ECHOCARDIOGRAPHIC STUDIES OF VALVULAR AND VENTRICULAR FUNCTION IN HORSES

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Thesis presented for the degree of

Doctor of Philosophy

University of Edinburgh

May 1993

DECLARATION

Miss L. E. Young performed the thermodilution studies in this work and Dr. J. D. Bonagura assisted in standardising the two-dimensional images. The rest of this thesis is my own work and has not been presented to any university other than the University of Edinburgh. A paper from this thesis has been published in the Equine Veterinary Journal and two abstracts have been published in the British Journal of Anaesthesia. The paper is included as Appendix 21.

May 1993

This work is dedicated to the memory of

David Mark Pogson

Blessed are those that have not seen and yet have believed

John 20 verse 29.

ECHOCARDIOGRAPHIC STUDIES OF VALVULAR AND VENTRICULAR FUNCTION IN HORSES.

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ACKNOWLEDGMENTS

I thank the Horserace Betting Levy Board for the financial support of this work and the Home of Rest for Horses whose generosity enabled the purchase of the Doppler ultrasound equipment.

Professor R. E. W. Halliwell provided research facilities in the Department of Veterinary Clinical Studies, and I thank Dr. P. G. G. Darke and Dr. C. M. MacGregor for their supervision and friendship. I am indebted to Dr. J. D. Bonagura for his enthusiastic teaching and patience during the early stages of this work. I acknowledge the support of colleagues in the Departments of Veterinary Clinical Studies and Preclinical Veterinary Sciences, especially Dr. D. F. Cottrell. Dr. G. R. Scott advised on the statistical analyses and I am grateful to Mr. J. A. Fraser for his judgement and guidance.

It is a pleasure to acknowledge the photographic expertise of Mr. R. Munro. Miss M. Kerr assisted with the typing of this manuscript and I thank Miss H. London for her invaluable help with the references. I thank Miss R. Muirhead, Miss L. E. Young and Mr. T. J. Anderson for assistance with the figures. I am indebted to the hospital staff of the Royal (Dick) School of Veterinary Studies who patiently held the horses as I learnt the echocardiographic techniques and carried out this work.

Miss L. E. Young performed the thermodilution studies recorded in this thesis, and I am grateful to the University of Liverpool, Department of Anaesthesia for the loan of the thermodilution computer and for their encouragement of this work.

I am especially grateful to Dr. Martyn Blissitt who advised, encouraged and supported me throughout this work. To all my friends I offer my sincere thanks. My final acknowledgment is to the horses whose trust and patience made this work possible.

ABSTRACT

This thesis evaluates echocardiography in the assessment of the equine heart. Echocardiography is employed extensively in human medicine to differentiate the origin of cardiac murmurs and to assess ventricular performance. Such a noninvasive method would be valuable in equine medicine where routine diagnostic techniques, used successfully in other species, are of little value. Publications concerning the origins of cardiac murmurs, the indications for echocardiography in human medicine, and the limited studies on echocardiography in horses have been reviewed.

The aims of Part 1 of this work were to standardise suitable images for two-dimensional, M-mode and Doppler echocardiography; to measure selected intracardiac dimensions from the standardised two-dimensional and M-mode images; and to record blood flow velocities from the heart and great vessels using Doppler echocardiography in a group of normal horses and in horses with valvular disease.

Eighteen standard images were defined. All but two of the images could be recorded within a narrow range of transducer location, rotation and angulation. Selected intracardiac dimensions were measured from M-mode studies derived from the standard two-dimensional images. Measurements of intracardiac dimensions were repeatable and were not significantly correlated to bodyweight or age. Horses with suspected aortic regurgitation had a significantly larger left ventricular internal dimension in diastole, measured from the right hemithorax, than normal horses. Although other significant differences were detected between groups, intracardiac dimensions were not sufficiently sensitive to differentiate horses with suspected valvular disease from normal horses.

Colour flow Doppler studies revealed that valvular regurgitation was present in many normal horses. Horses with murmurs indicating tricuspid and aortic regurgitation showed larger regurgitant signals at the tricuspid and aortic valves respectively, than normal horses. Horses with murmurs indicating mitral regurgitation had a regurgitant signal of longer duration than that of the control group,

but the size of the jet was not significantly different between groups. Colour flow Doppler echocardiography was a sensitive technique for the detection of valvular regurgitation in horses. The flow velocities recorded by pulsed wave Doppler echocardiography in horses were similar to those reported in other species. Flow velocities were significantly increased at elevated heart rates. The peak aortic velocity and acceleration were also significantly higher in horses with aortic regurgitation.

Part 2 of this thesis evaluates Doppler echocardiography in the assessment of ventricular performance in horses. Publications concerning measurement of cardiac output and the use of Doppler indices to assess ventricular function in other species have been reviewed. Cardiac output measured by Doppler echocardiography was compared to that measured by thermodilution in nine conscious horses. Cardiac output was modified by the infusion of inotropes and the administration of an alpha 2 agonist. The effects of these agents on selected Doppler indices were assessed.

