). After being fasted for 24 h, the ewes were anaesthetized by a bolus of ketamine (60–100 mg). Anaesthesia was maintained by continuous infusion of ketamine (25–30 mg/min) through an external jugular vein. The abdomen was prepared and draped, a mid-line incision was made, and the uterus exposed. The fetal neck was exposed by an incision in the uterus parallel to the fetal trachea. The fetal skin was incised, an electrocardiograph lead was implanted, and a catheter (HL-54, 0·04 in i.d. or N.I.H. angiographic, no. 5) was inserted into the external jugular vein and one into the common carotid artery, as discussed in Anderson *et al.* (1986).

A second uterine incision was made over the fetal left chest and a thoracotomy performed through the fourth intercostal space. The pericardium was opened, and the main pulmonary artery was dissected free from the ascending aorta. Piezoelectric ultrasonic transducers, made in our laboratory, were implanted. A cylindrical transducer (1 × 2 mm) was inserted into the ventricular septum through a puncture wound to the right of the anterior descending coronary artery. A 2·5 mm circular transducer was inserted through a puncture wound in the right ventricular wall and pulled back against the endocardium to measure a septum to right ventricular free wall dimension. In four lambs, a 2·5 mm circular transducer was inserted through a puncture wound in the left ventricular free wall in order to measure the dimension from septum to left ventricular free wall. An electromagnetic flow transducer (10 or 12 mm i.d.; Howell Instruments) was placed around the main pulmonary artery. Bipolar pacing electrodes were sutured to the right atrial appendage or to the inner surface of the pericardium overlying the junction of the superior vena cava and the right atrium. A cuffed fluid-filled catheter (N.I.H., no. 5) was introduced into the left pulmonary artery through a puncture wound and was held in place by a mattress suture. A fluid-filled catheter (HL-54, 0·04 in i.d.) was introduced into the left atrium through an incision in the left atrial appendage and was held in place by a mattress suture, and bipolar pacing electrodes were sutured to the left atrial appendage. The thoracotomy was closed. A subcutaneous electrocardiograph lead was implanted. The fetal skin was closed, and an amniotic catheter (HL-54, 0·08 in i.d.) was sutured to the incision. The catheters and cables were brought out through the uterine and peritoneal incisions. The uterus was closed. The peritoneum was closed. The catheters and cables were passed through a subcutaneous tunnel to exit through a stab wound in the left flank of the ewe. The abdominal incision was closed. The catheters and cables were placed in a pouch sewn to the flank of the ewe. Ampicillin (1·0 g) was infused through the amniotic catheter. The anaesthesia was discontinued, and the ewe was returned to her pen, located in a room adjoining the laboratory.

Each post-operative day, the ewe was brought into the laboratory to familiarize her with the rolling cage, the surroundings, and the experimenters. Ampicillin (0·5 g) was instilled daily into the amniotic space.

Data acquisition and recording

Right ventricular output was measured with an electromagnetic blood flow-rate meter (Howell Instruments HMS-1000, ±5% accuracy and ±2% reproducibility) using a band width of 30 Hz.

The right ventricular dimension was obtained with an ultrasonic micrometer (sonomicrometer), operating at 5 MHz with a pulse repetition rate of 1 kHz. The septal transducer was used to excite the right and left ventricular endocardial transducers. The sonomicrometer had a linearity of 0·5 % or better, a minimum resolution of 0·07 mm, and an electronic drift less than 0·05 mm/h (Kirkpatrick, Covell & Friedman, 1973). The flow probe excitation and the pulse repetition rate of the ultrasonic transmitter were derived from a common crystal-controlled oscillator to preclude interference. The relationship between the right ventricular dimension and right ventricular volume was examined *in vitro* as described below.

Pressure wave-forms were obtained by standard physiological pressure transducers (Hewlett-Packard 1280C, with 8805V carrier amplifiers). An electrocardiogram was obtained using an isolated amplifier (BioCom Bioamplifier, type 2122).

Measured data were recorded on a Brush oscillographic recorder at a chart speed of 5 in/s, or in digital form on video tape using a Sony Beta-format video cassette recorder and a 10-channel analog-to-digital converter at a sampling rate of 5 kHz.

The pressure transducers were calibrated by a mercury manometer. The blood flow-rate meter and the flow transducers were calibrated prior to implantation and were found to be linear over the range of values of physiological quantities here studied. Before and after the recording of data, the internal calibration references of the flow meter and of the sonomicrometer were recorded to verify system linearity and to provide calibration factors.

Experimental protocol

The lambs were studied *in utero* as early as 4 and as late as 17 days after surgery (7·1 ± 2·4 days). The arterial blood gas evaluations on the study days demonstrated P_{O_2} of 19–25 Torr, P_{CO_2} of 37–43 Torr. and pH of 7·36–7·41. Before carrying out an experimental perturbation, we recorded during spontaneous rhythm the right ventricular stroke volume and dimensions, aortic and pulmonary artery pressures, and an electrocardiogram.

Pacing was done at rates greater than the spontaneous rate. For each lamb, all of the pacing data obtained on the same day were grouped and designated an individual pacing study, within which the right and left atrial pacing data were grouped separately. The pacing rates used were 140, 150, 160, 171, 182, 200, 222, 240, 260, 280 and 300 beats/min. The slowest rate was used in four individual pacing studies. In all instances, the lambs could be paced as slowly as 200 beats/min.

The pacing protocols were performed as described in the study of fetal left ventricular output by Anderson *et al.* (1986). The period of atrial pacing ranged from 50 s to 30 min. In some of the lambs, the fastest rates (280 beats/min or greater) could not be maintained for over 60 s before atrioventricular block, of varying degree, occurred. Only those data which were obtained when the stroke volume and end-diastolic dimension at a given paced rate were in a quasi-steady state (beat-to-beat changes in stroke volume and end-diastolic dimension no longer occurring) were considered acceptable for analysis. Control periods of spontaneous rhythm followed each pacing period, during which the lamb's haemodynamic measures were monitored.

