J. Physiol. (1986), **372**, *pp.* 557–573
With 6 text-figures
Printed in Great Britain

THE EFFECT OF HEART RATE ON *IN UTERO* LEFT VENTRICULAR OUTPUT IN THE FETAL SHEEP

BY PAGE A. W. ANDERSON, KATHERINE L. GLICK, ALLEN P. KILLAM AND RICHARD D. MAINWARING

From the Departments of Pediatrics, Physiology, Biomedical Engineering, and Obstetrics and Gynecology, Duke Medical Center, Durham, NC, U.S.A.

(*Received 18 July 1985*)

SUMMARY

1. The effect of heart rate on left ventricular output was examined in seven fetal lambs at ages of 128 to 140 gestational days. The fetuses had been surgically instrumented at least 4 days previously with an ascending aortic flow probe, left ventricular dimension transducers, and left and right atrial pacing electrodes.

2. Natural variations in heart rate of the lambs taken as a group correlated positively with left ventricular output, and negatively with ventricular end-diastolic dimension and stroke volume ($P < 0{\cdot}0001$).

3. Rate did not affect output with right atrial pacing. With left atrial pacing, it correlated negatively with output ($P < 0{\cdot}0001$). At both pacing sites, rate correlated negatively with end-diastolic dimension and stroke volume ($P < 0{\cdot}0001$).

4. The introduction of a longer interval during each pacing rate circumvented the rate-related changes in dimension and allowed the ventricle to fill to the same end-diastolic dimension. The systole at the end of the longer interval had a greater stroke volume than the preceding systoles. The faster the preceding paced rate, the greater was the stroke volume ($P < 0{\cdot}0001$).

5. This study demonstrates that experimentally induced variations in heart rate produce changes in end-diastolic volume and contractility which prominently affect stroke volume. Over a broad range of rates, however, the effect of rate on left ventricular output is either negative or absent. With naturally occurring rate changes, there are additional changes in contractility and venous return which affect stroke volume. These combine to produce a positive relation between heart rate and left ventricular output. These effects of heart rate on output are qualitatively similar to those described for the adult animal.

INTRODUCTION

Investigators who have studied the mechanisms which control cardiac output in the developing fetus have drawn very different conclusions with respect to the importance of heart rate. For example, Rudolph and his associate (Heymann & Rudolph, 1973; Rudolph & Heymann, 1976) conclude that heart rate plays a major role in controlling fetal ventricular output. The studies of Kirkpatrick, Pitlick,

Naliboff & Friedman (1976), in contrast, show that left ventricular output remains constant during variations in rate owing to compensatory adjustments in end-diastolic volume and stroke volume. Though both groups rely on data from chronically instrumented animals, it is possible that their different approaches bring to light different aspects of how left ventricular output is modulated. Furthermore, the findings of Rudolph & Heymann (1976) and Pitlick, Kirkpatrick & Friedman (1976) that output depends on the site of atrial pacing, suggest that ventricular filling is an important modulator of stroke volume in the fetus.

In our study we have re-examined the relation of heart rate and left ventricular output. We have combined the experimental techniques used by the two groups just mentioned. We have used an electromagnetic flow transducer on the ascending aorta to monitor left ventricular stroke volume and output, as Rudolph & Heymann (1976) did. We have used a sonomicrometer to measure left ventricular dimensions, as did Kirkpatrick *et al.* (1976). We, like Rudolph & Heymann (1976), placed pacing electrodes on both the right and left atria; Kirkpatrick *et al.* (1976) employed only left atrial pacing electrodes. It was anticipated that such a combination of instruments would reveal how heart rate affects ventricular filling and stroke volume during natural variations in rhythm as well as during atrial pacing. Inasmuch as the data to be obtained would be comparable with those of the other two groups, it was expected that we might thereby determine why these two groups had reached different conclusions.

Our study demonstrates that heart rate interacts dynamically with ventricular filling and contractility to modulate fetal left ventricular output. Changing the rate by atrial pacing, for instance, causes changes in inotropy and end-diastolic volume, which cause changes in stroke volume which may alter output. In the case of natural variations in rate, additional mechanisms are brought into play. The stimulus which increases heart rate is associated with changes in contractility and venous return; these in turn modulate stroke volume and hence increase output. Variations in the extent of these interactions explain the apparently contradictory conclusions drawn by previous investigators. Furthermore, the presence of these mechanisms in the fetus, and their interactions, help to explain how output is able to increase following birth (Woods, Dandavino, Brinkman, Nuwayhid & Assali, 1978; Lister, Walter, Versmold, Dallman & Rudolph, 1979; and Breall, Rudolph & Heymann, 1984).

METHODS

Preparation

Seven fetal lambs from age-dated pregnancies underwent surgery *in utero* at 124 to 133 days of gestation (127 ± 2 days (mean $\pm$ S.D. of an observation)). The ewes were fasted for 24 h, and then anaesthetized by a bolus of ketamine. Anaesthesia was maintained by continuous infusion of ketamine (3 mg/ml) through the external jugular vein. The abdomen was prepared and draped, a mid-line incision was made, and the uterus exposed. A uterine incision parallel to the fetal trachea exposed the fetal neck. The fetal skin was incised, the external jugular vein and internal carotid artery were isolated, and a catheter (N.I.H. Angiographic, No. 5) was inserted into each vessel. The tip of one catheter was placed in the superior vena cava or right atrium, and the tip of the other was placed in the bracheocephalic artery. An electrocardiograph lead was implanted subcutaneously. The fetal skin incision and the uterine incision were closed.

A second incision was made over the fetal left chest and a thoracotomy performed through the fourth intercostal space. The pericardium was opened, and the ascending aorta was dissected free

from the pulmonary artery. Two 2·5 mm piezoelectric ultrasonic transducers were inserted through separate puncture wounds into the left ventricle in the plane of the minor axis and were then pulled back against the endocardium. A fluid-filled catheter (HL-54, 0·04 in. i.d.) was inserted into the left ventricle through an apical stab wound and was secured by a mattress suture. An electromagnetic flow transducer (8 or 10 mm i.d., Howell Instruments) was placed around the ascending aorta. 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 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. Bipolar pacing electrodes were sutured to the left atrial appendage. The thoractomy 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 brought through a subcutaneous tunnel to pass through a stab wound in the left flank of the ewe. The abdominal incision was closed. The catheters and cables were then placed in a pouch sewn to the flank of the ewe. Ampicillin (1·0 g) was infused into the amniotic cavity. The anaesthesia was discontinued, and the ewe was returned to her pen, located in a room next to 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

The left ventricular output was measured with an electromagnetic blood-flow-rate-meter (Howell Instruments HMS-1000) providing reproducibility within $\pm 2\%$; a band width of 30 Hz was used for all measurements.

