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The development of myocardial performance in lambs

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THE DEVELOPMENT OF MYOCARDIAL PERFORMANCE IN LAMBS

by

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DISSERTATION

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ABSTRACT

THE DEVELOPMENT OF MYOCARDIAL PERFORMANCE IN LAMBS

To evaluate postnatal development of myocardial performance in lambs, mean left atrial pressure was increased in a graded manner by venous infusions of 0.9% NaCl solution at body temperature in three groups of unanesthetized atropinized (0.2 mg/kg) lambs. Various measuring devices had previously been chronically implanted in each lamb. Under general anesthesia a precalibrated electromagnetic flow transducer had been placed around the ascending aorta, and catheters had been inserted into the left atrium, left ventricle, aortic arch, a peripheral vein and a large catheter for rapid infusion of saline had been introduced into the superior vena cava. Five lambs were prepared in this way 2-3 days after birth, five at 18-24 days and five at 38-42 days. After recovery from surgery for two days, daily two minute infusions of saline solution at a rate of 25 ml/kg/minute were given to each lamb for seven days. The maximum mean flow/kg observed during infusion was taken as a measure of myocardial performance in response to this uniform stress. Since histological studies of the lamb myocardium have shown that sympathetic innervation is not complete at birth, some of these infusions were carried out during beta adrenergic stimulation

by continuous isoproterenol administration at a rate of 1 ug/kg/min and some were carried out after beta adrenergic blockade with propranolol in a dosage of 1 mg/kg. Each lamb underwent at least two experiments of each kind. The lambs were all healthy, gained weight normally throughout the experiments, and had normal resting arterial blood gases. The results are summarized in the following table:

Average Age (days)		8	26	45
A = resting	ml/kg	425	355	147
B = after atropine	ml/kg	430	393	164
C = B + isoproterenol	ml/kg	553	570	265
D = B + propranolol	ml/kg	382	362	150
E = B + infusion	ml/kg	577	622	259
F = C + infusion	ml/kg	671	855	413
G = D + infusion	ml/kg	418	441	191
% increase of E over B		34.1	60.5	57.5
% increase of F over C		20.2	50.7	55.7
% increase of G over D		8.9	23.3	25.3

The most striking finding is that aortic flow relative to body weight fell progressively to about one-third of its initial level. This fall in flow/kg was associated with a consistent drop in resting heart rate and between 26 and 45 days after birth with a decrease in stroke volume/kg. The purpose of this high resting cardiac output/kg in one week old lambs is not clear. Such high flows/kg could be necessary to provide the greater oxygen supply needed by their tissues. It is possible that extraction of oxygen from the blood by the tissues is not as effective in the young lamb so that a greater tissue flow is needed. As a result of volume loading all three groups of lambs reached similar mean left atrial pressures and their mean arterial blood pressures increased 15-20%. The percentage increase in mean flow/kg with volume loading rose significantly between 8 and 26 days of age (regardless of the level of beta adrenergic activity), but showed no change beyond that time. Lambs of each age were able to respond to increasing beta adrenergic stimulation by increasing aortic flow. Since (unlike the older lambs) one week old lambs show no increase in resting flow after atropine, they apparently have less resting parasympathetic tone. Notice that propranolol decreased pre-infusion flow/kg significantly only in the youngest group suggesting that they may be more dependent on catecholamine support then or that propranolol may directly depress the young myocardium.

These findings suggest that the young myocardium is capable of maintaining a high cardiac output but resting requirements are so high at this age that there is little reserve capacity to increase output for additional demands.

ACKNOWLEDGMENTS

The time that was spent in doing this work was an exciting time for me. I am deeply indebted to Dr. Abraham Rudolph for his patience, his guidance, and for providing a remarkably stimulating atmosphere in which to work.

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TABLE OF CONTENTS

Page

INTRODUCTION

In the past it had generally been assumed that, despite its smaller size, the heart of the newborn had a similar capacity to perform work as did that of the adult. This assumption was first tested by Downing (1) in acute studies on anesthetized, open-chested newborn lambs. Left ventricular function curves on these lambs suggested that their hearts performed similarly to those of adults, but that hypoxia and acidemia together led to less depression of myocardial contractility (2). Cross' (3) observation that, unlike adult sheep, anesthetized lambs showed no increase in cardiac output in response to hypoxia led Dawes (4) to suggest that the cardiac output of newborn lambs may already be maximal. Friedman (5) studied the development of sympathetic nervous system innervation in the rabbit heart and found that the density of sympathetic nerve fibers and myocardial norepinephrine stores developed gradually and did not reach adult levels until three weeks after birth. A similar pattern in lambs has been described by Lebowitz (6). In view of the poor sympathetic nervous system development he had found in the newborn heart and Spann's (7) work stressing the importance of norepinephrine release on myocardial performance, Friedman questioned Downing's findings. It is difficult to evaluate Downing's studies since they were all done on anesthetized animals who had just undergone extensive surgical procedures. There have been several other attempts to assess myocardial performance using a variety of different approaches. Hopkins (8) obtained isometric ventricular pressure-volume curves and length-tension curves from excised perfused rat hearts. He found that the hearts of 10 day old rats had smaller peak active tensions and peak passive tensions than did hearts from older rats, but by 16 days of age they had reached adult levels. Friedman (9) found that adult sheep papillary muscle had a greater velocity and extent of shortening at any tension than did that of the fetus. The isometric resting and active tension at any length was also greater in the adult sheep. It is interesting that this progressive functional development in the young heart is associated with striking structural changes. Hopkins (8) observed that the poorly organized sparse myofibrils of the newborn rat became as numerous and as well-organized as the adult by 16-20 days of age. From Friedman's (9) work it appears that this general pattern is also present in sheep. Interestingly, Friedman found that the sarcomeres themselves seem to be histologically and functionally identical in fetal and adult sheep.

Unfortunately, most of the information available has been obtained from anesthetized animals or isolated preparations. Recently, in this laboratory and elsewhere, it has been possible to study the circulation in the unanesthetized fetal lamb _in utero_ after recovery from surgery. Using this technique, Vapaavouri (10) has shown that associated with the late appearance of myocardial sympathetic nervous system innervation in the lamb is the continuing development of beta adrenergic function throughout late gestation. In the same preparation, Heymann (11) has recently reported that late gestation fetal lambs respond poorly to an acute volume overload as compared to adults. The reasons for this have not been delineated but it may be due to the limited degree of sympathetic nervous system innervation of the myocardium or to the limited structural organization of the myocardium at that age.

Except for studies made in the period immediately after birth, there have been no studies describing the circulatory changes with growth in the intact unanesthetized neonate. Furthermore, no information is available describing the response of the heart to stress in young animals. In view of this, I set out to measure the changes in aortic blood flow, heart rate, and blood pressure in intact unanesthetized lambs at various times after birth. Since the adequacy of sympathetic nervous system control of myocardial performance has been questioned, I also examined the response to the stress of volume loading at various levels of beta adrenergic activity. I chose to work with lambs to facilitate comparisons to work others have already done in sheep.

METHOD

It was crucial to my experiments that the method used to measure myocardial performance gave repeatable values in the same animal from day to day. Although ventricular function curves as described by Sarnoff (12) have value in measuring the effects of acute interventions on ventricular function, Bishop (13) has shown that they vary markedly from day to day. After numerous preliminary experiments I found the method developed by Bishop, Stone and Guyton (13) for the measurement of ventricular output curves to be the best for my purposes. Left ventricular output curves are measured by rapidly increasing mean left atrial pressure with a brief rapid infusion of fluid into the superior vena cava. Left ventricular output measured during this maneuver increases initially but soon reaches a plateau at a maximum value. Stone (14, 15) has shown that this maximum left ventricular output is a measure of cardiac function.

Material - I studied 15 lambs. Various measuring devices were implanted in five 2-3 days after birth, five at 18-24 days and five at 38-42 days.

Surgical Procedure - I gave each lamb 2-3% fluothane in oxygen to breathe until endotracheal intubation with a cuffed tube 5-6 mm ID was completed. Then I connected the endotracheal tube to a Harvard pump which supplied positive pressure ventilation at a rate of about 20 ml/kg body weight. The respiratory rate was adjusted to between 13 and 16 per minute and the end expiratory pressure was held at 2 cm water by placing the exhalation tube under water. The tidal volume ranged between 70 and 200 ml depending on the weight of the lamb. About every 30 minutes during the surgical procedure, ½ ml samples of arterial blood were withdrawn and pH, PCO_2 and PO_2 were measured using a microcuvette blood gas analysis system (manufactured by the Radiometer Company, Copenhagen, Denmark, model numbers PHM27b - gas monitor, E5021a - micro electrode unit, E5046-0 -O_2 electrode, E5036-0 - CO_2 electrode). The respiratory rate was varied as needed to maintain the arterial PCO_2 between 38 and 40 mm Hg.