Over the range of paced rates, the end-diastolic dimension was found to change significantly (see e.g. Figs. 3, 4 and 5). To control for this variable, and to compare the effects of rate on stroke volume of systoles with the same end-diastolic dimension, we periodically but infrequently introduced longer paced intervals in the paced rate to blunt the fall in right ventricular end-diastolic dimension with rate (see Fig. 4). The longer paced interval was usually between 360 and 440 ms, and did not vary by more than 30 ms for a given study.

In vitro analysis

To examine the diastolic relationship between right ventricular volume and dimension, *in vitro* studies of hearts which had been instrumented *in vivo* were performed. Four hearts from animals which did not survive to be studied *in vivo* were included. Two of the hearts from lambs studied *in vivo* were not examined *in vitro* because of damage to one of the dimension transducers during necropsy.

Following necropsy, a lucite disk was sewn into the mitral valve annulus and another into the tricuspid valve annulus. A catheter passed through the centre of each disk into a compliant latex balloon. The left and right ventricles were distended by filling the balloons with fluid. The left ventricular balloon was filled with a known volume, and the right ventricular volume was varied to obtain a range of right ventricular dimensions. These measurements were repeated over a range of left ventricular volumes.

Data analysis

Oscillograms were manually digitized (DigiKitIzer; Talos, Inc.) and processed on a microcomputer; digital data recorded on video tape were read into the memory of the microcomputer for direct numerical analysis. Further analyses were performed using the statistical analysis program provided by SAS Institute, Inc. Data obtained less than 24 h before the onset of labour were excluded from the analysis. A two-way analysis of variance demonstrated that measurement error was not significant in any of the variables which showed an effect of heart rate.

The spontaneous heart rates and the rates during atrial pacing were determined by measuring the interval between peaks of the QRS complex. The stroke volume was determined by integrating the area under the flow wave-form between the onset of flow and the following zero crossing. The period during mid- to late diastole provided a biological zero-flow reference. The end-diastolic dimension was measured at the peak of the QRS complex.

The analysis of right ventricular stroke volume, end-diastolic dimension, and output revealed that significant differences in these variables were present from one lamb to another. Consequently, a multiple linear regression analysis was used on the group data to correlate the effects of heart rate on these variables. A class variable representing each lamb and an interaction term were used to incorporate the analysis of variance result whenever lamb-to-lamb variations were significant (Anderson, Glick, Manring & Crenshaw, 1984). Data from all of the lambs could by these means be compared. The data obtained during spontaneous rhythm, right atrial pacing, and left atrial pacing were considered separately. Two hypotheses, namely (1) that the regression slope for each lamb would be zero, and (2) that the average slope would be zero, were evaluated to test the significance of heart-rate effects.

A separate linear regression analysis was performed on the data obtained from each individual study. In addition, a linear regression analysis was performed on normalized data from all the individual studies in which the pacing rates extended from 180 to 300 beats/min (Anderson *et al.* 1986).

The *in vitro* data relating ventricular dimension to volume were analysed using a linear regression for the data obtained from individual lambs and a multiple linear regression for the group data with a class variable representing each lamb. The latter analysis was undertaken owing to lamb-to-lamb variations in heart size and in the placement of the septal and right ventricular free wall transducers.

Necropsy

Four lambs were delivered by caesarean section using ketamine anaesthesia in the same manner as in the initial surgery, and two lambs were delivered vaginally. Two lambs survived as living neonates with instrumentation intact and were followed for 4 to 20 days after birth, after which they were killed by intravenous pentobartitone. The neonatal data are not presented in this report. At necropsy effusions or ascites were not present. No evidence of infection was found. In all cases the placement of the right and left atrial pacing electrodes was confirmed. The right and left ventricular free wall dimension transducers were always on the endocardial surface, and the septal transducer was found to be from 1 to 6 mm from the right ventricular septal surface. Evidence of thinning of the main pulmonary artery wall from the flow transducer was found while evidence of pulmonary artery narrowing was not present.

RESULTS

Animals

The number of individual pacing studies for a lamb was $4{\cdot}0 \pm 1{\cdot}6$ (mean $\pm$ S.D.). The ages at the time of the studies ranged from 126 to 139 days of gestation. The spontaneous heart rate (166 ± 17 beats/min, mean $\pm$ S.D.) and pulmonary artery pressure (57 ± 4 systolic, 37 ± 5 diastolic (mean $\pm$ S.D., in mmHg)) were similar to those obtained in other chronically instrumented fetal lambs (Rudolph, 1979; Anderson, Bissonnette, Faber & Thornburg, 1981; Thornburg & Morton, 1983). The right ventricular output characteristics during spontaneous rhythm were likewise similar to those obtained in previous studies of fetal lambs instrumented with a main pulmonary artery flow transducer (Anderson *et al.* 1981; Thornburg & Morton, 1983): stroke volume of $1{\cdot}59 \pm 0{\cdot}56$ ml/kg, and right ventricular output of 256 ± 66 ml/min . kg.

Right ventricular dimension measurement

In vivo. In order to monitor changes in right ventricular end-diastolic volume, a dimension of the right ventricular chamber was measured from the ventricular septum to the right ventricular free wall (see Fig. 1). In four lambs, a left ventricular dimension was obtained simultaneously with the right by means of a left ventricular

free wall endocardial transducer which was excited also by the septal transducer (see Fig. 1). Representative septum to right ventricular free wall dimension and septum to left ventricular free wall dimension wave-forms are shown in Figs. 1 and 4.