The left ventricular dimension was measured with an ultrasonic sonomicrometer, operating at 5 MHz with a pulse repetition rate of 1 kHz. The sonomicrometer had a minimum resolution of 0·07 mm; electronic drift was equivalent to less than 0·05 mm/h (Kirkpatrick, Covell & Friedman, 1973). This method has been shown to be accurate and very sensitive to left ventricular end-diastolic volume (Bishop & Horwitz, 1970; Boettcher, Vatner, Heyndrickx & Braunwald, 1978; Sodums, Badke, Starling, Little & O'Rourke, 1984). The flow probe excitation and the pulse repetition rate of the ultrasonic transmitters were derived from a common crystal-controlled oscillator to preclude interference.

Pressure wave forms were obtained by standard physiological pressure transducers (Hewlett-Packard 1280C, with 8805 V carrier amplifiers). Electrocardiograms were obtained with a BioCom Bioamplifier (type 2122).

Measured data were recorded on a Brush oscillographic recorder at a chart speed of 5 in./s.

The blood-flow-rate-meter and the dimension transducers were calibrated before implantation and were found to be linear over the range of values of physiological quantities here studied. The pressure transducers were calibrated by a mercury manometer. Further calibration checks were made after implantation. The internal calibration reference of the flowmeter, and a series of internal crystal-controlled receive-delay intervals in the sonomicrometer, were recorded before and after the recording of data to verify system linearity and calibration factors.

Experimental protocol

The lambs were studied as early as 4 and as late as 12 days following surgery ($6·5 \pm 2·0$ days). The arterial blood gas evaluations on the study days demonstrated $p_{O_2} = 20$–26 torr, $p_{CO_2} = 38$–44 torr, and pH = 7·37–7·41. Before carrying out an experimental perturbation, we recorded the left ventricular stroke output and dimensions, the left ventricular and aortic pressures, and an electrocardiogram during spontaneous rhythm.

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

The atrium was stimulated with a programmed, crystal-controlled pacing system the timing accuracy of which was better than 0·1 %. An isolated stimulator (Digitimer) with a variable output

level provided a stimulating pulse of 5 ms duration. The period of atrial pacing ranged from 30 s to 10 min. The fastest rates (280 beats/min or greater) could not be maintained for over 60 s in many of the lambs before atrioventricular block, of varying degree, occurred. Only those data 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 followed each pacing period, during which the lamb's haemodynamic variables were monitored during spontaneous rhythm. Whenever the spontaneous heart rate differed by more than 10% between control periods, a change in the resting state of the lamb was assumed to have taken place; data from the intervening periods of pacing in such cases were excluded from the analysis. After one atrium was paced, the other atrium was paced; the order of the site pacing was reversed with each study. The protocol was repeated at least twice on each study day.

Over a range of heart rates, the end-diastolic volume was found to change (see Figs. 3 and 4). To control for this variable, and to compare the effects of all the heart rates on stroke volume, we periodically and infrequently introduced longer paced intervals in the pacing pattern. This permitted the left ventricle of the individual lamb to fill to the same end-diastolic dimension. The site of pacing for the systoles at the end of the interval was the same as that for the preceding systoles. The longer paced interval was usually approximately 400 ms, and did not vary by more than 30 ms for a given experiment. This ensured that, for each lamb, the aortic diastolic pressures that preceded these systoles with equal end-diastolic dimensions did not vary by more than 5 mmHg.

Data analysis

Oscillograms were manually digitized (DigiKitIzer; Talos, Inc.) and processed on an 8-bit microcomputer. Further analysis was performed at Triangle Universities Computing Center on an IBM 370/168 computer, 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 (ANOVA) demonstrated that measurement error was not significant in any of the variables which showed a heart rate effect.

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 time of rapid deflexion of the QRS. Percentage fractional shortening was obtained by subtracting end-systolic dimension from end-diastolic dimension, dividing this difference by end-diastolic dimension, and multiplying the quotient by 100%. This measure has been used to characterize the ejection characteristics of the left ventricle (Anderson, Manring, Glick & Crenshaw, 1982).

The analysis of left ventricular stroke volume, end-diastolic dimension, output, and percentage fractional shortening revealed that there were significant differences in these variables from one lamb to the next. Consequently, a multiple linear regression analysis was used on the group to correlate the effects of heart rate with these variables. A class variable representing each lamb and an interaction term were used to incorporate the ANOVA 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 pacing study. In addition, a linear regression analysis was performed on normalized data from those individual studies in which the pacing rates extended from 180 to 300 beats/min. In this latter analysis, the values measured at the slowest rate in an individual pacing study were defined as the 100% response. The values for stroke volume, end-diastolic dimension, percentage fractional shortening, and left ventricular output measured at other pacing rates were then expressed as percentages of these values. Such normalization allowed results from all of the lambs to be grouped together for illustrative purposes (see Figs. 3 and 4). Right atrial and left atrial pacing data were considered separately.

Necropsy

The lambs were delivered by caesarean section following onset of labour, using ketamine anaesthesia in the same manner as in the initial surgery. The lamb and ewe were killed by means of pentobarbitone. Effusions or ascites were not present. No evidence of infection was found. In all cases the placements of the right atrial and left atrial pacing electrodes were confirmed, and the dimension transducers were found to be at the level of the left ventricular minor axis and positioned against the endocardium. Evidence of aortic narrowing from the flow transducer was found in one lamb killed 19 days after surgery.

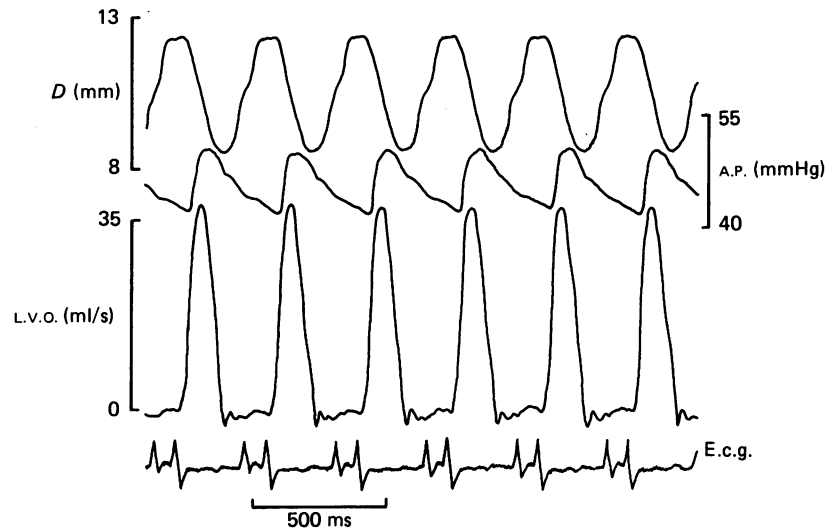

Fig. 1. Effects of spontaneous rhythm on left ventricular minor axis dimension (D), aortic pressure (A.P.), ascending aortic flow (L.V.O.), and electrocardiogram (e.c.g.). The data were obtained from a fetal lamb 7 days following surgery.