In preliminary experiments I found that lambs who had received more anesthetic than was necessary to prevent movement during surgery recovered poorly. Consequently I adjusted the amount of fluothane given during surgery to the lowest level necessary to prevent movement (generally 0.25 to 0.75% in 100% oxygen) and supplemented this with local injections of 1% lidocaine HCl on the rare occasions when it was needed.

Using strict sterile precautions and taking special care to minimize bleeding, I incised skin and muscle to expose the left third and fourth ribs from the costochondral junctions to the posterior axillary line. I opened the pleura, retracted the ribs, inserted a polyvinyl catheter (0.038 inch ID, 0.066 inch OD) into the left internal mammary artery and advanced it to the arch of the aorta. A similar catheter was inserted into the left internal mammary vein. The apex of the left lung was retracted to expose the pericardium and great vessels. I incised the pericardium and lifted the left ventricle out of the pericardial sac. Through a purse string suture placed at the apex, I inserted a polyvinyl catheter (0.038 inch ID, 0.066 inch OD) a short distance into the left ventricular cavity. Through a similar purse string suture in the left atrium an identical catheter was advanced into the cavity of the left atrium.

In lambs the ascending aorta is a short segment which gives rise to a common brachiocephalic artery a short distance above the aortic valve. I carefully dissected the ascending aorta free of surrounding tissues and placed a Statham precalibrated electromagnetic flow transducer around it just distal to the origin of the coronary arteries. Then the edges of the pericardium were approximated but were not closed. The electromagnet flow transducers I have used are calibrated by the Statham Instrument Company prior to delivery. Using 0.9% NaCl solution we checked these calibrations and found them to be stable. Further calibrations were not routinely done before and after each flow transducer was used.

I placed a polyvinyl catheter (0.1 inch OD) into the pleural cavity, brought all the catheters and flow transducer leads out of the left third intercostal space and threaded them subcutaneously to a nylon pocket sewn to the lamb's left flank. I brought the ribs together with heavy silk sutures and closed the muscle and skin in layers to provide an airtight seal. Then I removed air and fluid from the chest by applying gentle suction to the tube in the pleural cavity. I made a separate small incision in the left side of the neck, inserted a polyvinyl catheter (0.015 inches OD) into the external jugular vein and advanced it to the superior vena cava. This catheter was also passed subcutaneously to exit at the left flank and all catheters were filled with an aqueous heparin solution (500 U.S.P. units/ml) and plugged. I kept the lambs warm and fed them a milk formula overnight, occasionally drained their chest tubes, and returned them to their mothers in the morning. I allowed each lamb to recover from surgery for two days before beginning infusion studies.

Recording Equipment - All pressures were recorded using Statham P23Db or P23Dc pressure transducers (Statham Instrument Company, Hato Rey, Puerto Rico). These transducers have a frequency response of 185 HZ, an error of ±2.25 mm Hg at 7.5 volt excitation, and a sensitivity of 50 microvolt/volt/cm Hg.

The Statham precalibrated electromagnet flow transducer was connected to a Statham SP 2202 blood flowmeter. This combination has a frequency response of 100 HZ, repeatability of ±2% and a percent error of ±5%.

The output from each of the above systems was led to a Beckman Dynograph twelve channel oscillographic recorder (Beckman Instrument Company, Shiller Park, Illinois). The frequency response for this instrument is 80 HZ, the drift 1 mm equivalent input per hour at maximum gain, and the percent error is ±2%. The combined pressure measurement and oscillographic systems have a frequency response of 10 HZ, and show essentially no drift over a 90 minute period of observation.

With this equipment I recorded arterial blood pressure, mean arterial blood pressure, mean left atrial pressure, left ventricular pressure, left ventricular end diastolic pressure, aortic flow and mean aortic flow. Coronary flow was assumed to represent a small portion of left ventricular output and was not measured. Heart rate was recorded continuously using a cardiotachometer triggered from the left ventricular pressure signal.

Collection of Control Information - After recovery from surgery, each lamb was placed in an open straw-filled box and any fluid that had accumulated in the pleural cavity was drained. Heparin solution was removed from all other catheters and they were filled with saline. Pressure transducers were placed just outside the lamb's box and the zero reference level was adjusted to a height estimated to be the same as that of the left atrium. Catheters from the left atrium, left ventricle and aortic arch were each connected to pressure transducers and the flow transducer was connected to the blood flowmeter. After all recorded variables were stable for at least 5 minutes, normal control data was recorded as the lamb lay quietly in the box. In preliminary experiments I observed frequent large spontaneous changes in heart rate and flow which were abolished when a bolus of atropine (0.2 mg/kg) was given through the internal mammary vein catheter. In order to have a more constant baseline for subsequent measurements, I atropinized each lamb after control data were recorded.

Infusion of Saline Alone - the large venous catheter was connected to the outflow from a Sarns pump model #3500 (Sarns Inc., Ann Arbor, Michigan). The pump had previously been prepared to deliver 25 ml/kg of body weight/minute of 0.9% NaCl solution at body temperature. About 5 minutes after atropinization as pressures and flow were being measured, I infused saline for two minutes. Before and after each saline infusion arterial blood gases were measured as described previously.

To evaluate the role of the autonomic nerve supply during development, I measured myocardial performance at increased and minimal levels of beta adrenergic stimulation. To do this I infused saline when the lamb was under the influence of isoproterenol and also when the lamb was under the influence of propranolol. These types of infusions are described in detail below.

Saline Infusion During Isoproterenol Infusion - In these experiments I began an infusion of isoproterenol (0.1 ug/kg/minute) into the internal mammary vein using a Harvard variable speed infusion/withdrawal pump (Harvard Apparatus Company, Inc., Dover, Mass., model 600-900V). The infusion was maintained throughout the entire experiment. When pressures and aortic flow again reached a steady state, I gave 0.2 mg/kg atropine into the internal mammary vein and the saline infusion procedure was followed as before.

Saline Infusion After Propranolol - Experiments carried out when the lamb was under the influence of propranolol were similar to those in which only saline was infused except that prior to giving atropine I injected a bolus of propranolol (1 mg/kg) into the internal mammary vein. It has been found by Vapaavouri (10) and Harry (16) that the action of propranolol in the dose range of 1-2 mg/kg is primarily that of beta blockade and has no direct myocardial depressant effect. I tested the effectiveness of beta blockade by injecting a $\frac{1}{2}$-1 ug bolus of isoproterenol several minutes after giving propranolol (and again at the conclusion of the infusion). If the injection of isoproterenol caused no change in heart rate, left ventricular pressure, or arterial blood pressure, I proceeded with atropinization and saline infusion as usual.

The response to saline infusion was studied on each of seven consecutive days. The sequence of saline infusion alone, saline infusion during infusion of isoproterenol and saline infusion after propranolol was repeated twice for each lamb. A third infusion of saline was given on the seventh day.

A $p < 0.05$ was used as the criterion of statistical significance. The unpaired "students" t test was used to compare corresponding populations from different age groups. Each lamb received 3 infusions of saline alone, 2 infusions while receiving isoproterenol, and 2 infusions after propranolol. To compare the effects of these various types of experiments within any group, the data from like experiments in a single lamb were pooled and the paired "students" t test was used.

RESULTS

Recovery - The lambs were allowed two days for recovery after surgery before daily infusions were begun. There are several reasons to believe that this allowed sufficient time for recovery.

1) The lambs were all healthy and, as shown in Figure 1 and Table 1, each group of lambs gained weight normally during the study.

2) The resting arterial pH, blood gases and hematocrit for each group are listed in Table 2. I have not been able to find reports in the literature describing what the normal

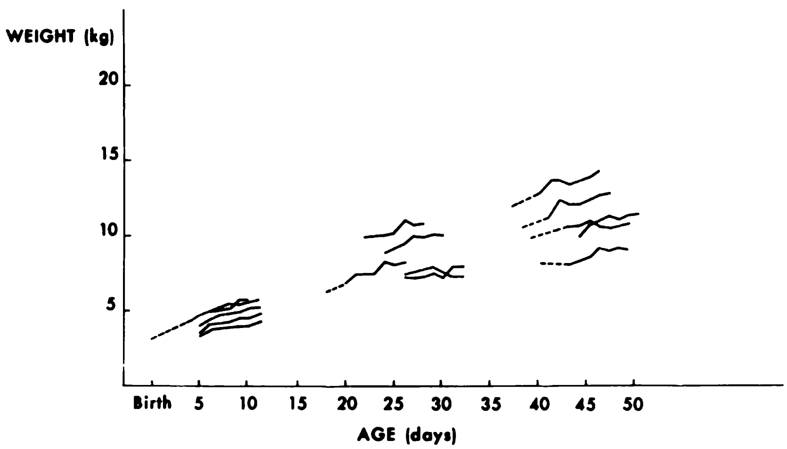


Figure 1. Each lamb was weighed before surgery and daily prior to each infusion study. Where present, dashed lines join a lamb's weight before surgery with his weight just prior to his first infusion. Solid lines join each lamb's daily weight.