Right ventricular dimension during diastole. The right ventricular dimension wave-form during diastole contained the usual, and anticipated, components of ventricular diastolic filling. A rapid increase in the dimension occurred during the first portion of diastole, the period during which ventricular volume would be expected

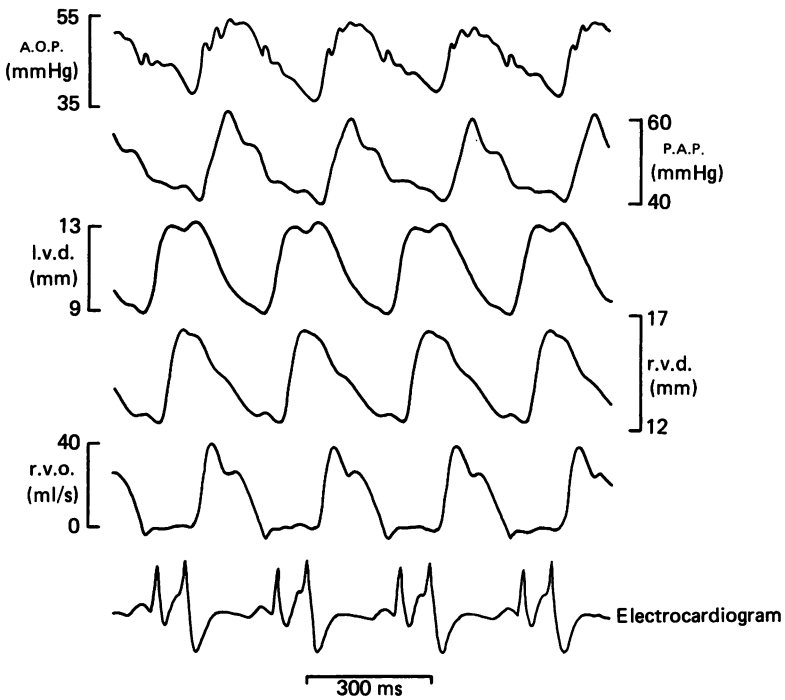

Fig. 1. Data obtained during spontaneous rhythm from a lamb at 131 days of gestation and 6 days after instrumentation. From the top of the illustration downward: bracheocephalic arterial pressure (A.O.P.); pulmonary artery pressure (P.A.P.); septum to left ventricular free wall dimension (l.v.d.); septum to right ventricular free wall dimension (r.v.d.); right ventricular output (r.v.o.); and the electrocardiogram.

to increase rapidly (Wiggers & Katz, 1922). Following this period, the dimension increased more slowly. Fig. 4 illustrates this slow increase better than Fig. 1 because of the longer diastolic period. In the last portion of diastole, a further increase in dimension occurred when the mechanical effects of atrial systole, which occur approximately coincident with the QRS complex of the electrocardiogram, would be expected to increase ventricular volume to its end-diastolic value.

In utero increase in right ventricular dimension. The effect of gestational age on right ventricular end-diastolic dimension was examined *in vivo*. Because of the effects of rate and pacing site on end-diastolic dimension (see below), only data obtained at the same pacing rate and site were used for comparison. Multiple linear regression analysis demonstrated that a significant and positive relationship existed between right ventricular end-diastolic dimension and gestational age: e.g. for data obtained at 200 beats/min the slope was 0·15, $r = 0{\cdot}98$ and $P < 0{\cdot}001$.

The increase in right ventricular dimension during gestation, similar to that described for fetal left end-diastolic dimension and gestational age (Kirkpatrick *et al.* 1973; Anderson *et al.* 1984) would seem to indicate the recovery of the lambs from the surgical manipulation and their growth *in utero*.

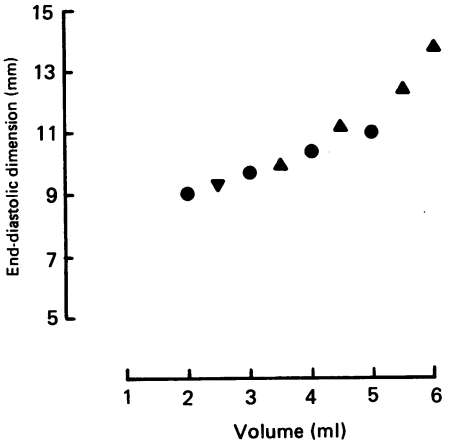

Fig. 2. *In vitro* relationship between right ventricular dimension and right ventricular volume of a heart obtained from a lamb studied *in utero*. The data were obtained at three left ventricular volumes: 5 ml (▼), 5·5 ml (▲) and 6 ml (●).

In vitro right ventricular dimension–volume relationship. Multiple linear regression analysis of the *in vitro* right ventricular dimension data obtained at different right ventricular volumes yielded, for the group of lambs, a linear relationship with a slope of 1·58, a coefficient of correlation (r) of 0·93 and $P < 0·0001$. Fig. 2 illustrates the relationship obtained from the heart of one lamb.

These observations *in vivo* and *in vitro* allowed us to use right ventricular end-diastolic dimension measurements to monitor *in vivo* changes in right ventricular end-diastolic volume.

The effects of heart rate during spontaneous rhythm

Seventeen spontaneous variations in heart rate of 10 % or more were observed. Such a change in rate was observed in each lamb; the largest change was 56 % (from 125 to 195 beats/min). The average change in rate was 30 beats/min.

The end-diastolic dimension, stroke volume and right ventricular output data at different spontaneous rates for all the lambs were compared by means of multivariate analysis (see Table 1). Heart rate was found (1) to have a positive effect on right ventricular output (slope, $2·77 \pm 0·46$); (2) not significantly to affect stroke volume, and (3) to have a positive effect on right ventricular end-diastolic dimension (slope, $0·028 \pm 0·006$). To examine why the spontaneous variations in rate affect output so differently from forced variations in rate using atrial pacing (see below), we examined the individual responses to spontaneous changes in rate to assess how stroke volume, end-diastolic dimension and output were affected.

The effects of heart rate on stroke volume were found to vary among the lambs and in the same lamb from day to day. In individual studies, stroke volume was found frequently to be related inversely to heart rate (eight out of seventeen

observations). It was related directly to heart rate in seven observations, and unaffected by it in two observations. This is in marked contrast to the effects of atrial pacing, in which rate had a consistently negative effect on stroke volume (see Fig. 3).

The individual responses of end-diastolic dimension to spontaneous variations in rate were also found to vary from lamb to lamb. End-diastolic dimension was unaffected by rate in six observations, positively related to rate in five observations, and inversely related to rate in six observations. This differs from the case with atrial pacing, in which end-diastolic dimension was consistently found to be inversely related to rate.