Doppler measurements of cardiac output correlated closely with measurements by thermodilution. Doppler measurements from the aortic outflow correlated more closely ($r = 0.89$) than those from the pulmonary artery ($r = 0.77$). The infusion of inotropes caused a significant increase in aortic peak velocity and peak acceleration, whereas the administration of detomidine and butorphanol resulted in a significant decrease in these variables. Other Doppler variables were also significantly altered by the administration of these agents.

In conclusion, echocardiography was found to be a sensitive technique for the diagnosis of cardiac murmurs and the evaluation of ventricular performance in horses.

SUMMARY

This thesis concerns the use of echocardiography to study the equine heart. Part 1 evaluates echocardiography in the assessment of cardiac murmurs. Part 2 evaluates echocardiography in the assessment of ventricular performance.

PART 1

Eighteen echocardiographic images for diagnostic imaging, M-Mode echocardiography, and Doppler echocardiography of the equine heart were standardised by relating the position of the axial beam to various intracardiac landmarks. The transducer orientation required for each image was recorded in fourteen adult horses by describing the degree of sector rotation and the orientation of the axial beam relative to the thorax. Repeatable images could be obtained within narrow limits of angulation and rotation for fourteen of the eighteen standardised images evaluated.

Intracardiac dimensions were measured from M-mode images recorded from four of the standard views. Further dimensions were measured from a standard two-dimensional image. Limitations of imaging depth prevented measurement of the left ventricular wall thickness from studies recorded from the right hemithorax in a high proportion of normal Thoroughbred and Thoroughbred cross horses (60% during systole, 87% during diastole). Depth limitations also prevented measurement of the left ventricular internal diameter during diastole in 15% of normal horses of this type. Left ventricular internal diameters were significantly larger when measured from images recorded from the left hemithorax than those measured from right sided images. Correlation between intracardiac measurements, bodyweight and age was examined. Studies from six horses on three consecutive days showed measurements to be repeatable. Horses with suspected aortic regurgitation had a significantly larger left ventricular internal dimension in diastole (measured from the right hemithorax) than normal horses. Although other significant differences were detected between

groups, intracardiac dimensions from two-dimensional and M-mode images were not sufficiently sensitive to differentiate horses with other cardiac murmurs from normal horses.

Colour flow Doppler studies were recorded from ten of the standard views, in the group of normal horses and in five groups of horses with specific cardiac murmurs. The predominant flow patterns within the cardiac chambers were described in relation to anatomical landmarks and the timing within the cardiac cycle. High velocity flow signals or jets were recorded at the cardiac valves in normal horses. These signals were either of brief duration associated with valve closure, or were regurgitant signals of longer duration. Horses with murmurs suggestive of tricuspid and aortic regurgitation showed larger regurgitant signals at the tricuspid and aortic valves respectively, when compared to normal horses. Horses with murmurs suggestive of mitral regurgitation had a regurgitant signal of longer duration than those of the control group, but the jets were not larger. Colour flow Doppler echocardiography is a sensitive technique for the detection of valvular regurgitation in horses.

Intracardiac flow velocities were recorded from the tricuspid and mitral inflow using pulsed wave Doppler echocardiography. The velocity of blood flow in the aorta and pulmonary artery was also recorded. The peak acceleration of flow was significantly higher in the aorta than in the pulmonary artery. The flow velocities recorded in horses were similar to those reported in other species. Flow velocities were significantly increased at elevated heart rates but were less affected by second degree atrioventricular block. Horses with functional murmurs showed a significant increase in specific flow variables compared to horses without flow murmurs. Horses with aortic regurgitation had a significantly higher aortic peak velocity and peak acceleration than normal horses. Horses with high grade tricuspid regurgitant murmurs had a higher rapid filling velocity at the mitral valve than normal horses. Horses with mild valvular regurgitation showed no differences in the flow velocities

recorded by Doppler echocardiography. The measurement of blood flow velocities appears to be useful in evaluating horses with more severe valvular regurgitation.

PART 2

Measurements of cardiac output by Doppler echocardiography were compared to measurements by thermodilution in nine conscious horses. Cardiac output was modified by the infusion of inotropes, dobutamine and dopamine, and the administration of the sedative combination, detomidine and butorphanol. The effects of these agents on selected Doppler indices were assessed.

Doppler measurements of cardiac output correlated closely with measurement by thermodilution. Measurements from the aortic outflow correlated more closely with thermodilution, than those from the pulmonary artery ($r = 0.89$ and $r = 0.77$ respectively). Doppler measurements agreed most closely with thermodilution measurements when the mean flow velocity was recorded from the aorta, and the flow area was measured from the ascending aorta using the leading edge method. The differences between the two methods increased with increasing cardiac output. In 95% of cases, the Doppler technique (leading edge aorta) resulted in an underestimation of the cardiac output measured by thermodilution, of up to 12.69 litres per minute or an overestimation of up to 11.83 litres per minute.