Table 1. Normal values recorded in resting lambs of each age group. Most of this information is illustrated in Figures 2a and 2b.

	Group Two	Group Three
Mean Systemic Arterial Pressure (mm Hg)	71 (±8.01)	80.1 (±10.2)
Group One 69.2 (±6.23)	NS	S
Group Two		S
Heart Rate (beats/min)	157 (±28.9	141 (±26.0)
Group One 210 (±27.4)	S	S
Group Two		S
Mean Left Atrial Pressure (mm Hg)	5.6 (±2.0)	4.4 (±1.9)
Group One 6.4 (±3.1)	NS	S
Group Two		S
$\overline{Q}$ in Ascending Aorta (ml/min)	3030 (±910)	1650 (±278)
Group One 2000 (±488)	S	S
Group Two		S
$\overline{Q}$/kg in Ascending Aorta (ml/min/kg)	355 (±116)	147 (±28.4)
Group One 425 (±86.3)	S	S
Group Two		S
Mean SV/kg (ml/kg)	2.28 (±0.673)	1.08 (±0.286)
Group One 2.09 (±0.541)	NS	S
Group Two		S
Average Body Weight (kg)	8.67 (±1.32)	11.5 (±1.73)
Group One 4.7 (±0.65)	S	S
Group Two		S

Notation: Mean (±Standard Deviation)
S = statistically significant at the 0.05 level
NS = not statistically significant at the 0.05 level

Table 2. Normal values recorded on arterial blood from resting lambs of each age group.

		Group Two	Group Three
Ph		7.46 (±0.049)	7.45 (±0.036)
Group One	7.42 (±0.041)	S	S
Group Two			NS
PO_2 (mm Hg)		79.7 (±7.39)	81.3 (±9.07)
Group One	71.1 (±9.64)	S	S
Group Two			NS
PCO_2 (mm Hg)		38.8 (±5.62)	37 (±3.02)
Group One	40.9 (±4.79)	NS	S
Group Two			NS
Hematocrit (percent)		30.6 (±2.79)	31.8 (±3.73)
Group One	28.1 (±2.39)	S	S
Group Two			NS

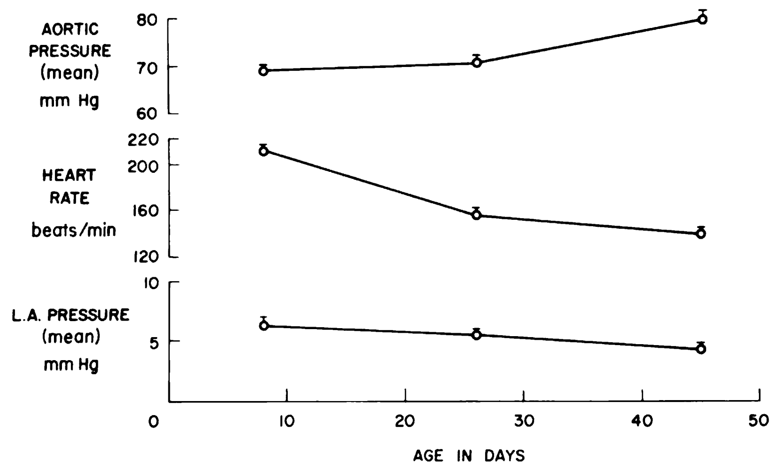
Notation: Mean (±Standard Deviation)
S = statistically significant at the 0.05 level
NS = not statistically significant at the 0.05 level

values are in resting, unanesthetized lambs of these ages. However, based on measurements that have been made on other lambs in our laboratory, the values in Table 2 are normal (17).

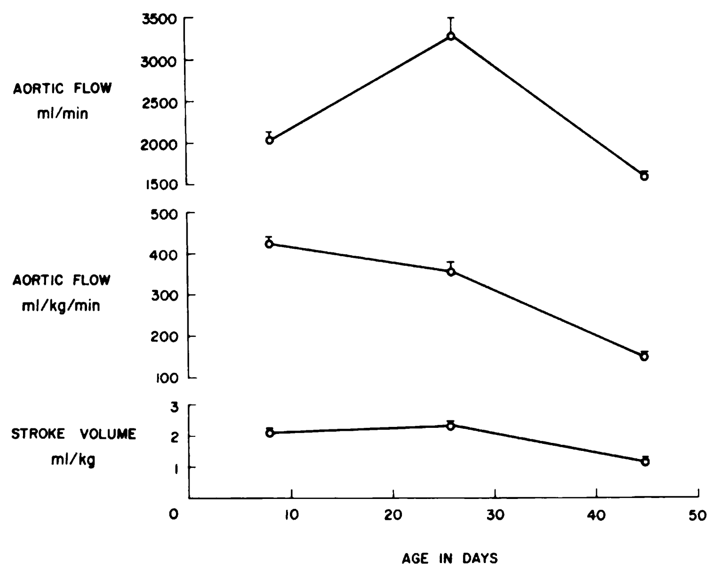
3) Resting output did not change significantly for any of the groups during the study. Since each type of infusions was repeated at least twice in each animal, it was also possible to compare maximum left ventricular output at various times after surgery. The maximum output observed during the infusion of saline alone three days after surgery was not significantly different than that observed six days later. Similarly, there was no change in the maximum output between the first and second experiments with isoproterenol or with propranolol.

Control Data - Observations in resting lambs before any drugs were given are shown in Table 1 and Figure 2. There were considerable changes in resting cardiovascular dynamics between lambs of these different ages. For each measurement shown in Figure 2 the mean and standard error are shown above the age of each group. The average age of group one was 8 days, of group two was 26 days and for group three it was 45 days. Mean arterial blood pressure increased significantly between 26 and 45 days while heart rate fell progressively from 8 to 45 days. The changes in mean left atrial pressure with growth were slight. Actual aortic

Figure 2. For each of these normal resting measurements, the mean plus one standard error of the mean are indicated above the average age of each group.



(a) Notice that while mean arterial blood pressure increased gradually, heart rate fell. Although mean left atrial pressure fell consistently the actual changes were small.



(b) The actual mean aortic flow increases dramatically and then falls. However, when expressed per kilogram of body weight, mean aortic flow falls dramatically to about one-third of its initial level. The rise in heart rate seen on the previous figure is associated with a fall in mean stroke volume per kilogram between 26 and 45 days of age.

flow increased between 8 and 26 days but then fell. The most striking finding was that aortic flow relative to body weight fell progressively to about one third its initial level. This fall in aortic flow/kg was due not only to a drop in resting heart rate but also to a significant decline in stroke volume/kg between 26 and 45 days.

Although resting arterial pH, PO_2 and hematocrit were significantly less in 8 day old lambs the actual differences were small. Resting arterial PCO_2 fell slightly between 8 and 45 days.

The General Effects of Infusion - As a result of infusion each group showed an increase in mean arterial blood pressure of 15-20% and similar mean left atrial pressures at maximum output.

I have measured the change in hematocrit during infusion in one of the older lambs (Figure 3). The lowest hematocrit in any of these infusions was 20%. If the blood volume in this lamb was 80 ml/kg and body weight was 10 kg, his total blood volume would have been 800 ml. The infusion increased this by 50 ml/kg or 500 ml. Therefore, his blood volume increased from 800 ml to 1300 ml in two minutes. If all of this fluid stayed in the vascular compartment his original hematocrit of 30% would be expected to change to 18.5% (ea. $30\% \times \frac{800}{1300}$). Actually the hematocrit changed

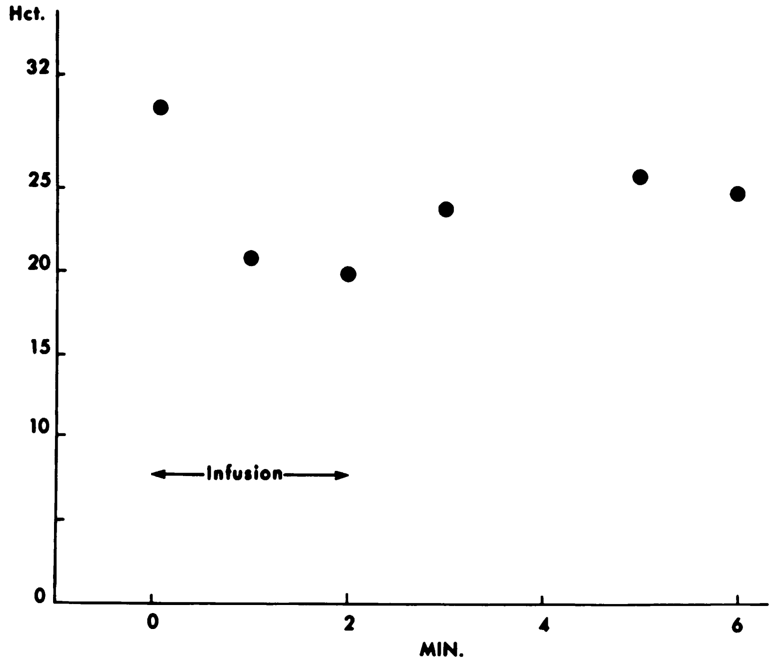


Figure 3. This figure illustrates the change in the hematocrit of arterial blood during infusion in one of the older lambs.

from 30% to 20% in this infusion. Very little saline seems to have been lost to the tissues during the two minute infusion. The change in plasma potassium with infusion was similar and was never seen to fall below 3 mg/100 ml.