TABLE 1. Results of multivariate analysis of the effects of an increase in heart rate

	Effect on right ventricular:		
Rhythm mode	Stroke volume	End-diastolic dimension	Output
Spontaneous	n.s. $P \leqslant 0{\cdot}96$ $r = 0{\cdot}96$	Increase $P < 0{\cdot}0001$ $r = 0{\cdot}96$	Increase $P < 0{\cdot}0001$ $r = 0{\cdot}98$
Right atrial pacing	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}98$	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}98$	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}93$
Left atrial pacing	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}98$	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}97$	n.s. $P \leqslant 0{\cdot}81$ $r = 0{\cdot}97$

Not significant, n.s.

Our ability to monitor right ventricular diastolic filling and stroke volume provided data for insights into the changes in ventricular filling and ejection which were associated with spontaneous variations in heart rate. In one spontaneous variation in heart rate, for example, end-diastolic dimension decreased minimally with rate, but stroke volume was larger while pulmonary artery pressure was unchanged. The greater stroke volume with little change in end-diastolic dimension requires that contractility must have increased at the faster heart rate.

The larger stroke volume required a greater amount of ventricular filling to maintain the end-diastolic dimension. Such increased filling occurred despite the decrease in filling time which accompanies an increase in rate. This demonstrates that an enhancement of venous return must have occurred with this spontaneous increase in heart rate.

In the seventeen individual spontaneous variations in heart rate, rate was positively related to ventricular output in sixteen of the observations, in agreement with the findings of Rudolph & Heymann (1976); but rate did not affect output in one observation. On this occasion, the associated change in pulmonary artery pressure during the change in rate could at least in part explain why output did not increase. Pulmonary artery pressure was higher at the fast rate (63/48 mmHg systolic and diastolic pressures, 207 beats/min) than at the low rate (57/35 mmHg, 167 beats/min). The inverse relationship between pulmonary artery pressure and the ability of the fetal right ventricle to eject (Thornburg & Morton, 1983) could explain why stroke volume fell at the faster rate, resulting in output being unaffected by rate.

Effects of heart rate during atrial pacing

Atrial pacing enabled us to examine how heart rate alters right ventricular output independently of the changes in venous return and contractility which are induced by the stimuli which cause spontaneous variations in heart rate. We were able also

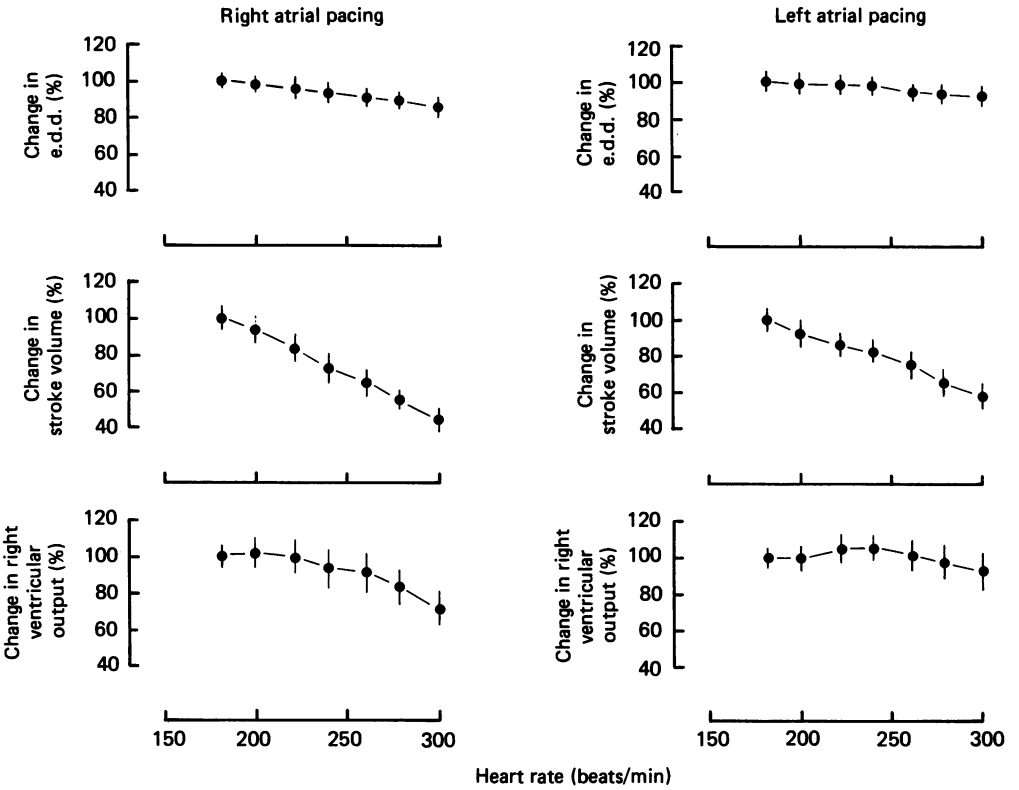

Fig. 3. Effects of right atrial and left atrial pacing in the lambs as a group. Values are normalized and expressed as percentages; 100% represents the slowest rate. Right ventricular end-diastolic dimension, e.d.d. The paced rates range from 182 to 300 beats/min. The vertical bars represent two standard deviations about the mean.

to examine how heart rate altered stroke volume and right ventricular end-diastolic dimension.

Right atrial pacing. Altering heart rate by means of right atrial pacing had a consistent effect on stroke volume and end-diastolic volume as compared to those associated with spontaneous variations in heart rate. When data from all the individual pacing studies were examined as a group by multivariate analysis, heart rate was found to correlate negatively with stroke volume (slope, $-0{\cdot}016 \pm 0{\cdot}001$) and end-diastolic dimension (slope, $-0{\cdot}028 \pm 0{\cdot}002$), as shown in Table 1. Because of the extent of the decrease in stroke volume with increase in heart rate, right ventricular output was affected negatively by rate (slope, $-1{\cdot}37 \pm 0{\cdot}20$).

The normalized responses for the entire group of lambs to heart rate for pacing

rates of 182 to 300 beats/min are illustrated in Fig. 3. In comparing the effects of different sites of atrial pacing, right atrial pacing produced a greater decrease in end-diastolic dimension and stroke volume than did left atrial pacing.