The infusion of inotropes caused a significant increase in aortic peak velocity and peak acceleration, whereas the administration of detomidine and butorphanol resulted in a significant decrease in these variables. Other Doppler variables were also significantly altered by the administration of these agents.

In conclusion echocardiography appears to be a sensitive, repeatable technique for the diagnosis of cardiac murmurs and the evaluation of ventricular performance in horses.

LIST OF ABBREVIATIONS

PART 1.

CHAPTER 1.	cm	centimetres
	gm/ml	grams per millilitre
	$dyne^{-s}$	dynes per second
	kg	kilograms
	ECG	Electrocardiograph
	mmHg	Millimetres of mercury
CHAPTER 2.	R.(D).S.V.S	Royal (Dick) School of Veterinary Studies
	MHz	Megahertz
CHAPTER 3.		
Tables 4 and 5.	RA	Right atrium
	RV	Right ventricle
	TV	Tricuspid valve
	IVS	Interventricular septum
	LA	Left atrium
	LV	Left ventricle
	MV	Mitral valve
	LVW	Left ventricular wall
	CT of MV	Chordae tendineae of the mitral valve
	Ao	Aorta
	AV	Aortic valve
	LVOT	Left ventricular outflow tract
	CT of TV	Chordae tendineae of the tricuspid valve
	RVOT	Right ventricular outflow tract
	PMs	Papillary muscles
	PM	Papillary muscle
	RVW	Right ventricular wall
	CT	Chordae tendineae
	SV crest	Supraventricular crest
	PA	Pulmonary artery
	PV	Pulmonary valve
Table 6.	CR	Cranial angulation of + or ++
	CD	Caudal angulation of + or ++
	D	Dorsal angulation of + or ++
	V	Ventral angulation of + or ++
	*	Angulation of ++ or +++
	()	Angulation of 0 or +
	MR	Mitral regurgitation
	AI	Aortic insufficiency
	TR	Tricuspid regurgitation
	PI	Pulmonary insufficiency

CHAPTER 4.

Tables 7-11.

- Max	Maximum measurement from five cardiac cycles
- Med	Median measurement from five cardiac cycles
- Min	Minimum measurement from five cardiac cycles
- s	Systolic measurement
- d	Diastolic measurement
LVID	Left ventricular internal diameter
LVW	Left ventricular wall
IVS	Interventricular septum
RVID	Right ventricular internal diameter
EF	Ejection fraction
FS	Fractional shortening
Aod	Aortic diameter in diastole
LIVS	Interventricular septum (Image recorded from the left hemithorax)
LLVW	Left ventricular wall (Image recorded from the left hemithorax)
LRVW	Right ventricular wall (Image recorded from the left hemithorax)
LRVW	Right ventricular wall (Image recorded from the left hemithorax)
LLVID	Left ventricular internal diameter (Image recorded from the left hemithorax)
LLVID	Left ventricular internal diameter (Image recorded from the left hemithorax)
LFS	Fractional shortening (Image recorded from the left hemithorax)
LEF	Ejection fraction (left hemithorax)

CHAPTER 5.

Tables 12-13.

m/s	metres per second
SAx	Short-axis
LAx	Long-axis
Syst 1	First half of systole
Syst 2	Second half of systole
E	Early diastole
M	Mid diastole
L	Late diastole

CHAPTER 6.

Tables 16-28.

- Max	Maximum measurement from five cardiac cycles
- Med	Median measurement from five cardiac cycles
- Min	Minimum measurement from five cardiac cycles
RRp	R-R interval preceding measurement
RR	R-R interval during measurement
E	Flow of rapid filling
A	Flow due to atrial contraction
QE	Time to onset of E waveform
QA	Time to onset of A waveform
dt	For mitral inflow tables = deceleration time. For aortic and pulmonary outflow tables = acceleration time
VMax	Peak velocity
dv/dt	Peak acceleration

CHAPTER 6.

Tables 16-28.	VTI	Velocity time integral
	PEP	Pre-ejection period
	EjT	Ejection time

PART 2.