Osgood (18) has shown that in humans the ratio of blood volume to body weight is very nearly constant after birth. If this is assumed to be true for sheep and if vascular permeability and plasma osmotic pressure are similar for each of the ages I studied then changes in hematocrit during infusion should not differ between groups.

<u>The Effects of Atropine</u> - These changes are shown in Tables 3, 4 and Figure 4. Lambs of all ages showed significant increases in heart rate and mean arterial blood pressure as mean stroke volume /kg fell. The rise in flow/kg was significant only in the two older groups.

When each of these changes is expressed as a percentage increase (Figure 4, Table 4) the change in heart rate in 8 day old lambs was significantly less than in the older lambs and the percentage decrease in mean stroke volume/kg was significantly less at one week than at four weeks of age. There were no other significant changes. Both heart rate and mean stroke volume/kg tended to change

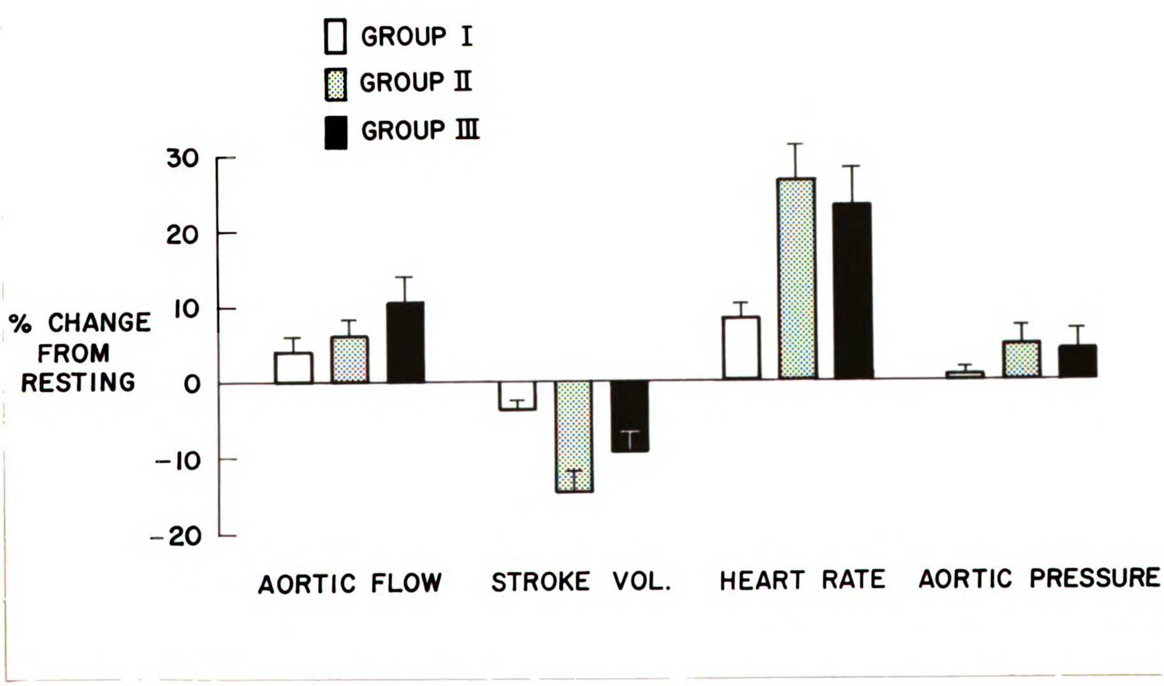


Figure 4. This figure illustrates the mean percentage change (+ SEM) in various cardiovascular measurements when atropine was given to resting lambs of each age group. Tables 3 and 4 list the actual values and the statistical significance of the changes shown here.

Table 3. This table shows the effect of atropine on various cardiovascular measurements in resting lambs of each age group. For each age the populations described here were those used to calculate the percentages illustrated in Figure 4.

	Pre	Signif	Post
$\overline{Q}$/kg (ml/min/kg)			
Group One	417(±88.8)	NS	430(±83.8)
Group Two	371(±127)	S	393(±136)
Group Three	150(±32.6)	S	164(±32.8)
$\overline{SV}$/kg (ml/kg)			
Group One	2.08(±0.555)	S	1.98(±0.468)
Group Two	2.45(±0.672)	S	2.07(±0.588)
Group Three	1.1(±0.308)	S	0.972(±0.227)
Heart Rate (beats/min)			
Group One	205(±25.4)	S	220(±19.8)
Group Two	151(±27)	S	188(±25.6)
Group Three	142(±27.3)	S	172(±31)
Mean Systemic Arterial Pressure (mm Hg)			
Group One	68.8(±5.9)	NS	69(±6.92)
Group Two	72.2(±8.21)	NS	75.4(±9.46)
Group Three	82.5(±12.0)	NS	85.3(±11.7)

Notation: Mean (±Standard Deviation)
S = statistically significant at the 0.05 level
NS = not statistically significant at the 0.05 level

Table 4. This table describes the percentage change in various cardiovascular measurements when atropine was given to resting lambs of each age group. This information is illustrated in Figure 4.

		Group Two	Group Three
$\overline{Q}$/kg (ml/min/kg)		6.12 (±8.4)	10.5 (±13.7)
Group One	3.97 (±8.02)	NS	NS
Group Two			NS
$\overline{SV}$/kg (ml/kg)		-14.8 (±11.7)	-9.29 (±11)
Group One	-3.79 (±5.23)	S	NS
Group Two			NS
Heart Rate (beats/min)		26.6 (±17.9)	23.3 (±19.8)
Group One	8.25 (±8.58)	S	S
Group Two			NS
Mean Systemic Arterial Pressure (mm Hg)		4.77 (±10.1)	4.09 (±11)
Group One	0.244 (±6.22)	NS	NS
Group Two			NS

Notation: Mean (±Standard Deviation)
S = statistically significant at the 0.05 level
NS = not statistically significant at the 0.05 level

less after atropine in 8 day old lambs suggesting that the young lambs had less resting parasympathetic tone.

<u>The Effects of Propranolol with Atropine</u> - It is interesting that when propranolol was given to atropinized lambs only the youngest age group showed a statistically significant decline in aortic flow/kg (Figure 5, Tables 5 and 6). This suggests that 8 day old lambs were more dependent on catecholamine support at rest or that propranolol directly depressed the young myocardium. As before, the oldest lambs had a significantly lower flow/kg and the two younger groups were very similar to each other.

<u>The Effects of Isoproterenol with Atropine</u> - Figure 5 and Tables 5 and 6 show that at each age isoproterenol increased mean aortic flow/kg significantly in atropinized lambs. It is apparent that lambs of each age were able to respond to increasing beta adrenergic stimulation with an increased mean aortic flow/kg. In this situation, as with atropine alone, there was no significant difference in aortic flow/kg between the 8 and 26 day old lambs. As before, flow/kg in the 45 day old lambs was significantly lower.

<u>Effects of Infusion</u> - By referring to Figures 5 and 6 and to Table 5 we can compare the effects of various types of infusions at each age.