When the right atrial pacing studies were examined singly, heart rate was found to correlate negatively with stroke volume. Similarly, right ventricular end-diastolic dimension declined with an increase in rate. However, the extent to which these measures were affected by rate varied from study to study. This produced qualitative variations in the relationship between output and rate in our individual pacing studies.

For example, although right ventricular output decreased with an increase in rate in the majority of studies, output was on occasion found to be unaffected by an increase in rate, or even to increase. Day-to-day variations in the effects of rate on output occurred in the same animal: e.g. output in a lamb would decrease significantly on one day, yet on another day output would be unaffected by rate. These differences appeared to be related to the extent to which end-diastolic dimension and stroke volume were affected by the alterations in rate.

When we examined each individual pacing study to determine the largest increase in output at any paced rate as compared to the output at the slowest rate (similar to the approach of Rudolph & Heymann, 1976), we found that the largest increase during any right atrial pacing study was 20%. This occurred only twice, and in two different lambs. The maximum increases occurred at a rate of 200 beats/min in one lamb and 260 beats/min in the other. As an example of the variability of the effects of rate, on other study days the outputs at those same rates for these two lambs were 2% greater and 1% less than the outputs at the lowest paced rates. Among the other lambs, individual pacing studies could be found in which the output never increased at any given paced rate above the slowest paced rate: i.e. at each of the faster pacing rates output either remained constant or decreased.

Left atrial pacing. Multivariate analysis of the responses of the lambs as a group to left atrial pacing revealed that, just as with right atrial pacing, rate correlated negatively with stroke volume (slope, $-0{\cdot}010 \pm 0{\cdot}001$) and end-diastolic dimension (slope, $-0{\cdot}014 \pm 0{\cdot}002$); see Table 1. The effects on output engendered by diminished stroke volume were offset by an increase in heart rate such that, using left atrial pacing, heart rate did not affect right ventricular output.

Fig. 3 shows the normalized data from pacing studies in all the lambs for rates from 182 to 300 beats/min. No significant effect on output was found, whereas stroke volume and end-diastolic dimension were negatively related to rate ($P < 0{\cdot}0001$).

When individual pacing studies were analysed singly, rate was always found to be correlated negatively with stroke volume. However, the extent of the decrease varied, as did the effects of rate on end-diastolic dimension. Consequently, the effects of rate on right ventricular output were found to vary qualitatively among the studies. In the majority, output was not significantly affected by rate; in four it was related inversely to rate; and in two it was related positively to rate. The effects on output were found, moreover, to vary from study to study in the same lamb; output could, for example, be unaffected by rate on one day yet would increase with an increase in rate on another.

When individual pacing studies were examined to find the greatest increase in

output as compared to the output at the slowest pacing rate, the highest value found was 29 % in one study, and the next highest was 23 % in another study. In these two lambs, the pacing rates which were used ranged from 150 to 300 beats/min and 140 to 300 beats/min respectively; the largest increase in output was found at rates of 200 and 180 beats/min. In other lambs, the maximum increase at any paced rate over that of the slowest rate was less than 1 %. We therefore conclude that, for the lambs as a group, there is no significant effect of rate on output when using left atrial pacing.

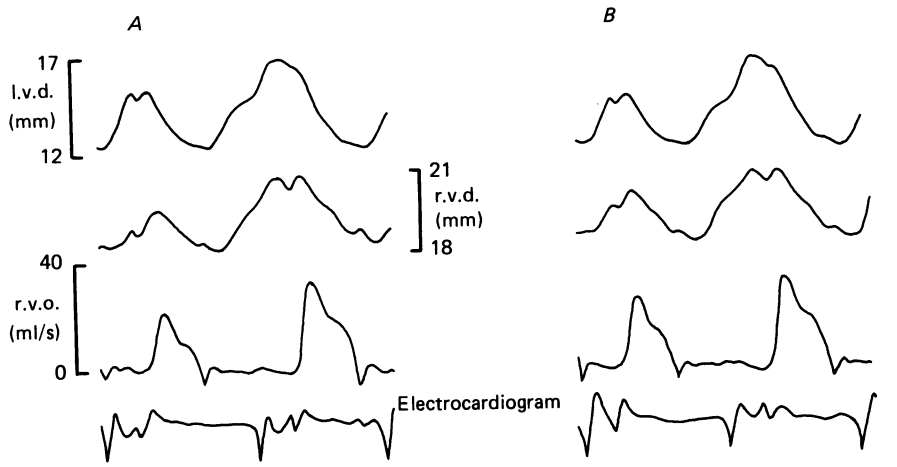

Fig. 4. The effect of introducing a longer pacing interval during pacing at two different constant rates (*A*, 280 beats/min; *B*, 240 beats/min). Right ventricular end-diastolic dimension and stroke volume were smaller at the faster rate. By introducing the same longer pacing interval, the right ventricular end-diastolic dimensions were increased to a similar value at both rates as were the left ventricular end-diastolic dimensions. Right ventricular stroke volumes were also increased. The data were obtained from a lamb at 130 days of gestation. From the top of the illustration downward: septum to left ventricular free wall dimension (l.v.d.); septum to right ventricular free wall dimension (r.v.d.); right ventricular output (r.v.o.); and the electrocardiogram. The data were obtained using right atrial pacing.

The effect of modulating end-diastolic dimension

To examine the effects of heart rate on stroke volume independently of variations in preload and afterload, longer paced intervals were introduced from time to time into the faster rates. By this means we could examine, among different paced rates, systoles which began contraction from the same end-diastolic dimension (see Fig. 4).

Figs. 4 and 5 show the stroke volumes of systoles before and after the interposition of a longer paced interval. In those systoles where the rate-induced fall in end-diastolic dimension was not circumvented, end-diastolic dimension and stroke volume became progressively smaller as the rate increased. These effects on stroke volume were the same for both right and left atrial pacing. For the systoles with equal end-diastolic dimension, in contrast, stroke volume was found not to fall, but to be greater the faster the preceding paced rate (Fig. 5).