RESULTS

Animals

The number of individual pacing studies for a lamb averaged $4{\cdot}0 \pm 2{\cdot}6$ (mean $\pm$ S.D.). The gestational ages at the time of these studies ranged from 128 to 140 days. The spontaneous heart rates, 168 ± 8 beats/min, and arterial pressures, 53 ± 8 mmHg (systolic), were similar to those obtained in other chronically instrumented fetal lambs (see, e.g. Kirkpatrick, Covell & Friedman, 1973; Rudolph & Heymann, 1976; and Kirkpatrick *et al.* 1976). The left ventricular characteristics during spontaneous rhythm (see Fig. 1) were likewise similar to those obtained in previous studies (Rudolph & Heymann, 1970; Heymann, Creasy & Rudolph, 1973; Anderson, Bissonnette, Faber & Thornburg, 1981; Anderson *et al.* 1984): stroke volume, $0{\cdot}91 \pm 0{\cdot}11$ ml/kg body weight; left ventricular output, 178 ± 60 ml/kg; end-diastolic dimension, 17 ± 3 mm; and percentage fractional shortening, 32 ± 4 %.

Effects of heart rate during spontaneous rhythm

There were nineteen spontaneous variations in heart rate of 10 % or greater. Such changes in rate were observed in every lamb; the largest change was 49 % (from 138 to 206 beats/min).

End-diastolic dimension, stroke volume, and left ventricular output data at different spontaneous rates for the entire group of lambs were compared by means of multivariate analysis (see Table 1). It was found that heart rate was (1) related directly to left ventricular output (slope, $1{\cdot}62 \pm 0{\cdot}25$); (2) related inversely to stroke volume (slope, $-0{\cdot}013 \pm 0{\cdot}001$); (3) likewise inversely related to end-diastolic dimension (slope, $-0{\cdot}014 \pm 0{\cdot}004$), and (4) had no significant effect on percentage fractional shortening (slope, $0{\cdot}006 \pm 0{\cdot}009$). These effects of rate on left ventricular output are consistent with those of Rudolph & Heymann (1976), and the effects on stroke volume and end-diastolic dimension are in agreement with those of Kirkpatrick *et al.* (1976).

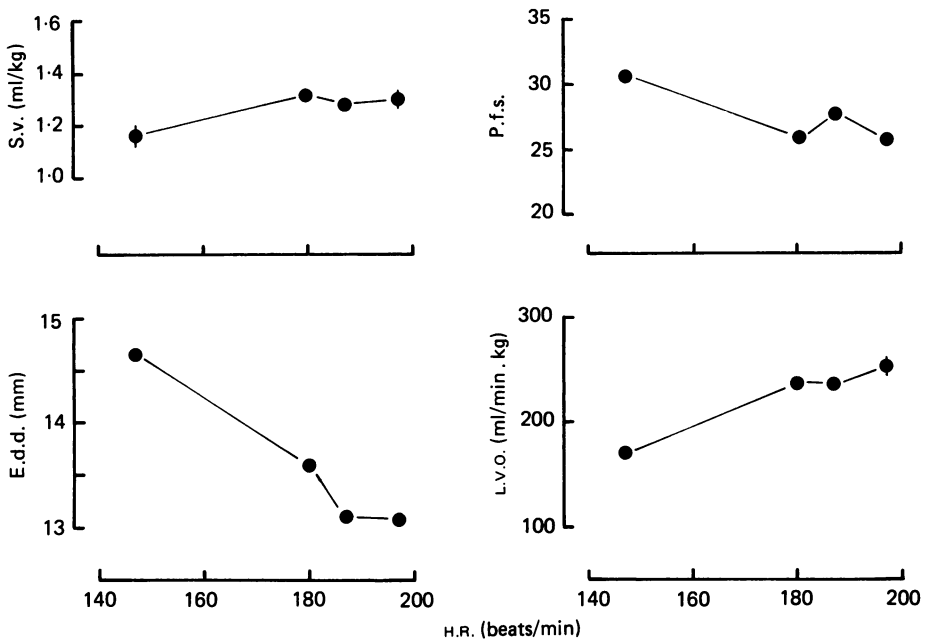

Fig. 2. The effects of heart rate (H.R.) during spontaneous rhythm. In this example obtained from a lamb 8 days post-surgery, end-diastolic dimension (e.d.d.) fell with an increase in rate while stroke volume (s.v., corrected for body weight) increased. The increase in stroke volume and rate resulted in a marked increase in left ventricular output (L.V.O., corrected for body weight). Percent fractional shortening (p.f.s.):

$$\left(\frac{[\text{end-diastolic dimension} - \text{end-systolic dimension}]}{\text{end-diastolic dimension}} \times 100\,\%\right)$$

fell with an increase in rate despite the increase in stroke volume. This demonstrates a limitation in using systolic excursion of the left ventricular minor axis to characterize stroke volume during spontaneous rhythm. The vertical bars represent two standard deviations of the mean.

When the responses in an individual lamb to changes of rate were examined, the extent of such effects was found to vary. Stroke volume, for example, could be related positively or negatively to a change in rate, or unaffected by it (see Fig. 2). It was usually the case for stroke volume to be smallest at the highest rate.

The effects of a spontaneous variation in rate on end-diastolic dimension differed from those found during pacing. Rate and end-diastolic dimension were usually

inversely related, as in Table 1. In the example illustrated in Fig. 2, stroke volume was larger at the higher rate, despite the large decrease in end-diastolic dimension at the faster rate. This larger stroke volume from a smaller end-diastolic volume demonstrated an enhancement of contractility at the faster rate which was greater than the usual rate-induced increase in contractility (for example in Fig. 6). On other occasions, the end-diastolic dimension remained unchanged or, rarely, was larger at the faster rates. A constancy of dimension at different rates implies that venous return was increased during the faster rate in order for there to have been a similar amount of ventricular filling despite a shorter diastole. (Compare the atrial pacing data in Figs. 3 and 4). This interaction of changes in venous return and contractility resulted in percentage fractional shortening being unaffected by rate.

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

Rhythm mode	Effect on left ventricular:			
	S.v.	E.d.d.	P.f.s.	Output
Spontaneous	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}94$	Decrease $P < 0{\cdot}0002$ $r = 0{\cdot}98$	N.s. $P \leqslant 0{\cdot}53$ $r = 0{\cdot}88$	Increase $P < 0{\cdot}0001$ $r = 0{\cdot}95$
Right atrial pacing	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}94$	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}94$	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}85$	N.s. $P \leqslant 0{\cdot}49$ $r = 0{\cdot}93$
Left atrial pacing	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}94$	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}98$	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}87$	Decrease $P < 0{\cdot}0001$ $r = 0{\cdot}88$

S.v., stroke volume; e.d.d., end-diastolic dimension; p.f.s., percentage fractional shortening; n.s., not significant.