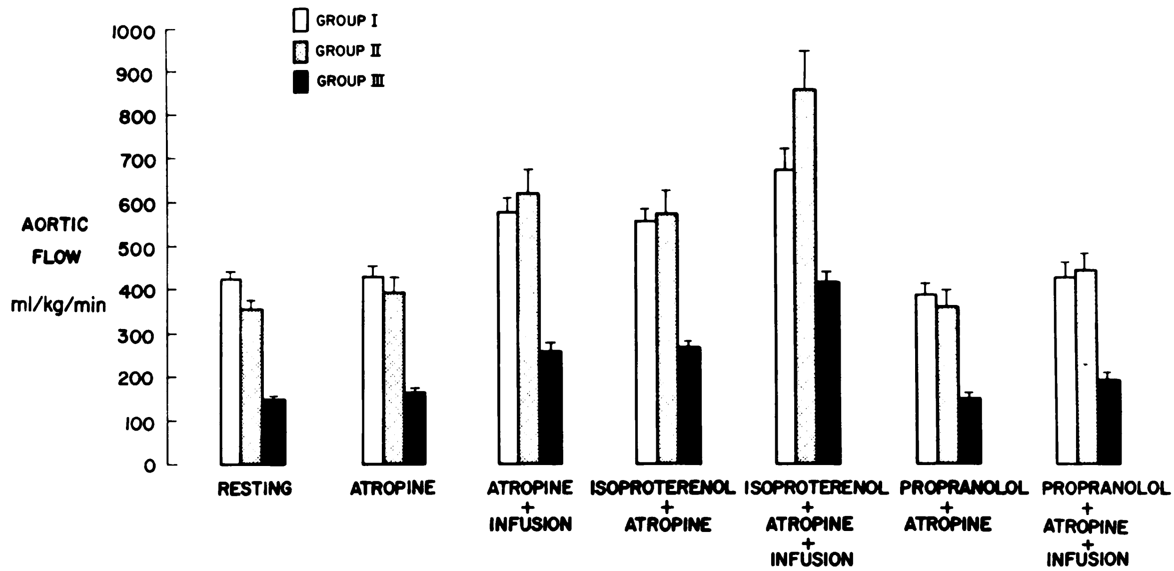


Figure 5. The mean aortic flow per kilogram (+ SEM) in various situations is shown here. Although the data are arranged to facilitate comparisons between age groups, comparisons within any group are also possible. The actual values shown here and their statistical significance are tabulated in Tables 5 and 6.

For all of these ages the flows per kilogram observed during infusion were greatest with isoproterenol, intermediate with saline alone, and least after propranolol. All of these differences were significant at maximum flow and at most mean left atrial pressures. For group one there was no significant difference in aortic flow between saline infusion alone and infusion of saline after propranolol at a mean left atrial pressure of 10 mm Hg or between saline infusion alone and saline infusion with isoproterenol at a mean left atrial pressure of 20 mm Hg. In both groups two

Table 5. The significance of differences in mean aortic flow per kilogram in various situations within each group is shown here. This information is illustrated in Figure 5. (In some instances it was necessary to pool data prior to the statistical calculations.)

	Resting	Atropine	Atropine + Infusion	Isoproterenol + Atropine	Isoproterenol + Atropine + Infusion	Propranolol + Atropine	Propranolol + Atropine + Infusion
WITHIN GROUP ONE:	425	430	577	553	671	382	418
Resting		NS					
Atropine			S	S		S	
Atropine + Infusion					S		S
Isoproterenol + Atropine					S	S	
Isoproterenol + Atropine + Infusion							S
Propranolol + Atropine							S
WITHIN GROUP TWO:	355	393	622	570	855	362	441
Resting		S					
Atropine			S	S		NS	
Atropine + Infusion					S		S
Isoproterenol + Atropine					S	S	
Isoproterenol + Atropine + Infusion							S
Propranolol + Atropine							S

Table 5 (Continued)

	Resting	Atropine	Atropine + Infusion	Isoproterenol + Atropine	Isoproterenol + Atropine + Infusion	Propranolol + Atropine	Propranolol + Atropine + Infusion
WITHIN GROUP THREE:	147	164	259	265	413	150	191
Resting		S					
Atropine			S	S		NS	
Atropine + Infusion					S		S
Isoproterenol + Atropine					S	S	
Isoproterenol + Atropine + Infusion							S
Propranolol + Atropine							S

Notation: S = statistically significant at the 0.05 level
NS = not statistically significant at the 0.05 level
all flows are in ml/min/kg

Table 6. The significance of differences in mean aortic flow per kilogram within each group in various situations is shown here. This information is illustrated in Figure 5.

		Group Two	Group Three
Resting		355 (±116)	147 (±28.4)
Group One	425 (±86.3)	S	S
Group Two			S
Atropine		393 (±136)	164 (±32.8)
Group One	430 (±83.8)	NS	S
Group Two			S
Atropine + Infusion		622 (±214)	259 (±72.4)
Group One	577 (±135)	NS	S
Group Two			S
Isoproterenol + Atropine		570 (±170)	265 (±47.9)
Group One	553 (±90.3)	NS	S
Group Two			S
Isoproterenol + Atropine + Infusion		855 (±284)	413 (±87)
Group One	670 (±156)	NS	S
Group Two			S
Propranolol + Atropine		362 (±117)	150 (±32.2)
Group One	391 (±78.7)	NS	S
Group Two			S
Propranolol + Atropine + Infusion		441 (±129)	191 (±58)
Group One	428 (±99.9)	NS	S
Group Two			S

Notation: Mean (±Standard Deviation)
S = statistically significant at the 0.05 level
NS = not statistically significant at the 0.05 level
All flows are in ml/min/kg

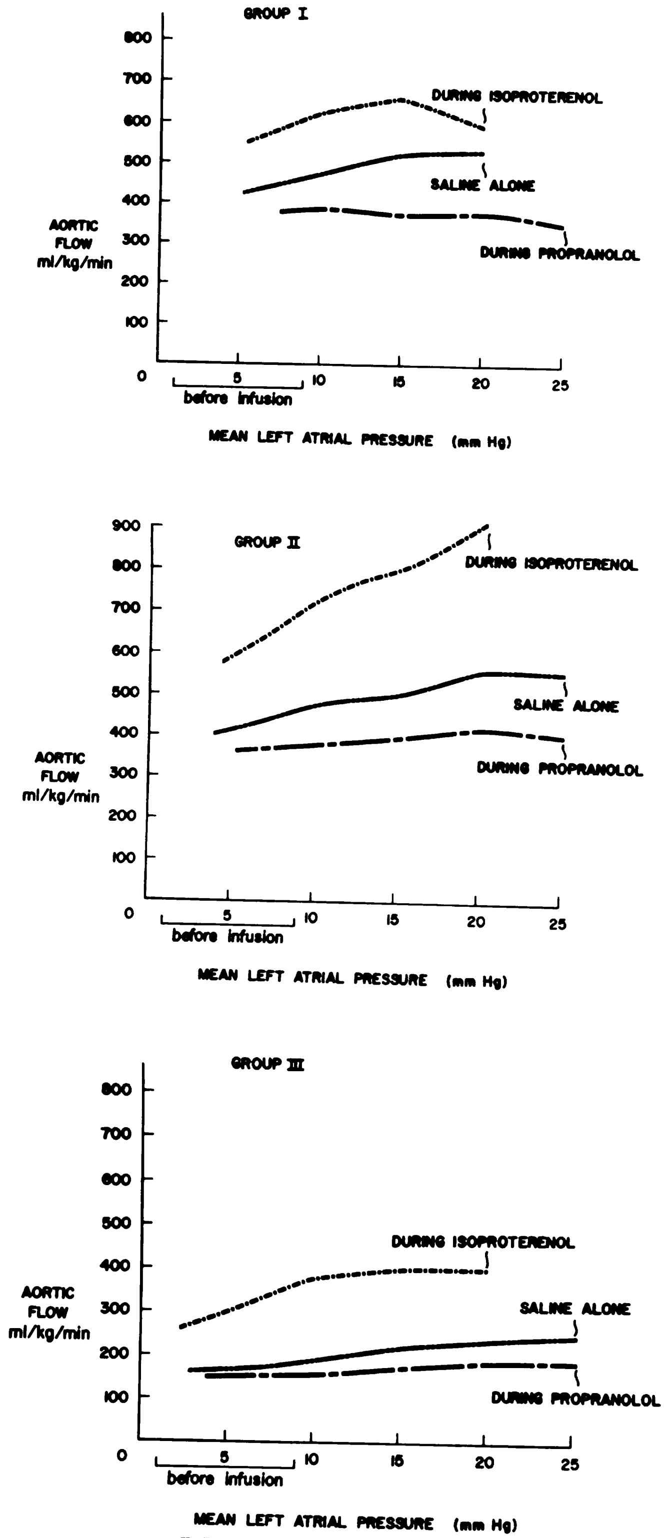


Figure 6. The mean aortic flow per kilogram has been recorded just before infusion and at mean left atrial pressures of 10, 15,20, and 25 mm Hg. Each illustration contains the values for a single age group.

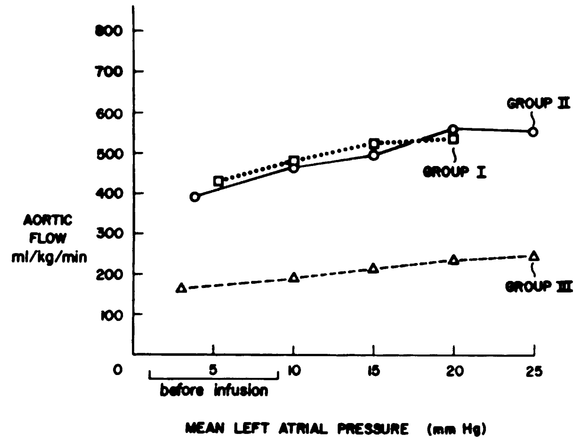
and three, there was no significant difference in aortic flow between saline infusion alone and infusion of saline after propranolol at a mean left atrial pressure of 25 mm Hg.