CHAPTER 1.	mls	Millilitres
	min	Minute
	cc	Cubic centimetres
	^{o}C	Degrees centigrade
CHAPTER 2.	mg	Milligrams
CHAPTER 3.	l/min	Litres per minute
	µg	Micrograms
Table 29-32.	Leading edge	Diameter of the ascending aorta measured by the leading edge method.
	Inner edge	Diameter of the ascending aorta measured by the trailing edge leading edge method.
	Leading valve	Aortic diameter measured between the valve tips by the leading edge method.
	Trailing valve	Aortic diameter measured between the valve tips by the leading edge trailing edge method.
	Sinus	Aortic diameter measured at the sinus of Valsalva by the leading edge method.
	M-mode	Aortic diameter measured from an M-mode study by the leading edge method.
	Both walls	Pulmonary artery diameter measured by the leading edge trailing edge method
	Right wall	Pulmonary artery diameter measured to include the thickness of the right vessel wall
	Inner edge	Pulmonary artery diameter measured by the trailing edge leading edge method

GENERAL INTRODUCTION

This thesis evaluates echocardiography in the assessment of the equine heart. Echocardiography, the examination of the heart using ultrasound, is employed extensively in human medicine. It is especially useful in the differentiation of functional murmurs from those associated with valvular disease.

There is a high incidence of functional and pathological murmurs in horses. The accurate diagnosis of cardiac murmurs is essential to advise on the safety of the horse for riding, or to offer a prognosis for the future athletic performance of the horse. Many horses have been wrongly condemned due to the presence of a cardiac murmur. The lack of a sensitive noninvasive technique to evaluate the equine heart, has resulted in a paucity of objective data on the progression of valvular disease and its relationship to performance.

Haemodynamic monitoring in equine medicine is also limited by the lack of suitable noninvasive techniques. Such techniques would be valuable in the assessment of cardiac function in horses with a history of poor athletic performance. The use of echocardiography in human medicine has greatly improved understanding of blood flow in the normal and diseased heart. It is used for monitoring critically ill patients and also for the assessment of ventricular function.

Little work has been done to evaluate echocardiography in horses. It is hoped that this technique will improve our diagnostic capabilities in cases with valvular disease and provide a means of assessing cardiac performance in this species.

PART 1

CHAPTER 1. REVIEW OF THE LITERATURE

INTRODUCTION

"A horse with valvular disease is certainly unsound, but he is not useless. The general impression seems to be that such an animal will almost certainly fall dead. This is a mistake" (Stockman 1894).

Determining the usefulness and safety of horses with valvular disease is a problem (Holmes 1977). This is due to difficulties in diagnosis (Holmes 1968; Bonagura 1990), and lack of objective data on the progression of valvular disease and its relationship to performance (Brown 1985). Cardiac murmurs can be indicative of valvular disease (Smetzer, Bishop and Smith 1966) but are also present in a large number of clinically normal horses (Patterson, Detweiler and Glendinning 1965).

Routine techniques for the diagnosis and assessment of valvular disease in human medicine are of limited value in horses (Reef 1991a). The use of thoracic radiography, to evaluate cardiac size in mature horses, is difficult (Koblik and Hornof 1985), as only lateral views of the heart can be obtained (Reef 1991a; Farrow 1981), and often the entire heart cannot be fitted onto a single film (Koblik and Hornof 1985; Carlsten 1986). Angiocardiography, although useful in the diagnosis of congenital lesions in foals (Tadmoor, Fischel and Shem Tov 1983; Hinchcliff and Adams 1991), is of limited value in horses whose bodyweight exceeds 400kg (Carlsten, Kvart and Jeffcott 1984). Electrocardiography gives limited information on cardiac disease in horses compared to humans and dogs, because of differences in the ventricular activation process in ungulates (Hamlin and Smith 1965).

Two-dimensional echocardiography uses ultrasound technology to record dynamic images of the heart noninvasively (Feigenbaum 1986). More specialised ultrasound techniques utilizing the Doppler principle, can be used to record the velocity and direction of blood flow in targeted organs (Goldberg, Allen, Marx and Donnerstein 1988). Doppler echocardiography has been shown to be a sensitive

indicator of abnormal blood flow in humans, and is the only noninvasive method of recording valvular regurgitation (Krayenbuehl and Jenni 1985).

Part 1 of this thesis evaluates echocardiography in the assessment of equine heart murmurs.

CARDIAC MURMURS

AETIOLOGY

Murmurs have been described as prolonged audible vibrations occurring during a normally silent period of the cardiac cycle (Reef 1985a) and have been attributed to the presence of turbulent blood flow (Rushmer 1970; Braunwald 1988). In 1883 Reynolds demonstrated that the development of turbulence in fluid systems comprising non-pulsatile flow was dependent on the velocity of flow (v), the diameter of the tubes, and the density (d) and viscosity (v) of the fluid. It was shown that when a critical value was exceeded laminar flow became turbulent. Rushmer (1970) using the following equation, gave the critical value (Reynolds number) for the development of turbulence in blood as 970+/-80.

$$\text{Reynolds number } R = \frac{\text{vessel radius (cm) x velocity (cm/s) x density (gm/ml)}}{\text{viscosity (dyne}^{-s}/\text{cm}^2)}$$

Hatle and Angelsen (1982), using vessel diameter in the above equation instead of radius, quoted a Reynolds number of 2650 for blood in the human aorta.