The effect on ventricular output of changes in stroke volume and rate in nineteen observations was found to be (1) an increase in fourteen observations, in agreement with the findings of Rudolph & Heymann (1976); (2) no change in four observations, demonstrating the relation reported by Kirkpatrick *et al.* (1976) in which rate-induced changes in stroke volume and the extent of rate change were so matched as to produce no change in output; and (3) a decrease in one observation. In this observation, the fall in stroke volume and the fall in end-diastolic dimension associated with the rate increase were so large that output fell whilst arterial diastolic pressure remained unchanged. When all of the responses for the group were analysed, heart rate was related directly and with statistical significance ($P < 0{\cdot}0001$) to left ventricular output (see Table 1).

Effects of heart rate during atrial pacing

Atrial pacing enabled us to examine, independently of those stimuli which induced spontaneous variations in heart rate, how heart rate alters stroke volume, end-diastolic volume, percentage fractional shortening, and left ventricular output.

Right atrial pacing. Altering heart rate by means of right atrial pacing had a more consistent effect on ventricular performance than did spontaneous variations in heart rate. When data from all of the individual pacing studies were examined as a group

by multivariate analysis, heart rate was found to correlate negatively with stroke volume, end-diastolic dimension, and percentage fractional shortening (Fig. 1). The slopes were $-0{\cdot}012 \pm 0{\cdot}0007$, $-0{\cdot}026 \pm 0{\cdot}003$, and $-0{\cdot}115 \pm 0{\cdot}009$ respectively, with $P < 0{\cdot}0001$. Because of the balance achieved between the extent of the change in rate and the stroke volume, left ventricular output was not significantly altered by a change in rate, as shown in Table 1.

An example of normalized responses for the entire group for pacing rates of 182 to 300 beats/min is shown in Fig. 3.

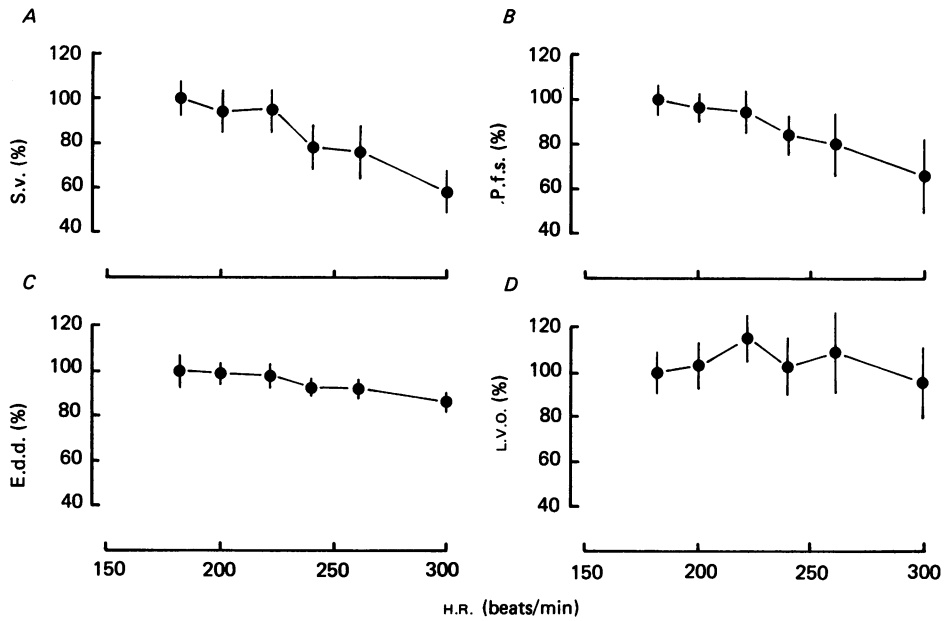

Fig. 3. Effects of right atrial pacing in the lambs as a group. Values are expressed as a percentage of the value obtained at the slowest rate, the latter being considered as 100%. The paced rates range from 182 to 300 beats/min. *A* shows the percentage change in stroke volume (s.v.); *B*, the percentage change in percent fractional shortening (p.f.s.); *C*, the percentage change in end-diastolic dimension (e.d.d.); and *D*, the percentage change in left ventricular output (L.V.O.). The vertical bars represent two standard deviations of the mean.

In each individual study, heart rate correlated negatively with stroke volume, end-diastolic dimension, and percentage fractional shortening, although the extent to which these variables were affected varied from study to study.

The extent to which stroke volume was altered determined whether the variation in output was inverse, direct, or negligible. The extent of changes in left ventricular output in the individual pacing studies were therefore analysed further. It was found that some lambs exhibited an increase in output, the greatest instance being 38%; but in others there was either no increase or a fall in output for all rates above the slowest paced rate. Among those lambs whose output did increase, the rate of pacing at which the maximum output obtained was found to vary from animal to animal: e.g. 200 beats/min in one and 260 beats/min in another. There was also a day-to-day

variation in the same animal such that on one day an increase might be found, but on another day no effect of rate on output would be seen as end-diastolic dimension and stroke volume were maximally affected by rate.

Left atrial pacing. Multivariate analysis of the response of the lambs as a group to left atrial pacing revealed that, as in right atrial pacing, rate correlated negatively with stroke volume, end-diastolic dimension, and percentage fractional shortening (see Table 1). (The slopes were $-0{\cdot}017 \pm 0{\cdot}003$, $-0{\cdot}020 \pm 0{\cdot}002$, and $-0{\cdot}076 \pm 0{\cdot}007$, respectively with $P < 0{\cdot}0001$.) The effect of rate on stroke volume was very great at faster rates; consequently, the data obtained during rates of 200 beats/min or more were so weighted as to yield an inverse relation between rate and left ventricular output.

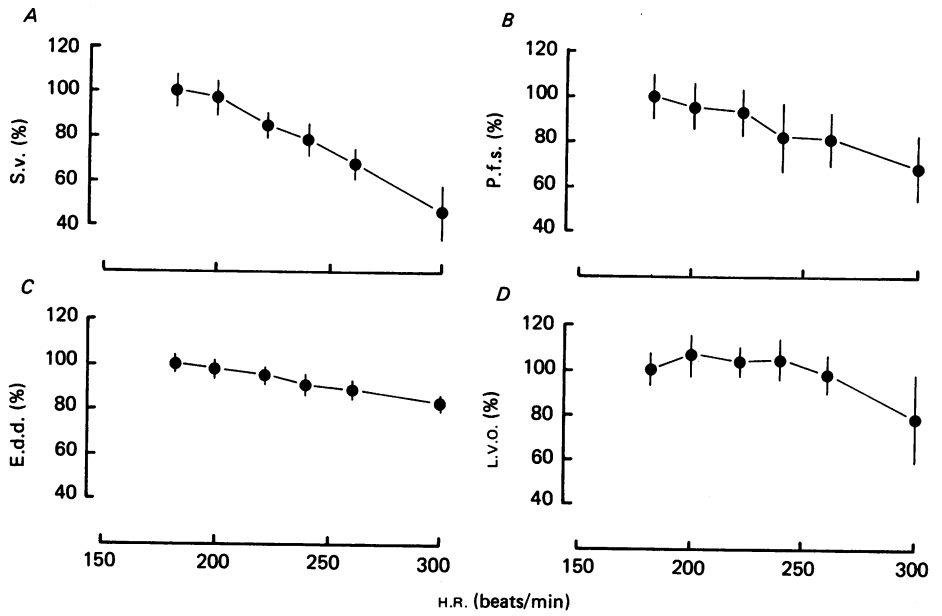

Fig. 4. Effects of left atrial pacing in the lambs as a group. See legend to Fig. 2 for a description of the variables. The paced rates range from 182 to 300 beats/min.