Figures 5 and 7 and Table 6 compare the effect of volume loading on lambs of different ages at each level of beta adrenergic activity. Lambs in group three had significantly lower mean flows per kilogram than did the younger two groups. This was true at maximum flow per kilogram, and at almost all mean left atrial pressures. (The aortic flow/kg during infusion of saline and isoproterenol was not significantly different between groups one and three at a mean left atrial pressure of 20 mm Hg.) The mean flows per kilogram for the younger lambs never differed significantly from each other.

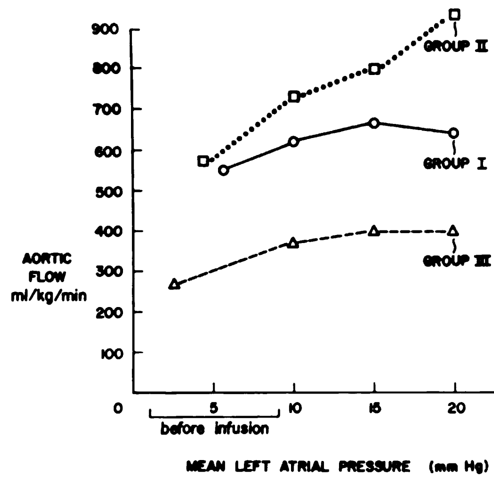
With infusion there was an increase in mean aortic flow in all lambs but this increase was proportionately greater in groups two and three. This can be seen more clearly in Figure 8 in which I have shown the percentage increase of maximum flow over the preinfusion level. Notice that group one lambs responded less than the other lambs regardless of the level of beta adrenergic activity. The responses of the older two groups were almost identical.

Notice too that in the two older groups the percentage increase for infusions with saline alone and for saline infusions with isoproterenol were the same and were

(a) infusion of saline alone



(b) infusion of saline during isoproterenol infusion



(c) infusion of saline after administration of propranolol

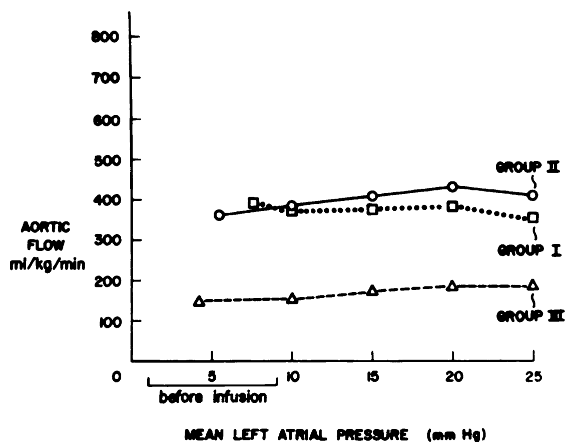


Figure 7. The mean aortic flow per kilogram has been recorded just before infusion and at mean left atrial pressures of 10,15,20, and 25 mm Hg. Each illustration contains the values for a single type of infusion.

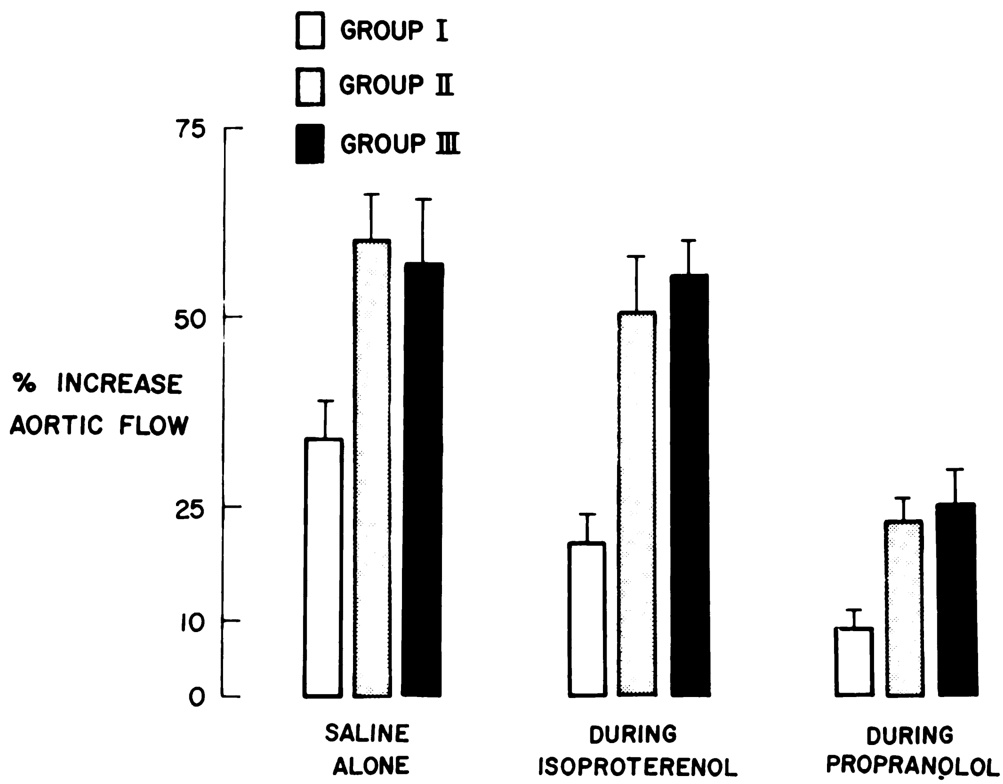


Figure 8. This figure depicts the mean percentage increase (+SEM) of peak mean aortic flow per kilogram over the corresponding value just before infusion. Notice that the younger lambs responded less than the others regardless of the degree of beta adrenergic activity. The older two groups showed almost identical responses.

significantly greater than for infusions after propranolol. On the other hand, in group one lambs the only significant difference was between infusions of saline alone and infusions after propranolol.

DISCUSSION

General Comments - Finding a suitable method to measure changing myocardial performance in growing animals was a difficult problem. I preferred a method which could be used in a chronic, unanesthetized preparation and which would reflect the overall performance of the intact heart. Of course, it was essential that measurements made in the same animal be repeatable from day to day. My preliminary efforts soon centered on the relative merits of left ventricular function curves (12) and ventricular output curves (13). Since the maximum aortic flow observed with ventricular output curves was much less variable from day to day than values obtained from ventricular function curves, I chose to use ventricular output curves. In doing so I had to accept several disadvantages inherent in applying this method to growing animals. To continuously measure aortic flow during infusion an electromagnet flow transducer was placed around the ascending aorta. I found that this rigid annular device began to constrict the growing aorta in about two weeks. Therefore, experiments had to begin as soon as the lamb recovered from surgery and, if possible, had to be repeated daily. To minimize the volume of fluid infused, a very rapid rate of infusion was used. I chose to infuse 0.9% NaCl solution knowing that it would cause the hematocrit and serum potassium to change during infusion. If whole blood or plasma had been used the blood volume would not have returned to normal rapidly enough to allow the procedure to be repeated the following day. Although Barger (19) has shown in adult dogs that a sodium load may be excreted within 8 hours, I did not know if the potassium load which would be given if Tyrode solution were used for infusion could be as quickly excreted. When saline was used for infusion the lambs did not show an excessive daily increase in body weight. Although the percentage increase in blood volume caused by infusion was not trivial, if it were the same from day to day for all animals they would all be exposed to a uniform stress. This assumes that vascular permeability and protein osmotic pressure are similar at each age. Since blood volume/kg of body weight was probably constant in the range of ages I studied (18), infusing saline at a fixed rate of 25 ml/kg for the same period of time should have increased the blood volume of all animals proportionately. In fact, my observation that the changes in mean left atrial pressure and mean arterial blood pressure were uniform during infusion for all ages, coupled with the close agreement between the observed and calculated change in hematocrit during infusion show that this was so.

Although Carlson (20)has recently reported that in dogs several weeks may be needed in some cases for complete recovery from cardiac surgery, surgical manipulation of the heart in these lambs was minimal. The left ventricular catheter was placed at the apex of the heart. In lambs the myocardium in this region is thin and the procedure resulted in very little damage to myocardial tissue. The lambs were healthy, gained weight normally throughout the experiments, and had normal resting arterial blood gases. The maximum ventricular output recorded at varying times after surgery for similar infusions showed no significant change. For these reasons I feel that the lambs had recovered from surgery before infusion experiments were started.

The ductus arteriosus is usually closed within the first day after birth in lambs. At the time of surgery the great vessels were examined to confirm that the ductus arteriosus was well constricted and that no thrill was present in the pulmonary artery.