McDonald (1957) proposed that murmurs were caused by eddy formation rather than turbulence and for murmurs to be loud enough to be heard outside the body, the eddies must cause vibration of solid structures (the chordae tendineae,valve cusps or vessel walls). He further suggested that sound may be produced by the formation and collapse of gas bubbles within the circulation (cavitation).

Bruns (1959) suggested that it was theoretically unlikely that turbulent blood flow, due to its random nature, could generate sufficient acoustical energy to produce murmurs. He also excluded cavitation as a cause of cardiac murmurs, because experimental evidence showed that cavitation did not occur in flowing blood unless

the driving pressure exceeded 350mmHg. He proposed that murmurs were caused by pressure fluctuations in the wake produced as blood flows over an obstacle. Vortices shed as blood flows over a cylinder cause the wake to fluctuate periodically. Brun suggested that this non random flow pattern was an efficient method of sound production and that small non circular obstacles were capable of shedding vortices and thus producing cardiac murmurs. He concluded that sound was produced by fluctuations in the wake itself, and that any vibrations in the structures of the cardiovascular system were secondary.

The theoretical mechanisms for generating cardiovascular sound have been summarised (Rushmer and Morgan 1968). These workers believed that the most common cause of murmurs were eddies occurring at the boundaries of turbulent jets produced by blood flowing at high velocity through a narrow orifice into a wide channel. The eddies strike the vessel walls resulting in vibrations, which are transmitted to the body surface. Murmurs were also thought to be produced by jets directly striking vessel or chamber walls. They believed that vortex shedding and periodic wake fluctuations would produce musical murmurs with fundamental frequencies, rather than the more common noisy murmurs which have a wide spectra of frequencies. As musical murmurs are rare, they discounted Bruns' theory that periodic wake fluctuation was generally the cause of murmurs. Bruns (1959) however had suggested that where an obstruction or orifice had slight variations in its diameter, or was not smoothly constructed, noise comprising many frequencies would be generated, rather than a clear note with fundamental frequencies.

Sabbah and Stein (1976) investigated the association of turbulent blood flow and cardiac murmurs. Intra-arterial sound recorded during left ventricular ejection in thirteen humans was always associated with highly turbulent or disturbed flow, whereas no intra-arterial sound was recorded when minor flow disturbances were present. Having demonstrated an association between turbulent flow and ejection murmurs, the authors used Reynolds' equation to explain the characteristic crescendo

- decrescendo shape of murmurs produced during ventricular ejection, and the effects of increased flow states (anaemia and exercise) on sound production.

PHYSIOLOGICAL MURMURS

Systolic murmurs

Highly disturbed flow velocities have been recorded in the aorta of conscious and anaesthetised horses (Nerem, Rumberger, Gross, Hamlin and Geiger 1974). Phonocardiograms recorded from the surface of the pulmonary artery and aorta of lightly anaesthetised horses have revealed a murmur during the first third to two thirds of systole (Patterson, Detweiler and Glendenning 1965). In the same study similar systolic murmurs were recorded transthoracically over the pulmonary, aortic and mitral valve areas in a number of clinically healthy horses. Low intensity systolic murmurs could be heard in a high percentage of the horses studied, but these were not detected using the phonocardiograph. This probably reflects the extreme sensitivity of the human ear (Wirt 1965). Brown and Holmes (1979a) recorded systolic murmurs intra-arterially at the root of the aorta and pulmonary artery in conscious horses. Murmurs were recorded from the aorta in all six horses studied and from the pulmonary artery in five of six horses studied. External phonocardiography demonstrated a systolic murmur in only one of these horses (Brown and Holmes, 1979b). It was not stated whether systolic murmurs were audible on auscultation in the remaining horses. Other workers have described the presence of systolic murmurs in clinically normal horses. Niemetz (1924) found that of 418 horses studied, 66.2% (sic) had a systolic murmur. Perevezentsev (1940) also described functional systolic murmurs audible over the aorta and pulmonary artery. These murmurs were found to change in character following exercise, some disappearing and some becoming more accentuated. Other workers have described the appearance of, or increase in amplitude of, systolic murmurs during excitement (Patterson, Detweiler and Glendenning 1965). Littlejohn and Button (1982) described such murmurs as normal heart sounds of the horse and advised against the use of the word murmur due to

unnecessary doubts raised about a horse's soundness when the word murmur is used. Sudakov (1959) considered a systolic murmur to be a normal finding in Russian thoroughbreds.