The fall in stroke volume with decreasing end-diastolic dimension, and the absence of such a fall in stroke volume when the end-diastolic dimension was maintained,

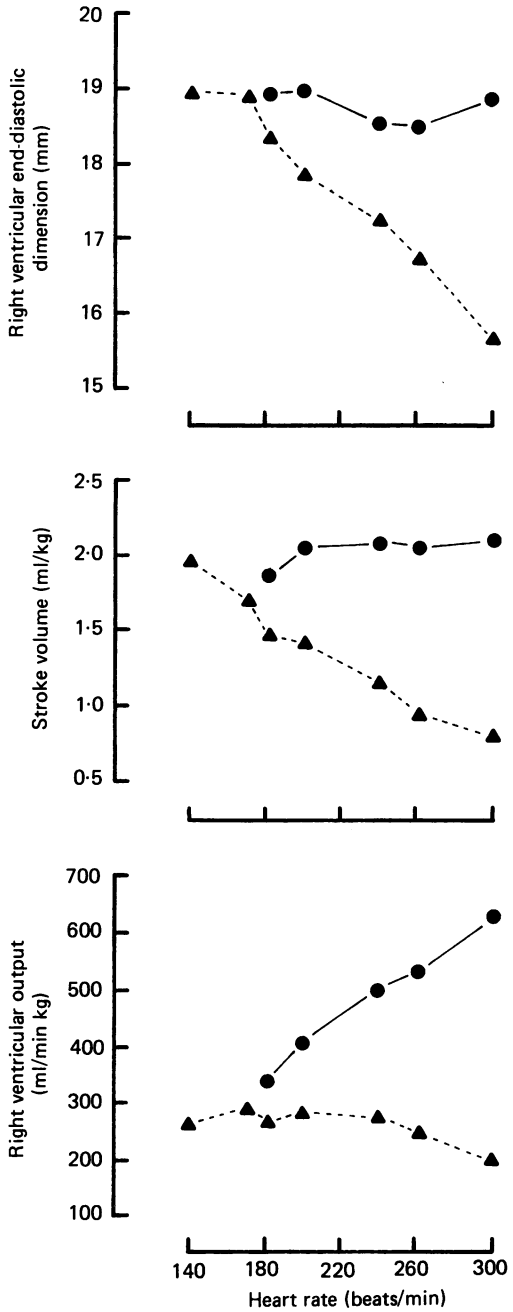

Fig. 5. The effects of introducing a longer pacing interval (see Fig. 4) on right ventricular end-diastolic dimension, stroke volume (corrected for body weight), and output (corrected for body weight) during pacing over a broad range of rates. Data obtained (▲) during pacing at a constant rate; and (●) following the introduction of a longer pacing interval (415 ms). When the fall in right ventricular dimension was circumvented by introducing the pause, stroke volume did not decrease with increasing heart rate. If these measures had been maintained at the faster heart rates, right ventricular output would have increased markedly. Left atrial pacing was used in a lamb at 137 days of gestation, 10 days after instrumentation.

demonstrate the presence of the Frank–Starling relationship in the fetal right ventricle. They also demonstrate that right ventricular output would increase markedly with an increase in rate if rate-induced changes in end-diastolic dimension could be avoided.

DISCUSSION

The relationship between heart rate and right ventricular output in the fetal lamb *in utero* depends on the interaction of diastolic filling time, venous return, and contractility. Because of the complexity of the interaction, heart rate can have different effects on right ventricular output in the same fetus. During right atrial pacing, for example, rate has a negative effect on output; but in the case of spontaneous variations of rate, there is a positive effect on output. These findings demonstrate that the mechanisms which modulate fetal right ventricular output are the same as those through which fetal left ventricular output (Kirkpatrick *et al.* 1976; Anderson *et al.* 1986) and adult cardiac output (Ross *et al.* 1965; Sugimoto *et al.* 1966; Cowley & Guyton, 1971) are affected by rate.

Our conclusions regarding the mechanisms through which rate affects right ventricular output in the chronically instrumented *in utero* lamb were based on data obtained from the monitoring of ventricular ejection and filling. Right ventricular output and stroke volume were measured with an electromagnetic flow transducer placed around the main pulmonary artery, similar to the fetal study of rate effects performed by Rudolph & Heymann (1976). Ventricular filling was monitored using right ventricular free wall and septal ultrasonic dimension transducers to measure an end-diastolic dimension of the right ventricle. Although sonomicrometry has been used to measure a right ventricular dimension in the intact adult dog (e.g. by Sodums, Badke, Starling, Little & O'Rourke, 1984), this study is the first to apply the technique in the fetus. It is an extension of the established use of dimension transducers to monitor fetal left ventricular chamber size *in utero* developed by Kirkpatrick and his associates (Kirkpatrick *et al.* 1973). By measuring right ventricular filling as well as stroke volume, we were able more completely to assess the mechanisms through which rate might alter output.

The ability to alter heart rate using right and left atrial pacing allowed us to contrast how spontaneous variations in rate and those induced by pacing could affect fetal right ventricular output.

Right ventricular end-diastolic volume

Our use of dimension transducers to monitor changes in end-diastolic volume required an examination of the relationship between right ventricular volume and dimension. The shape of the right ventricle, in contrast to the left, does not lend itself to approximation by a simple geometric model (Sodums *et al.* 1984). However, it appeared reasonable to assume that changes in right ventricular end-diastolic volume would be reflected by changes in a right ventricular free wall to septum dimension. This assumption was tested by our *in vitro* studies (Fig. 2), and an excellent relationship between dimension and volume was found.

The value of right ventricular end-diastolic dimension measurements was confirmed *in vivo* by our ability to relate changes in stroke volume to changes in

ventricular volume, and by allowing us to perform experiments in which heart-rate effects on end-diastolic volume were circumvented by introducing longer diastolic filling times during the various paced rates. The day-to-day increase in right ventricular end-diastolic dimension furthermore provided evidence of the recovery of the lambs from surgery and of their growth *in utero*.