Observations in Resting Lambs - The rapid postnatal decline in cardiac output/kg which I observed has also been described by Cross (3) in lightly anesthetized lambs. The reasons for this dramatic fall in aortic flow/kg and for the extremely high levels found at birth have not been explained. It could be related to the higher oxygen consumption/kg in the newborn (3), a change in oxygen uptake from blood perfusing the tissues or to a change in the oxygen capacity of the blood.

Acute studies on anesthetized newborn lambs by Downing (1) and on lightly anesthetized newborn lambs (3) suggest that the maximum left ventricular output in the newborn lamb may be about 300 or 325 ml/kg/minute. These values are less than those I observed in week old lambs at rest and are considerably below the aortic flows/kg found during isoproterenol administration or infusion studies. This discrepancy may be due to the chronic nature of my studies and the fact that my lambs were not anesthetized.

The Need for Oxygen:

Young mammals of various species have been known for some time to have higher metabolic rates (as measured by oxygen consumption per kilogram) than adults of the same species (21). Dawes (22) found that the oxygen consumption per kilogram in fetal sheep is similar to that in adults but then triples at birth. Surface area per kilogram in lambs is also about three times greater than in the adult. If the increased surface area per kilogram were the only factor increasing heat loss, the increase in oxygen consumption would be adequate to maintain the lamb's body temperature at adult levels. Of course, the lamb's increased activity after birth must also account for some increase in oxygen consumption.

The Release of Oxygen to the Tissues:

Arterial PO_2 for resting group one, two and three lambs were 70.5, 79.7 and 81.3 mm Hg respectively. Hall (23) found that the normal arterial PO_2 for adult sheep is about 99 mm Hg at sea level. It does not follow, however, that the lamb's hemoglobin is less saturated than the adult's. Karvonen (24) reported that at birth lambs have equal amounts of fetal hemoglobin and adult hemoglobin. Four weeks later less than a quarter of this fetal hemoglobin is said to remain. Bartels (25, page 94) found levels of fetal hemoglobin at birth in lambs of about 5% decreasing to about 2% by 45 days after birth. In our laboratory levels of fetal hemoglobin as high as 100% have been found in newborn lambs. Whatever the level may be there is general agreement that some fetal hemoglobin is present at birth and is gradually replaced with adult hemoglobin after birth. Fetal hemoglobin has a much lower affinity for organic phosphates than adult hemoglobin and since organic phosphates compete with oxygen for sites on hemoglobin the oxygen dissociation curve for fetal blood is shifted to the left of the adult curve (26). Therefore, the oxygen affinity of the blood at birth is greater than in the adult. Blood in the newborn is more easily oxygenated but since the oxygen is more firmly bound, release to the tissues is difficult. This assumes that pH temperature, and PO_2 in the lamb tissues are similar to those of the adult and that the effect of pH and temperature on fetal hemoglobin is similar to the effect on adult hemoglobin *in vivo*. Bartels (25, page 94) reported that the PO_2 at which lamb blood is half saturated with oxygen (i.e., the P_{50} of lamb blood) although initially low reaches adult levels at about 16 days after birth. This is apparently due to both replacement of fetal hemoglobin with adult hemoglobin and to an increase in the concentration of the organic phosphate diphosphoglyceric acid in red blood cells as described by Battaglia (27). Lambs' P_{50} values continue to increase from 16 to 50 days of age.

The Oxygen Capacity of the Blood:

Reports in the literature describing the oxygen capacity of the blood at different ages do not agree. Barron (28) has reported that although the oxygen capacity of lamb blood initially exceeds that in the adult, it falls to adult levels within a month. Cross (3) found that the fall takes more time, Bartels (25, page 94) described fluctuations above the adult level, and Friedman (9) reported that the blood oxygen capacity is constant after birth. An increased blood oxygen capacity in young lambs could partially compensate for a decreased tissue oxygen availability. This view is supported by work done in piglets by Delivoria-Papadopoulos (29) which indicates that although artificially decreasing oxygen affinity or increasing oxygen capacity lead to decreased cardiac output, they have no effect on oxygen consumption.

Of course, oxygen delivery to the tissues could also be impaired if the capillary density were less in young lambs or if barriers to diffusion were more severe than in more mature animals.

I feel it is likely that the changing pattern of resting flow associated with growth in lambs results from a decreasing oxygen demand per kilogram of body weight as the ability of the blood to release oxygen to the tissues increases.

The Response to Infusion - Although the hearts of the youngest group of lambs were able to provide sufficient flow at rest, the percentage increase in their flow during infusion was less than in the older animals. Unfortunately, the catheter system used in recording arterial blood pressure had a frequency response too low to allow for the calculation of vascular impedance. Consequently, the data do not allow a description of how vascular impedance may change with age or how it may have changed during infusion. However, the fact that the increase in mean arterial blood pressure during infusion was 15-20% for each group suggests that the effect of infusion on vascular impedance was similar at each age. It may be that the higher resting flow/kg in week old lambs places a relatively greater burden on their hearts restricting their reserve capacity.

Of course, the heart of the one week old lamb may be faced with intrinsic disadvantages as compared to older lambs. Aside from the obvious increase in heart size with growth, other changes occur which may affect myocardial performance. As I will describe below, the hearts of young lambs:

(1) may not be able to generate as much force as do older lambs;

(2) are incompletely innervated by the sympathetic nervous system; and,

(3) may be mechanically limited by a lower compliance than is present in more mature sheep.

<u>The Generation of Force</u>:

No information is available in the literature which would allow the mechanics of myocardial contraction in lambs to be compared to that in adults. However, information is available for older gestation fetuses and for adults. Friedman (9) found that using right ventricular moderator band preparations the extent of shortening, velocity of shortening, and isometric force development were less in the older fetus than in adult sheep. However, in glycerinated cardiac muscle fibers at equivalent sarcomere lengths the same tensions and ATPase activities were observed in both fetus and adult. So, although the late gestation fetus does not perform as well in terms of extent or velocity of shortening and isometric force development, it has been suggested that the individual muscle fibers behave as do those of the adult. This could be due to the relatively low concentration of contractile material in the fetal myocardium as compared to the adult. Friedman (9) has estimated that whereas contractile elements constitute 60% of the cardiac muscle mass in adults they account for only 30% of the fetal myocardium. Whatever the explanation, the late gestation fetus must function with a myocardium which apparently is unable to generate the levels of isometric tension found in the adult.

Sympathetic Nervous System Innervation:

Both Friedman (9) and Lebowitz (6) have found that sympathetic innervation of the ventricular myocardium, although extensive at birth, is probably not complete for several more weeks. Vapaavouri (10) has reported that full responsiveness to parasympathetic and alpha adrenergic blockade develops by 120 days gestation in the lamb but beta adrenergic response to blockade continues to develop during late gestation. Downing (30) has shown that newborn lambs do respond to sympathetic nervous system stimulation but the response relative to the adult has not been assessed.

Of course, the relative importance of the various mechanisms available for control of the circulation may vary throughout development. It is possible that young lambs rely on circulating catecholamines but in older lambs, as sympathetic myocardial innervation matures, it becomes more important. According to Friedman (9), norepinephrine dose response curves of sheep right ventricular papillary muscle indicate that fetal hearts are more sensitive to norepinephrine than adults, and lambs less than 3 days old are more sensitive than lambs aged 4 days to 3 weeks (9). It is interesting that in each case the hearts with the least sympathetic innervation are the ones which are the most sensitive to the sympathetic postganglionic neurotransmitter. Uptake and binding of norepinephrine by sympathetic fibers is an important mechanism limiting its action on the myocardium. If fewer fibers were present in immature hearts (as has been shown histologically) of if their ability to take up norepinephrine were limited, one would expect to see a greater response to a given dose of norepinephrine than is found in more mature hearts. Notice that this argument assumes that the concentration of beta adrenergic receptors is similar at all ages. Isoproterenol also stimulates beta adrenergic receptors in the myocardium but is not taken up or bound as is norepinephrine. To see if the increased responsiveness to exogenous norepinephrine in young hearts is due to an increased beta adrenergic receptor sensitivity or to a limited uptake and binding of norepinephrine, Friedman (9) measured fetal and adult isoproterenol dose response curves. He found that the sensitivity to isoproterenol was the same in both the fetus and in the adult. On this basis, beta receptor sensitivity to catecholamines was assumed to be similar in fetal and adult myocardium.

In summary, beta receptors are present in the developing heart and apparently are as sensitive to catecholamines as in the adult. The young hearts' increased sensitivity to norepinephrine could result from a decreased uptake or removal of norepinephrine and may help to compensate for an incomplete sympathetic innervation. It is also possible that the hearts of young lambs are more dependent on circulating catecholamines than are those of older lambs.