Diastolic murmurs

Diastolic murmurs have also been recorded in apparently normal horses. These have been reported as presystolic or early diastolic. In a study of 94 clinically healthy horses by Glendinning (1964), a characteristic early diastolic murmur was heard in ten Thoroughbred horses. The murmur was described as a faint high pitched squeak which occurred just after the second heart sound during the rapid filling phase of the left ventricle. Other authors have recorded the presence of diastolic murmurs in the absence of demonstrable heart disease (Smith, Smetzer, Hamlin and Watsabaugh 1962). They are most often reported as being of short duration (Muylle and Oyaert 1980), beginning with or immediately following the second heart sound (Detweiler and Patterson 1972) and extending to or just beyond the third heart sound (Patterson, Detweiler and Glendenning 1965). Nerem *et al.* (1974) recorded highly disturbed flow in the left ventricle of conscious horses during the rapid filling phase of the ventricle.

Presystolic murmurs occurring between the fourth and first heart sound have also been reported in clinically healthy horses (Patterson, Detweiler and Glendenning 1965; Detweiler and Patterson 1972; Muylle and Oyaert 1980; Reef 1989). It has been suggested that this sound is associated with blood flow through the atrioventricular orifice during atrial systole (Patterson, Detweiler and Glendenning 1965).

PATHOLOGICAL MURMURS

Systolic murmurs

Systolic murmurs have been classified as either ejection murmurs audible in midsystole, or regurgitant murmurs audible throughout systole (Leatham 1955).

Ejection murmurs

Ejection murmurs occur when there is obstruction to ventricular outflow (valve stenosis), increased flow through the aortic and pulmonary valve, valvular damage without stenosis or dilation of the vessel beyond the valve (Leatham 1958). Aortic and pulmonary stenosis although common in man (Braunwald 1988) and dogs (Olivier 1988) have been rarely reported in horses (Bonagura 1990). Pulmonary stenosis has been reported in foals with multiple cardiac abnormalities (Reynolds and Nicholl 1978; Bayly, Reed, Leathers, Brown, Traub, Paradis and Palmer 1982) but has only recently been reported as an isolated finding (Hinchcliff and Adams 1991).

There is confusion in the veterinary literature as to the incidence of aortic stenosis in horses. Congenital stenosis of the aortic valve is considered to be a rare occurrence (Patterson, Detweiler and Glendenning 1965; Muylle and Oyaert 1980; Glazier 1984; Reef 1989). However stenosis may develop in cases of endocarditis (Reef 1989; Bonagura 1990). In a post-mortem study of 1557 horses (Else and Holmes 1972), lesions were found on the aortic valves in 331 horses (20%). The types of lesions identified were nodules, fibrous bands or fenestrations. Nodules ranged from simple smooth nodules in the commisural angles, to extensive large nodules occupying the bodies of the cusps. Fibrous bands ranged from minimal to large. The latter group caused severe cusp distortion. By correlation of these post-mortem findings to clinical auscultation Holmes and Else (1972) considered 21 of the 195 horses examined (11%) to have aortic stenosis. It was not stated however, whether the valve lesions resulted in narrowing of the aortic orifice, or whether the murmurs auscultated were caused by normal blood flow, or turbulence produced as the blood flowed over the irregular valve surface. Holmes (1966) reported a case of aortic stenosis in a pony, causing obstruction to blood flow which was confirmed at post-mortem. A systolic murmur caused by relative aortic stenosis, due to increased blood flow through the valve, has been reported in a case with severe aortic insufficiency (Brown and Holmes 1979b).

Regurgitant murmurs

Systolic regurgitant murmurs are caused by the flow of blood from a chamber or vessel that is at a higher pressure throughout systole than the receiving vessel or chamber (Leatham 1958). Systolic regurgitant murmurs result from regurgitation of blood through the mitral valve, tricuspid valve and through ventricular septal defects. Leatham (1958) proposed that because ventricular pressure is elevated throughout systole, regurgitant systolic murmurs were always maintained throughout systole (pansystolic). Recent knowledge has indicated that regurgitant murmurs are not always pansystolic but may vary greatly (Braunwald 1988).

Mitral regurgitation has been reported to be a cause of systolic murmurs in horses (Patterson, Detweiler and Glendenning 1965; Muylle and Oyaert 1980) Causes of mitral regurgitation include rupture of the chordae tendineae of the valve (Brown, Bell, Paradis and Breeze 1983; Holmes and Miller 1984), thickening and rounding of the valve leaflets (Miller and Holmes 1985) and papillary muscle dysfunction (Miller and Holmes 1984). Miller and Holmes (1985) proposed that the increased volume of blood flowing into the left ventricle during diastole results in compensatory dilation of the ventricle which further aggravates the mitral insufficiency by changing the position of the papillary muscles relative to the mitral valve. In cases of severe mitral regurgitation the third heart sound is exaggerated and a pronounced flow murmur is heard in diastole due to the increased inflow into the left ventricle (Littlewort 1977; Holmes and Miller 1984; Miller and Holmes 1985).