Fig. 4 shows the effects of left atrial pacing on ventricular filling and ejection. The data are from those pacing studies in which the lower and upper limits of pace rate were 182 and 300 beats/min, the same range used by Kirkpatrick *et al.* (1976), and are normalized. Although left ventricular output fell significantly with an increase in rate in the multivariate analysis of all pacing studies, the fall was not significant in this subgroup of studies. The absence of an effect on output is the same as that described for left atrial pacing in general by Rudolph & Heymann (1976) and Kirkpatrick *et al.* (1976).

When individual pacing studies were analysed singly, rate was found in all cases to be correlated negatively with stroke volume, end-diastolic dimension, and percentage fractional shortening. The extent of the decrease was, however, variable: consequently, in some studies, output did not vary significantly with changes in rate, but in others output was related inversely to rate. When the extent to which output

increased during individual studies was examined, in no instance was an increase greater than 11 % found for any given rate.

The effect of modulating end-diastolic volume by varying diastolic filling time

When heart rate was being controlled by atrial pacing, longer paced intervals could be introduced from time to time. This allowed longer periods for ventricular filling, and allowed us to examine systoles which occurred during periods of different pacing rates, yet had the same end-diastolic dimension (see Fig. 5). The controlled duration of the interval permitted aortic diastolic pressure during each individual study to be the same before each one of these systoles. By this means, the effect of heart rate on contractility could be examined free of the effects of end-diastolic volume and aortic pressure. Furthermore, the effects of a rate-induced fall in end-diastolic dimension could be assessed.

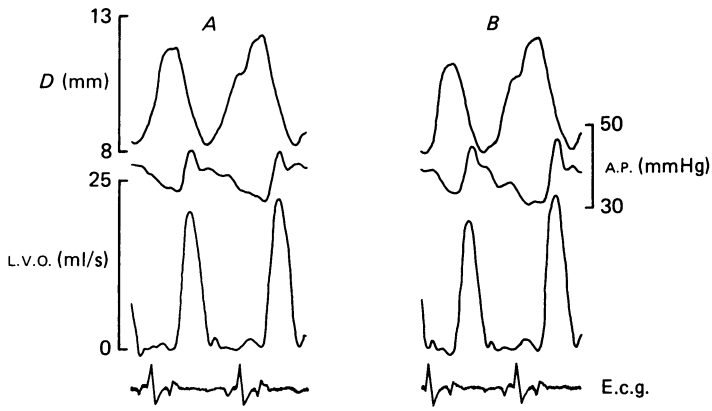

Fig. 5. Effects of introducing into the paced rate a longer paced interval. In *A*, the first systole is in response to a paced rate of 240/min (pacing interval 250 ms), the second systole follows a paced interval of 400 ms. In *B*, the first systole is in response to a paced interval of 280/min (paced interval 215 ms), and the second systole again follows a paced interval of 400 ms. Note that at the faster rate the end-diastolic dimension and stroke volume are smallest. Following the 400 ms interval, stroke volume is larger following the faster paced rate. The right atrium is being paced. From the top of the illustration down are left ventricular minor axis dimension (*D*), aortic pressure (A.P.), ascending aortic flow (L.V.O.) and electrocardiogram (e.c.g.).

Fig. 6 shows the effects of heart rate on systoles before and after such a longer paced interval. For the systoles with equal end-diastolic dimensions, stroke volume was found to increase significantly with faster preceding paced rates. But for those preceding systoles whose end-diastolic dimension was allowed to decrease, the stroke volume became progressively smaller as rate was increased. If such rate-induced changes in end-diastolic dimension could be avoided, left ventricular output would indeed increase markedly with an increase in rate. These effects on stroke volume and their potential effects on output were the same for pacing from the left and the right atrium.

The increase in stroke volume with increased pacing rate for systoles with equal

end-diastolic dimensions demonstrates that there is a rate-induced enhancement of contractility which exists in addition to the influence of end-diastolic dimension on stroke volume.

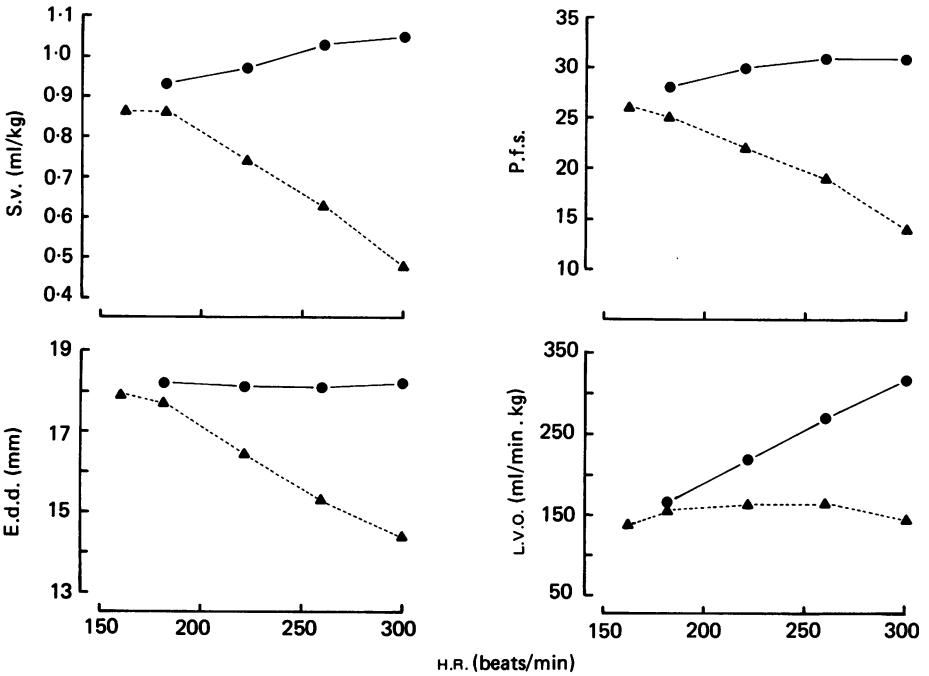

Fig. 6. A comparison of ventricular filling and ejection during right atrial pacing for the systoles during pacing rates from 150 to 300/min (▲), and the subsequent systoles (●, see Fig. 5) that follow the introduction of a longer paced interval (400 ms). Left ventricular end-diastolic dimensions (e.d.d.) following the longer paced intervals were within 0·1 mm while the dimensions during continuous pacing at a constant rate became progressively smaller with each increase in rate. For the systoles with comparable end-diastolic dimensions (●), stroke volume (s.v., corrected for body weight), percent fractional shortening (p.f.s.), and left ventricular output (L.V.O., corrected for body weight) were larger the faster the preceding paced rate. The data were obtained from a lamb 10 days following surgery.