I have attempted to assess the importance of beta adrenergic stimulation on myocardial performance at each age by both the addition of exogenous catecholamines and by beta adrenergic blockade. Lambs of all of the ages I studied were able to respond to increasing beta adrenergic activity by increasing aortic flow. However, as Figure 8 shows, the ability of the myocardium to increase peak mean flow per kilogram over control levels in response to the stress of volume loading was less in the youngest group regardless of the degree of beta adrenergic activity. In atropinized lambs, propranolol decreased aortic flow only in the youngest group. This suggests either that younger lambs may be more dependent on catecholamine support than older lambs or that propranolol may directly depress the young myocardium.

<u>Possible Mechanical Limitations</u>:

Since the Frank-Starling effect (31, 32) has been well-documented in anesthetized lambs by Downing (1), the changing response to volume loading I observed could have been caused in part by differences in myocardial compliance between lambs of different ages. The uniform increase in left atrial pressure during infusion would cause a compliant heart to dilate more rapidly and lead to an increase in output. This is possible since mean arterial blood pressure changed in the same way at each age during volume loading. There have been no in vivo studies describing compliance in lambs of these ages but in freshly excised hearts Romero (33) has reported the compliance of newborn lambs to be less than that of adult sheep. Since I have not measured compliance in my lambs I do not know to what extent this may account for the differences in response to volume loading I observed between age groups.

CONCLUSIONS

1) Resting aortic flow per kilogram decreased dramatically after birth; however, differences in flow per kilogram between one and four weeks of age were not seen after the lambs had received atropine, isoproterenol, propranolol or during infusion of saline. The higher resting aortic flow per kilogram seen in one week old lambs could have been related to their higher oxygen consumption per kilogram or it could have been due to a change in oxygen uptake from blood perfusing the tissues.

2) The ability of the myocardium to increase aortic flow during volume loading at varying levels of beta adrenergic stimulation rose significantly between one and four

weeks after birth, but showed no further change beyond that time. This suggests that although the myocardium in week old lambs was capable of maintaining a high cardiac output, resting requirements were so high at this age that there was little reserve capacity to increase output for additional demands.

3) Lambs of all ages were able to respond to increasing beta adrenergic stimulation by increasing aortic flow.

4) Since (unlike the older lambs) week old lambs showed no increase in resting flow after atropine, they apparently had less resting parasympathetic tone.

5) In atropinized lambs, propranolol decreased aortic flow only in the youngest lambs suggesting that they were more dependent on catecholamine support than were older lambs or that propranolol directly depressed their young hearts.

6) Calculated resting mean stroke volume per kilogram of body weight did not change between one and four weeks of age but fell slightly thereafter.

7) Resting heart rate fell gradually with age.

8) Resting mean arterial blood pressure increased gradually with age but the increase between one and four weeks was small and not statistically significant.

9) Although differences between the age groups in

resting mean left atrial pressure and in hematocrit were occasionally statistically significant, the actual changes were small.

LITERATURE CITED

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1. Downing, S. E., Talner, N. S. and Gardner, T. H.: Ventricular function in the newborn lamb. Amer. J. Physiol. 208:931, 1965.
2. Downing, S. E., Talner, N. S. and Gardner, T. H.: Influences of arterial oxygen tension and pH on cardiac function in the newborn lamb. Amer. J. Physiol. 211:1203, 1966.
3. Cross, K. W., Dawes, G. S. and Mott, J. C.: Anoxia, oxygen consumption and cardiac output in newborn lambs and adult sheep. J. Physiol. 146:316, 1959.
4. Dawes, G. S.: Changes in the circulation at birth. Brit. Med. Bull. 17:148, 1961.
5. Friedman, W. F., Pool, P. E., Jacobowitz, D., Seagren, S. C. and Braunwald, E.: Sympathetic innervation of the developing rabbit heart. Circ. Res. 23:25, 1968.
6. Lebowitz, E. A., Novick, J. S. and Rudolph, A. M.: Development of myocardial sympathetic innervation in the fetal lamb. Pediatr. Res. 6:887, 1972.
7. Spann, J. F., Sonnenblick, E. H., Cooper, T., Chidsey, C. A., Willman, V. L. and Braunwald, E.: Cardiac norepinephrine stores and the contractile state of heart muscle. Circ. Res. 19:317, 1966.
8. Hopkins, S. F., McCutcheon, E. P., Wekstein, D. R.: Postnatal changes in rat ventricular function. Circ. Res. 32:685, 1973.
9. Friedman, W. F.: The intrinsic physiologic properties of the developing heart. In Friedman, W. F., Lesch, M., Sonnenblick, E. H. (Ed.): Neonatal Heart Disease, New York, Grune and Stratton, 1972.

10. Vapaavouri, E. K., Shinebourne, E. A., Williams R. L., Heymann, M. A. and Rudolph, A. M.: Development of cardiovascular responses to autonomic blockade in intact fetal and neonatal lambs. Biol. Neonate 22:177, 1973.

11. Heymann, M. A. and Rudolph, A. M.: Effects of increasing preload on right ventricular output in fetal lambs _in utero_. Circulation 7 and 8, supplement 4:IV-37, 1973. (Abstract number 143.)

12. Sarnoff, S. J. and Berglund, E.: Ventricular function 1. Starling's law of the heart studied by means of simultaneous left and right ventricular function curves in the dog. Circulation 9:706, 1954.

13. Bishop, V. S., Stone, H. L. and Guyton, A. C.: Cardiac function curves in conscious dogs. Amer. J. Physiol. 207:677, 1964.

14. Stone, H. L., Bishop, V. S. and Guyton, A. C.: Cardiac function after embolization of coronaries with microspheres. Amer. J. Physiol. 204:16, 1962.

15. Stone, H. L., Bishop, V. S. and Guyton, A. C.: Ventricular function following radiation damage of the right ventricle. Amer. J. Physiol. 211:1209, 1966.

16. Harry, J. D., Kappagoda, C. T., Linden, R. J. and Snow, H. M.: Action of propranolol on the dog heart. Cardiovasc. Res. 7:729, 1973.

17. Rudolph, A. M.: Personal communication.

18. Osgood, E. E.: Development and growth of hematopoietic tissues. Pediatrics 15:733, 1955.

19. Barger, A. C., Ross, R. S., Price, H. L.: Reduced sodium excretion in dogs with mild valvular lesions of the heart, and in dogs with congestive failure. Amer. J. Physiol. 180:249, 1955.

20. Carlson, E., McCutcheon, E. and Sandler, H.: Variations in post-operative recovery response to lower body negative pressure in chronically instrumented dogs. Fed. Proc. 34 #3:450, 1975. (Abstract number 1298.)
21. Clark, A. J.: Comparative physiology of the heart. New York, MacMillan Company, 1927.
22. Dawes, G. S. and Mott, J. C.: The increase in oxygen comsumption of the lamb after birth. J. Physiol. 146:295, 1959.
23. Hall, F. G., Dill, D. B. and Guzman Barron, E. S.: Comparative physiology in high altitudes. J. of Cell. and Comp. Physiol. 8:301, 1936.
24. Karvonen, M. J.: In Roughton, F. J. W. and Kendrew, J. C. (Ed.): Haemoglobin. New York, Interscience Publishers, 1949, p. 279.
25. Bartels, H.: Prenatal respiration. Amsterdam, North-Holland Publishing Company, 1970.
26. Miller, W. W.: Erythrocyte oxygen transport in normal infants and in infants with cardiovascular disease. In Friedman, W. F., Lesch, M., Sonnenblick, E. H. (Ed.): Neonatal Heart Disease, New York, Grune and Stratton, 1972.
27. Battaglia, F. C., McGaughey, H., Makowski, E. L. and Meschia, G.: Postnatal changes in oxygen affinity of sheep red cells: a dual role of diphosphoglyceric acid. Amer. J. Physiol. 219:217, 1970.
28. Barron, D. H.: Postnatal changes in the oxygen capacity and dissociation curve of the blood of lambs. Yale J. of Biol. and Med. 24:191, 1951.
29. Delivoria-Papadopoulos, M., Martens, R. J., Oski, F. A. and Forster, R. E.: Pediatr. Res. 6:342/382, 1972.

30. Downing, S. E., Talner, N. S., Campbell, A. G. M., Halloran, K. H. and Wax, H. B.: Influence of sympathetic nerve stimulation on ventricular function in the newborn lamb. Circ. Res. 25:417, 1969.

31. Frank, O.: zur dynamik des herzmuskels. Z. Biol. 32:370, 1895.

32. Patterson, S. W., Piper, H. and Starling, E. H.: The regulation of the heart beat. J. Physiol. 48:465, 1914.

33. Romero, T., Covell, J. and Friedman, W. F.: A comparison of pressure-volume relations of the fetal, newborn and adult heart. Amer. J. Physiol. 222:1285, 1972.