Tricuspid regurgitation also causes a systolic murmur in horses (Patterson, Detweiler and Glendenning 1965; Muylle and Oyaert 1980; Reef 1989). In the post-mortem study of Else and Holmes (1972) lesions found on the tricuspid valve and mitral valve were similar. These consisted primarily of fibrous thickenings of the valve cusps, which ranged from localised nodules to generalised diffuse thickening of the valve. The incidence of lesions on the tricuspid valve (2%), was lower than for the mitral valve (5%). Comparison of post-mortem findings to clinical auscultation,

suggested that only six horses of 195 horses examined, had tricuspid regurgitation, whereas fifteen horses were thought to have mitral regurgitation. However murmurs were only attributed to a particular valve if lesions were present on the valve at post-mortem (Holmes and Else 1972). In an earlier report, Stockman (1894) suggested that dilation of the right heart without anatomical changes of the valves, resulted in tricuspid regurgitation, which in his opinion was "by far the most common affection of the heart in the horse". Reef (1990b) supports this view, stating that murmurs of tricuspid regurgitation are the most common finding in horses presenting for cardiac evaluation.

Murmurs associated with ventricular septal defects

Flow of blood through a ventricular septal defect also results in a systolic murmur (Braunwald 1988). The ventricular septal defect is the commonest congenital heart defect in horses (Reef 1985b). Blood flows from the left side of the heart across the defect to the right side causing a systolic murmur. The increased blood flow into the right ventricle leads to increased flow through the pulmonary artery causing an ejection murmur (Reef 1985b). This is a murmur of relative pulmonary stenosis (Reef 1989).

Diastolic murmurs

Diastolic murmurs can be divided into three categories, atrial systolic murmurs (presystolic), ventricular filling murmurs and regurgitant murmurs from incompetent aortic and pulmonary valves (Leatham 1958).

Atrial systolic murmurs and ventricular filling murmurs, have been reported in clinically healthy horses (page 7). Ventricular filling murmurs have also been reported in association with severe mitral regurgitation (page 9). Mitral and tricuspid stenosis are a recognised cause of diastolic murmurs in man (Braunwald 1988), but occur rarely in horses (Glendinning 1972; Detweiler and Patterson 1972) although

they have been reported in foals as part of complex congenital disorders (Bayly *et al.* 1982).

Aortic insufficiency is a common condition in horses, especially in older animals (Glazier 1984; Bonagura 1985; Reef 1989). In the post-mortem study by Else and Holmes (1972), severe distortions of the aortic valve were associated with jet lesions, indicative of significant valvular regurgitation. Bishop, Cole and Smetzer (1966) studied fifteen equids with aortic insufficiency and also demonstrated thick fibrous bands on the insufficient valve cusps. These authors described nodular lesions similar to those reported by Else and Holmes (1972) but considered that these did not contribute to valvular insufficiency. Pulmonary insufficiency can occur in horses but is rarely associated with an obvious murmur (Reef 1989).

DIAGNOSIS OF CARDIAC MURMURS

The differentiation of the various cardiac murmurs in horses is difficult (Glendinning 1972; Reef 1991a), especially systolic murmurs (Miller and Holmes 1985) which are the most common murmurs and can arise from a number of sites. Difficulty in diagnosis of systolic murmurs has also been reported in humans (Hoffmann and Burckhardt 1983).

Location

The point of maximum intensity of a cardiac murmur is generally located over the site of turbulence (Braunwald 1988), and gives an indication of the origin of the murmur (Stockman 1894; Littlewort 1962; Reef 1985a; Bonagura 1990). The direction in which a murmur radiates over the body surface is also thought to be helpful in localizing the site of origin of a murmur (Detweiler and Patterson 1972). The systolic murmur associated with a ventricular septal defect is most commonly heard on the right side of the horse, cranially at the third or fourth intercostal space radiating ventrally towards the sternal border. An associated systolic ejection murmur

is often heard over the base of the left heart at the level of the pulmonary valve (Reef 1985b).

The point of maximum intensity of a murmur of tricuspid regurgitation is described as over the third or fourth intercostal space on the right side of the thorax (Glazier 1984; Reef 1989; Bonagura 1990) and is rarely most intense over the left cranial heart border (Bonagura 1990). Other authors describe the murmur as being most obvious over the left side of the thorax at the third intercostal space (Stockman 1894; Detweiler and Patterson 1972; Holmes and Else 1972). Glendinning (1972) disagreed with Holmes that the tricuspid valve is situated cranially and on the left of the thorax, and stated that "the tricuspid valve was undoubtedly on the right side of the midline". In a case of tricuspid regurgitation described by Holmes and Else (1972) the murmur of tricuspid regurgitation could be heard on both sides of the thorax. Holmes (1966) considered that auscultation of the right side of the thorax provided less useful information on cardiac disease but was of value in detecting, or confirming, lesions involving the tricuspid valve. As Reef (1990b) considered tricuspid regurgitation to be the most common finding in horses presenting for cardiac evaluation, auscultation of the right side of the thorax should be informative.