DISCUSSION

The relation between heart rate and left ventricular output appears to change with development from fetus to adult. Ventricular output in adult animals and man is unaltered over a broad range of heart rates (Ross, Linhart & Braunwald, 1965; Sugimoto, Sagawa & Guyton, 1966). In the fetus, however, a positive relation has been reported by Rudolph & Heymann (1976). The range of changes in ventricular end-diastolic dimension and stroke volume that we have observed during spontaneous variations in heart rate suggests that the underlying processes which cause changes in heart rate must have effects on fetal haemodynamics which are additional to rate-induced ones. In order to separate the effect attributable to rate from other effects, we, like Rudolph & Heymann (1976) and Kirkpatrick *et al.* (1976), controlled heart rate by atrial pacing.

Relation of stroke and end-diastolic volumes to rate

The effects of heart rate on stroke volume and on end-diastolic volume during right and left atrial pacing appeared to be straightforward: the shorter the pacing interval, the shorter the diastole and the smaller the ventricular end-diastolic dimension and stroke volume. To clarify the association between heart rate and left ventricular output, we examined the effects of altering end-diastolic dimension. This manipulation revealed that it is the *extent* of change in end-diastolic dimension which determines, in large part, whether and in what manner left ventricular output will be related to heart rate. Such interaction can account for much of the conflict among the conclusions reached in previous fetal studies.

In our examination of individual pacing studies, our observations of the effects of heart rate during left atrial pacing are in agreement with those of Kirkpatrick *et al.* (1976) or Rudolph & Heymann (1976). The extent of the effect of rate on stroke volume which we observed in some lambs led us to the same conclusion as that of Kirkpatrick *et al.* (1976): namely, that heart rate has no significant effect on left ventricular output. But when we analysed the responses of all of the lambs in all of the left atrial pacing experiments, we found that heart rate and left ventricular output were inversely related. These findings are similar to some which had been described by Rudolph & Heymann (1976), who also found an inverse relation between heart rate and output in some experiments. Such changes in output are a result of the large effects of rate on stroke volume, which are associated with large changes in end-diastolic volume. This association suggests that stroke volume must in large part depend on end-diastolic volume.

Kirkpatrick *et al.* (1976) also related the significant changes in end-diastolic volume to changes in stroke volume. Their observation is consistent with the hypothesis that in the fetus, stroke volume is modulated by end-diastolic volume over a physiological range of filling pressures. We sought to elucidate the relation between stroke volume and end-diastolic volume by the introduction of longer paced intervals during different pacing rates. This allowed the left ventricle to fill to the same end-diastolic dimension for systoles which had been immediately preceded by different paced rates. When this was done, the prominent rate-related changes in stroke volume were eliminated. This demonstrates that a large part of the effect of rate on stroke volume is due to the contraction of the left ventricle from different end-diastolic volumes at different rates. These findings furthermore confirm the concluson of Kirkpatrick *et al.* (1976) that, over a physiological range of filling pressures, end-diastolic volume is an important modulator of fetal stroke volume.

Interactions of rate with stroke volume and end-diastolic volumes

During right atrial pacing, as during left atrial pacing, the interaction of rate with end-diastolic volume and stroke volume was a major determinant of left ventricular output. The range of such interaction was greater in the case of right atrial pacing, and so a wider range of effects on left ventricular output was observed: positive, negative, or no effect. These effects, in fact, varied from day to day in the same lamb. It is reasonable to suppose that such variability during right atrial pacing might be the reason we found no effect of rate on output whilst Rudolph & Heymann

(1976) found a positive one. Differences in the level of venous return from one experiment to another will alter the extent to which rate changes end-diastolic dimension, and can yield different relations between rate and output.

Another, and more likely, reason for our finding no effect where Rudolph & Heymann (1976) did, is to be found in the differences in how we each report our studies. Rudolph & Heymann observed that an increase in left ventricular output occurred, but they did not present the responses of the group nor the extent of the change in ventricular output. The latter is of particular importance, for although we found in some lambs that output increased as much as 10% over the output at the slowest rate, we did not consider this amount of change to be significant. Perhaps Rudolph & Heymann would have.

Our analysis is consistent with that of Rudolph & Heymann (1976) to the extent that right atrial pacing is able to produce a positive relation in the fetus between heart rate and left ventricular output. In general, however, we found no such positive relation when we used right atrial pacing. An absence of such an effect during right atrial pacing is also found in the adult animal. Studies in the adult animal (Sugimoto *et al.* 1966; Cowley & Guyton, 1971) and in the intact human (Ross *et al.* 1965) demonstrate that ventricular output is unaffected over a broad range of rates. At the highest rates, in fact, output decreases. These effects on output in the adult likewise reflect an interaction between the extent of the rate change and its effects on stroke volume. Thus, the present study demonstrates that there is no developmental change in the relation between heart rate and left ventricular output from late gestation through adulthood.

Our finding that changes in end-diastolic volume are an important factor in the changes in stroke volume and left ventricular output with changes in rate is consistent with the results of studies in the adult. In the dog, for example, stroke volume falls when end-diastolic volume is allowed to decrease during an increase in right atrial pacing. But if end-diastolic volume is maintained during increases in rate, the left ventricular output increases markedly. This was demonstrated by Sugimoto *et al.* (1966) using volume infusions and atrial pacing. In another study of the adult dog, a fistula was placed between the aorta and the pulmonary artery (Cowley & Guyton, 1971). When the fistula was open, left ventricular output continued to increase with an increase in heart rate; but when it was closed, output remained constant. The increase in left atrial venous return secondary to the patent fistula allowed stroke volume to be maintained or to increase as heart rate was increased.