Further considerations for measuring right ventricular dimension

Although changes in right ventricular end-diastolic volume can be monitored by such measures as mean atrial or ventricular end-diastolic pressures, we chose to use a ventricular end-diastolic dimension because it is a more direct measure of beat-to-beat changes in ventricular end-diastolic volume.

The need to consider the effects of ventricular compliance when relating changes in pressure to changes in volume is avoided by the use of a dimension measurement. In an *in vitro* study of fetal, neonatal, and adult sheep hearts, Romero, Covell & Friedman (1972) demonstrated that a small change in pressure is associated with a relatively large change in end-diastolic volume when ventricular filling pressures are low; but over higher pressure ranges, large changes in pressure are associated with only small changes in volume.

The likelihood that right ventricular filling and end-diastolic pressures are affected by the filling and end-diastolic pressure of the left ventricle further supports the use of a right ventricular dimension measurement. The presence of ventricular interaction in the *in vivo* developing heart is probable for at least two reasons. First, it is known to exist in the *in vivo* adult heart (Laks, Garner & Swan, 1967; Taylor, Covell, Sonnenblick & Ross, 1967; Oboler, Keefe, Gaasch, Banas & Levine, 1973; Santamore, Lynch, Meier, Heckman & Bove, 1976; Badke, 1982). Secondly, it is suggested by the direction of maturational changes in right ventricular compliance found *in vitro*; the fetal right ventricle is less compliant than the adult (Romero *et al.* 1972). Alterations in left ventricular filling *in vitro* have a greater effect on fetal than on adult right ventricular pressure when the left ventricle is filled to a greater extent. By measuring a right ventricular dimension, we avoid this potential complexity in relating changes in pressure to those in volume.

Interaction of rate, stroke volume and end-diastolic dimension

The effects of heart rate on right ventricular stroke volume and end-diastolic dimension were straightforward when heart rate was controlled by atrial pacing. When the rate was increased and diastolic filling time fell, stroke volume and end-diastolic dimension decreased. This occurred with either site of pacing.

This observation that stroke volume declines with a decrease in end-diastolic dimension supports the hypothesis that the Frank–Starling relationship is present in the fetal right ventricle. This conclusion is consistent with the results of Thornburg & Morton (1983) and of Gilbert (1980), who demonstrated that right ventricular output is significantly affected by right atrial pressure over a physiologic range. Such dependence of fetal right ventricular stroke volume and output on end-diastolic volume is similar to that described in the fetal left ventricle by Kirkpatrick *et al.* (1976) and by ourselves (Anderson *et al.* 1986).

The effects of rate on stroke volume through the Frank–Starling relationship, and

ultimately on right ventricular output, was further demonstrated by the experiments in which we introduced longer paced intervals during the various paced rates. By thus forcing longer diastolic filling times we were able to compare systoles that began from the same end-diastolic dimension, but which occurred during different average pacing rates. When the rate-related decrease in end-diastolic dimension was prevented, stroke volume no longer fell. These findings suggest that if venous return were increased during the pacing studies and the rate-induced fall in end-diastolic volume circumvented, a markedly positive relationship between rate and output would result. When venous return is enhanced in the adult heart, a positive relationship between heart rate and output has indeed been found (Sugimoto *et al.* 1966; Cowley & Guyton, 1971).

By comparing systoles at the same end-diastolic dimension during different paced rates, rate-related changes in inotropy can be isolated. We found that stroke volume increased with paced rate, showing that rate-related changes in inotropy have a significant effect on right ventricular stroke volume provided that end-diastolic volume is controlled. Such dependence of right ventricular inotropy on rate is similar to that described for the fetal left ventricle (Kirkpatrick *et al.* 1975; Anderson, Manring, Glick & Crenshaw, 1982) and for the adult heart (Mahler *et al.* 1974).

Comparison of the effects of right and left atrial pacing

The site of atrial pacing was found to be important when the relationships between rate and output were examined. With right atrial pacing, rate was inversely related to output; but with left atrial pacing, rate did not affect output. A differential effect of the pacing site on right ventricular output has also been described by Rudolph & Heymann (1976) and by Pitlick *et al.* (1976).

In the present study, we were able to assess the effects of the pacing site on right ventricular filling by measuring end-diastolic dimension. The decrease in right ventricular end-diastolic dimension with an increase in rate was greater during right atrial pacing than during left atrial pacing. The difference in ventricular filling, produced by changing the site of atrial pacing, may be the result of alterations in the phasic pressure relationships between the atria consequent to changes in the timing of the contraction of each atrium. During left atrial pacing, for example, the flow of blood from the right atrium to the left atrium might be decreased by the change in the relative timing of left and right atrial systole, and so result in a relatively larger right ventricular end-diastolic volume as compared to that which is obtained during right atrial pacing at the same rate.

Such possible effects on the relative filling of the right ventricle would be consistent with the way in which the site of atrial pacing affects fetal left ventricular filling and produces the observed effects on left ventricular output (Pitlick *et al.* 1975; Anderson *et al.* 1986). Fetal left ventricular output is affected by atrial pacing site in the opposite sense to that of right ventricular output; i.e. during left atrial pacing, rate is inversely related to left ventricular output, but has no effect on left ventricular output with right atrial pacing.

Other effects of the atrial pacing site, such as the timing of atrial systole relative to the onset of ventricular systole, do not seem to be a likely cause of the differential effects. In a fetal study of left ventricular output and rate (Anderson *et al.* 1986), we

suggested that the decrease in fetal left ventricular output with an increase in rate, using left rather than right atrial pacing, might result from the longer conduction time from the left atrial pacing site to the left ventricle. Relative differences in conduction time could result in the appropriate timing of ventricular presystolic stretching by atrial systole – a physiologic factor known to modulate ventricular contraction (Linden & Mitchell, 1960; Brady, 1965) – being lost first with left atrial pacing. Such an argument would apply equally in the case of right ventricular output, but in fact the differential effects of atrial pacing site on right ventricular output are reversed. It follows that this factor, though important in itself, does not appear to be a significant contributor to the effects of the site of atrial pacing on right ventricular output.