The absence of qualitative change in the relations among heart rate, end-diastolic dimension, and stroke volume during development suggests that these interrelations may be utilized after birth by the left ventricle to increase its output. This would be achieved by interactions among (1) neonatal increase in venous return to the left atrium (Dawes, 1968); (2) the larger end-diastolic dimension of the neonatal left ventricle as against that of the fetus at the same heart rate (Anderson *et al.* 1982, 1984); (3) the neonatal increase in spontaneous heart rate (Comline & Silver, 1972; Anderson *et al.* 1982, 1984); and (4) the neonatal increase in circulatory catecholamines (Geis, Tatooles, Priola & Friedman, 1975; Eliot, Lam, Leake, Hobel & Fisher, 1980). A marked increase in left ventricular output is in fact observed in the new-born (Woods *et al.* 1978; Lister *et al.* 1979; Breall *et al.* 1984).

Changes in contractility

Another way in which heart rate may alter left ventricular output is through a change in contractility. Previous studies by Kirkpatrick, Naliboff, Pitlick & Friedman (1975), Anderson, Manring & Crenshaw (1980*a*, *b*) and Anderson *et al.* (1982) have demonstrated that, in both the isolated fetal myocardium and in the intact fetal left ventricle, a positive relation exists between contractility and heart rate, similar to that observed in the intact adult animal (Mahler, Yoran & Ross, 1974). When we compared the systoles from equal end-diastolic volumes following different pacing rates, we found that faster pacing evoked greater stroke volumes. Such an increase in stroke volume demonstrates, again, that rate has a positive effect on contractility. This effect is, however, masked when venous return is not altered from one rate to another, thus allowing end-diastolic volume to change with rate.

Effects of pacing site

Our study confirms the important influence of the site of atrial pacing on the observed quantities which was described by Rudolph & Heymann (1976). The differential effect of pacing site on left ventricular output may have several causes.

Rudolph & Heymann suggest that different pacing sites change the phasic pressure relation between the atria; this implies that pre-load, and therefore end-diastolic volume, is altered. We observed that end-diastolic dimension was usually greater with right atrial pacing than it was with left atrial pacing, which may be a consequence of different pacing sites. A second reason could be the existence of an interaction between the right and left ventricles. This has been observed in the adult animal (Laks, Garner & Swan, 1967; Taylor, Covell, Sonnenblick & Ross, 1967; Oboler, Keefe, Gaasch, Banas & Levine, 1973; Santamore, Lynch, Meier, Heckman & Bove, 1976): e.g. a greater filling of one ventricle interferes with the systolic contraction of the other. Such an interaction would, indeed, be expected to be more prominent in the fetus than in the adult because of the developmental change in right and left ventricular compliances (Romero & Friedman, 1979). A third explanation may lie in the timing of the atrial contraction and its effects on presystolic stretching of the ventricle and on the subsequent contraction (Linden & Mitchell, 1960; Brady, 1965). With left atrial pacing, there is a longer interval between atrial systole and ventricular systole than there is with right atrial pacing. This interval increases progressively at higher rates, perhaps as a result of changes in conduction velocity. Ultimately, the appropriate timing between these two mechanical events could be lost, and left ventricular contraction affected.

Although all of these effects may contribute to the observed differences between right and left atrial pacing, the major effect appears to be the differences in ventricular filling. The evidence for this is that allowing the left ventricle to fill to the same end-diastolic dimension, by the introduction of a longer paced interval, eliminates the differential effect of the atrial pacing site.

Spontaneous variations in rate

The effects of spontaneous variations of heart rate on stroke volume, and thus on left ventricular output, are more complicated than those observed when the heart

rate is controlled by atrial pacing. During pacing, stroke volume and end-diastolic dimension are always inversely related to rate. We found this to be the case during spontaneous variations in heart rate for the lambs as a group, but we also observed many individual exceptions. Such exceptions reveal that contractility can be enhanced out of proportion to what is seen with atrial pacing at the same rate: e.g. larger stroke volume at a smaller end-diastolic dimension. In addition, venous return can be increased so that end-diastolic dimension does not fall with an increase in rate. In view of these changes in contractility and end-diastolic volume which occur during a spontaneous change in rhythm, it is not surprising to find that left ventricular output is positively correlated with rate.

Summary

It is for these reasons – and in particular, the complex interactions of end-diastolic volume and contractility – that we found for the group of lambs as a whole responses to natural variations in rate which agree with those described by Rudolph & Heymann (1976), yet found in some individual studies no significant change in left ventricular output with spontaneous changes in heart rate. The latter instances agree with the findings reported by Kirkpatrick *et al.* (1976) for two fetal lambs. The range of responses in our lambs is thus in agreement with both of these prior studies, and indicates their commonality in the intact fetal lamb. Furthermore, our findings in the present study demonstrate that the relation between spontaneous changes in heart rate and left ventricular output are in general qualitatively the same as in the adult animal (Rushmer, 1959; Khouri, Gregg & Rayford, 1965), confirming the lack of developmental change from late gestation to the adult.

This study demonstrates that heart rate has a major effect on ventricular filling and stroke volume in the fetus. During spontaneous rate changes, superimposed alterations in venous return and contractility can have a prominent effect on output. The similarity of characteristics for the relation of rate to output in fetus and adult suggests that, at birth, when heart rate and venous return to the left atrium are increased, the left ventricle of the new-born can make use of these changes to produce the large neonatal increase in cardiac output.

The authors wish to thank Chris Brown and Annette Oakeley for their assistance in performing the experiments, analysing the data and illustrating it, Jeffrey Johnson for reviewing the manuscript, and Deborah Hudgins for preparing the manuscript. The work was supported in part by National Institutes of Health Grants HL28470, HL20749-07A1, HL33680, HL07063, and by funds provided by the American Heart Association and in part by the North Carolina Affiliate, Inc. Funds were provided by the Michael J. Synan Memorial Fund.

REFERENCES

ANDERSON, D. F., BISSONNETTE, J. M., FABER, J. J. & THORNBURG, K. L. (1981). Central shunt flows and pressures in the mature fetal lamb. *American Journal of Physiology* **241**, H60–66.

ANDERSON, P. A. W., GLICK, K. L., MANRING, A. & CRENSHAW JR, C. (1984). Developmental changes in cardiac contractility in the fetal and postnatal sheep: *In vitro* and *in vivo*. *American Journal of Physiology* **247**, H371–379.

ANDERSON, P. A. W., MANRING, A. & CRENSHAW JR, C. (1980*a*). Biophysics of the developing heart (I): The force–interval relationship. *American Journal of Obstetrics and Gynecology* **138**, 33–43.

ANDERSON, P. A. W., MANRING, A. & CRENSHAW JR, C. (1980*b*). Biophysics of the developing heart (II): The interaction of the force–interval relationship with inotropic state and muscle length (preload). *American Journal of Obstetrics and Gynecology* **138**, 44–54.

ANDERSON, P. A. W., MANRING, A., GLICK, K. L. & CRENSHAW JR, C. (1982). Biophysics of the developing heart (III): A comparison of the left ventricular dynamics of the fetal and neonatal lamb heart. *American Journal of Obstetrics and Gynecology* **143**(2), 195–203.

BISHOP, V. S. & HORWITZ, L. D. (1970). Left ventricular transverse internal diameter: Value in studying left ventricular function. *American Heart Journal* **80**, 507–514.

BOETTCHER, D. H., VATNER, S. F., HEYNDRICKX, G. R. & BRAUNWALD, E. (1978). Extent of utilization of the Frank–Starling mechanism in conscious dogs. *American Journal of Physiology* **234**, H338–345.

BRADY, A. J. (1965). Time and displacement dependence of cardiac contractility: Problems in defining the active state and force–velocity relations. *Federation Proceedings* **24**, 1410–1420.

BREALL, J. A., RUDOLPH, A. M. & HEYMANN, M. A. (1984). Role of thyroid hormone in postnatal circulatory and metabolic adjustments. *Journal of Clinical Investigation* **73**, 1418–1424.

COMLINE, R. S. & SILVER, M. (1972). The composition of foetal and maternal blood during parturition in the ewe. *Journal of Physiology* **222**, 233–256.

COWLEY, A. W. & GUYTON, A. C. (1971). Heart rate as a determinant of cardiac output in dogs with arteriovenous fistula. *American Journal of Cardiology* **28**, 321–325.

DAWES, G. S. (1968). Changes in the circulation after birth. In *Foetal and Neonatal Physiology; A comparative study of the changes at birth*, pp. 160–176. Chicago: Year Book Publishers, Inc.

ELIOT, R. J., LAM, R., LEAKE, R. D., HOBEL, C. J. & FISHER, D. A. (1980). Plasma catecholamine concentrations in infants at birth and during the first 48 hours of life. *Journal of Pediatrics* **96**, 311–315.

GEIS, W. P., TATOOLES, C. J., PRIOLA, D. V. & FRIEDMAN, W. F. (1975). Factors influencing neurohumoral control of the heart in the newborn dog. *American Journal of Physiology* **228**, 1685.

HEYMANN, M. A., CREASY, R. K. & RUDOLPH, A. M. (1973). Quantitation of blood flow patterns in the foetal lamb in utero. In *Proceedings of the Sir Joseph Barcroft Centenary Symposium: Foetal and Neonatal Physiology*, pp. 129–135. Cambridge: Cambridge University Press.

HEYMANN, M. A. & RUDOLPH, A. M. (1973). Effects of increasing preload on right ventricular output in fetal lambs in utero. *Circulation* **48**, suppl. IV, 37.

KHOURI, E. M., GREGG, D. E. & RAYFORD, C. R. (1965). Effect of exercise on cardiac output, left coronary flow and myocardial metabolism in the unanesthetized dog. *Circulation Research* **17**, 427–437.

KIRKPATRICK, S. E., COVELL, J. W. & FRIEDMAN, W. F. (1973). A new technique for the continuous assessment of fetal and neonatal cardiac performance. *American Journal of Obstetrics and Gynecology* **116**, 963–972.

KIRKPATRICK, S. E., NALIBOFF, J., PITLICK, P. T. & FRIEDMAN, W. F. (1975). Influence of post-stimulation potentiation and heart rate on the fetal lamb heart. *American Journal of Physiology* **229**, 318–323.

KIRKPATRICK, S. E., PITLICK, P. T., NALIBOFF, J. & FRIEDMAN, W. F. (1976). Frank–Starling relationship as an important determinant of fetal cardiac output. *American Journal of Physiology* **231**, 495–500.

LAKS, M. M., GARNER, D. & SWAN, H. J. C. (1967). Volumes and compliances measured simultaneously in the right and left ventricles of the dog. *Circulation Research* **20**, 565–569.

LINDEN, R. J. & MITCHELL, J. H. (1960). Relation between left ventricular diastolic pressure and myocardial segment length and observations on the contribution of atrial systole. *Circulation Research* **8**, 1092–1099.

LISTER, G., WALTER, T. K., VERSMOLD, H. T., DALLMAN, P. R. & RUDOLPH, A. M. (1979). Oxygen delivery in lambs: Cardiovascular and hematologic development. *American Journal of Physiology* **237**, H668–675.

MAHLER, F., YORAN, C. & ROSS JR, J. (1974). Inotropic effect of tachycardia and poststimulation potentiation in the conscious dog. *American Journal of Physiology* **227**, 569–575.

OBOLER, A. A., KEEFE, J. F., GAASCH, W. H., BANAS JR, J. S. & LEVINE, H. J. (1973). Influence of left ventricular isovolumic pressure upon right ventricular pressure transients. *Cardiology* **58**, 32–44.

PITLICK, P. T., KIRKPATRICK, S. E. & FRIEDMAN, W. F. (1976). Distribution of fetal cardiac output: Importance of pacemaker location. *American Journal of Physiology* **231**, 204–208.

ROMERO, T. E. & FRIEDMAN, W. F. (1979). Limited left ventricular response to volume overload in the neonatal period: A comparative study with the adult animal. *Pediatric Research* **13**, 910–915.

ROSS JR, J., LINHART, J. W. & BRAUNWALD, E. (1965). Effects of changing heart rate in man by electrical stimulation of the right atrium. Studies at rest, during exercise, and with isoproterenol. *Circulation* **32**, 549–558.

RUDOLPH, A. M. & HEYMANN, M. A. (1970). Circulatory changes during growth in the fetal lamb. *Circulation Research* **26**, 289–299.

RUDOLPH, A. M. & HEYMANN, M. A. (1976). Cardiac output in the fetal lamb: The effects of spontaneous and induced changes of heart rate on right and left ventricular output. *American Journal of Obstetrics and Gynecology* **124**, 183–192.

RUSHMER, R. F. (1959). Constancy of stroke volume in ventricular responses to exertion. *American Journal of Physiology* **196**, 745–750.

SANTAMORE, W. P., LYNCH, P. R., MEIER, G., HECKMAN, J. & BOVE, A. A. (1976). Myocardial interaction between ventricles. *Journal of Applied Physiology* **41**, 362–368.

SODUMS, M. T., BADKE, F. R., STARLING, M. R., LITTLE, W. C. & O'ROURKE, R. A. (1984). Evaluation of left ventricular contractile performance utilizing end-systolic pressure–volume relationships in conscious dogs. *Circulation Research* **54**, 731–739.

SUGIMOTO, T., SAGAWA, K. & GUYTON, A. C. (1966). Effect of tachycardia on cardiac output during normal and increased venous return. *American Journal of Physiology* **211**, 288–292.

TAYLOR, R. R., COVELL, J. W., SONNENBLICK, E. H. & ROSS JR, J. (1967). Dependence of ventricular distensibility on filling of the opposite ventricle. *American Journal of Physiology* **213**, 711–718.

WOODS JR, J. R., DANDAVINO, A., BRINKMAN III, C. R., NUWAYHID, B. & ASSALI, N. S. (1978). Cardiac output changes during neonatal growth. *American Journal of Physiology* **234**, H520–524.