Effect of heart rate on output using atrial pacing

Although there is agreement between the present study and that of Rudolph & Heymann (1976) that right ventricular output is affected differentially by the site of pacing, the conclusions reached regarding the effects of rate on output appear to differ. Rudolph and Heymann found an increase in right ventricular output with rate during right and left atrial pacing, but in our group of lambs we found no effect of rate on output with left atrial pacing, and an inverse effect with right atrial pacing.

When the gestational ages, the experimental approaches, and the physiological variables are compared, no major differences can be found in the two studies. In both studies, the lambs were allowed to recover from the surgical procedure before examination of any effects of rate on right ventricular output. Moreover, when we investigated whether the interval following surgery affected the relationship between rate and output, we found no such effect. The animals appeared to have recovered from the instrumentation; for example, their right ventricular outputs were similar to those reported in other studies using electromagnetic flow transducers (Anderson *et al.* 1981; Thornburg & Morton, 1983).

Bearing in mind that stroke volume is altered by rate, diastolic filling time, and pulmonary artery pressure (Thornburg & Morton, 1983), we wondered if significant differences in heart rate or different effects of heart rate on pulmonary artery pressure existed between our study and that of Rudolph & Heymann (1976). The mean spontaneous rate of our lambs was approximately 167 beats/min, within the range of resting rates of 160 to 180 beats/min observed in the study by Rudolph & Heymann (1976). Data obtained by Rudolph and Heymann using vagal stimulation to slow heart rate were excluded from our comparison of spontaneous rates, for such stimulation can depress ventricular function (DeGeest, Levy, Zieske & Lipman, 1965), complicating the interpretation of any associated fall in output. The similarity of the spontaneous heart rates allowed pacing over a range of 182 to 300 beats/min in both groups. In addition, pulmonary artery diastolic pressure was maintained over the range of rates used in both studies. Accordingly, these variables do not appear to be potential contributors to the apparently different findings of the two studies.

When we examined the effects of heart rate on output in individual lambs from day to day, the differences between our study and the study by Rudolph & Heymann (1976) appeared to be largely resolved. Rudolph and Heymann found, for example, that right ventricular output increased on average by 15 to 20% at the paced rates

of 240 to 270 beats/min, using left atrial pacing. We found an individual study in our series in which output increased by 29 % for a given rate. In addition, during right atrial pacing, we found individual studies in which output increased by as much as 20 % over that at the slowest paced rate. Some of our individual left atrial pacing studies, however, yielded an increase in output of less than 10 %, and some of our right atrial pacing studies demonstrated that output never increased with an increase in rate. Although we had some individual studies whose results agreed with those of Rudolph and Heymann, the variability of the effect of rate on output from lamb to lamb and from day to day were such that heart rate had no significant effect on right ventricular output during left atrial pacing, and a negative effect on output during right atrial pacing, for the group of animals as a whole.

Based on the present study, the responses of the fetal right ventricle to alterations in heart rate are similar to those of the fetal left ventricle (Kirkpatrick *et al.* 1976; Anderson *et al.* 1986) and to those of the adult heart (Ross *et al.* 1965; Sugimoto *et al.* 1966; Cowley & Guyton, 1971). Rate does not increase output. Such similarity among the effects of rate at different stages of development would support the hypothesis that the dependence of the right and left ventricle on heart rate does not undergo a maturational change from late gestation to adulthood.

Spontaneous variations in heart rate

When heart rate changes spontaneously in the fetus, a complex interaction is found to exist between rate and the ways in which stroke volume is altered by contractility, end-diastolic dimension and pulmonary artery pressure. In almost all of our observations we found a positive relationship between heart rate and output, similar to the findings of Rudolph & Heymann (1976), but we also found no significant effect on right ventricular output on one occasion. The differences in responses between these during spontaneous variations in rate and those during atrial pacing were due to the interaction of the variables which appear to control stroke volume in the fetus.

As an example, stroke volume and end-diastolic volume usually did not decrease with a spontaneous increase in rate to the same extent as they did with atrial pacing. Indeed, end-diastolic dimension often increased at the higher rates. In view of the decrease in diastolic filling time at faster rates, the increase in end-diastolic dimension suggests that venous return was increased in response to that underlying stimulus which brought about the rate change. Other examples demonstrate that contractility was enhanced at the faster rate: although end-diastolic volume decreased, stroke volume increased. The increase in contractility often exceeded the increase found in association with changes in rate using atrial pacing. Furthermore, the effect of right ventricular afterload was also noted. On one occasion, right ventricular output did not increase with rate as pulmonary arterial pressure rose and stroke volume fell. This demonstrated the expected effect of afterload on fetal right ventricular stroke volume (Thornburg & Morton, 1983).

In summary, the stimuli which produce spontaneous variations in rate are associated with changes in the physiological variables, preload, afterload, and contractility which affect fetal right ventricular stroke volume. Consequently, spontaneous variations in heart rate can produce a range of effects on fetal right ventricular output. For the group of lambs we studied, rate was positively related

to right ventricular output. This exhibits the same general relationship found between spontaneous variations in rate and fetal left ventricular output (Rudolph & Heymann, 1976; Anderson *et al.* 1986), and it is in agreement with the effect of spontaneous changes in rate on fetal right ventricular output described by Rudolph & Heymann (1976).

The modulation of fetal right ventricular output by the interaction of venous return, contractility and heart rate demonstrates that these mechanisms are physiologically important *in utero* during late gestation and are available at the time of birth to bring about the enhanced neonatal ventricular output (Woods, Dandavino, Brinkman, Nuwayhid & Assali, 1978; Lister, Walter, Versmold, Dallman & Rudolph, 1979; Breall, Rudolph & Heymann, 1984). The birth-related changes in inotropy (e.g. secondary to circulating catecholamines or sympathetic stimulation (Geis, Tatooles, Priola & Friedman, 1975; Eliot, Lam, Leake, Hobel & Fisher, 1980)), the fall in right ventricular afterload with ventilation (Dawes, 1968), the increase in heart rate (Comline & Silver, 1972; Anderson *et al.* 1982, 1984), and the increase in venous return would allow the right ventricle to increase its output in response to the requirements of neonatal life.

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