The murmur of mitral insufficiency is most often heard over the left side of the thorax in the mitral area or left apex (Glazier 1984; Reef 1989; Bonagura 1990). In some cases the murmur radiates over a wide area on the left side of the chest (Brown *et al.* 1983; Holmes and Miller 1984; Miller and Holmes 1985). The murmur of mitral regurgitation radiates dorsally to the upper third of the thorax (Detweiler and Patterson 1972; Muylle and Oyaert 1980) or caudally (Stockman 1894) and in some cases can be heard on the right side of the thorax (Holmes and Miller 1984).

The systolic ejection murmur described in normal horses (page 6) is most commonly auscultated at the left heart base over the aortic and pulmonary areas (Patterson, Detweiler and Glendenning 1965; Detweiler and Patterson 1972). When ejection murmurs are loud however, they can be transmitted to the cardiac apex,

where they must be distinguished from the murmur of mitral regurgitation (Leatham 1958).

Timing

Further differentiation of the causes of cardiac murmurs is gained from their timing within the cardiac cycle (Littlewort 1962; Holmes 1968; Detweiler and Patterson 1972). Ejection murmurs are midsystolic and end before the second heart sound (Leatham 1958; Braunwald 1988) whereas regurgitant murmurs may occur at any time during systole and may extend beyond the second heart sound. Diastolic murmurs associated with ventricular filling end, at or just beyond the third heart sound (page 7), whereas the murmur of aortic insufficiency is often present throughout diastole (Bishop, Cole and Smetzer 1966; Smetzer, Bishop and Smith 1966).

Frequency

The frequency or quality of a murmur may also indicate the underlying cause (Littlewort 1962; Reef 1985a). Smetzer, Bishop and Smith (1966) described the murmur associated with aortic insufficiency in 24 equids. The diastolic murmur was completely or predominantly musical in thirteen cases (54%) and was described as noisy in the remainder. The diastolic murmur observed in healthy young horses by Glendinning (1964) was described as a high pitched squeak.

Intensity

Murmurs may also be differentiated by their intensity (Littlewort 1962; Reef 1985a). The murmur of aortic insufficiency decreases in intensity throughout diastole and may increase in intensity immediately following the fourth heart sound (Smetzer, Bishop and Smith 1966; Bishop, Cole and Smetzer 1966). Systolic ejection murmurs often increase in intensity reaching a crescendo in midsystole and then decrease (Leatham 1958; Patterson, Detweiler and Glendenning 1965), whereas regurgitant murmurs are usually of constant intensity (Detweiler and Patterson 1972) and are

described as band shaped (Reef 1985a). The intensity of heart murmurs is graded either using a grade 1-6 system (Levine and Harvey 1949) or a 1-5 system (Detweiler and Patterson 1972). Grade 4-5/5 and 5-6/6 murmurs are associated with a palpable precordial thrill and are thought always to be associated with significant cardiovascular dysfunction (Fregin 1978). The significance of the intensity of a murmur is debatable. Some authors believe that innocent murmurs in healthy horses are always grade 3/5 or less (Fregin 1978; Reef 1985a). However, Brown (1985) proposed that not all serious valvular lesions cause loud murmurs and not all loud murmurs arise from serious valvular lesions. Brown suggested that widely radiating murmurs are likely to arise from significant lesions, whereas very localized murmurs are often not associated with significant lesions. This concept is supported by Holmes (1977), who also attributes significance to the timing of murmurs within the cardiac cycle. He suggested that murmurs which are present throughout systole are more likely to be significant than those present only in early or late systole. Else however, guarded against placing too much significance on the timing of murmurs (Glendinning 1972).

Although auscultation remains one of the most valuable methods of identifying equine heart disease (Holmes 1968; Bonagura 1990), auscultation cannot always differentiate between pathological and non-pathological murmurs (Littlewort 1962). Brown (1985) suggests that the recent expansion of echocardiography will hasten diagnosis and improve our understanding of equine valvular disease.

TWO-DIMENSIONAL AND M-MODE ECHOCARDIOGRAPHY

HISTORY AND DEVELOPMENT

The term echocardiography, describes the examination of the heart using ultrasound (Braunwald 1988). The history of the development of ultrasound in medical diagnosis has been reviewed by Edler (1961; 1990). Keidel (1950) first used ultrasound to study the heart. He directed an ultrasound beam through the chest, and recorded the residual intensity of the sound on the opposite side of the thorax. He was able to show variations in intensity of the sound coinciding with each heart beat but was unable to quantify these changes.

In modern echocardiographic techniques, the transmitter and receiver are positioned on the same side of the thorax combined in a single transducer (Feigenbaum 1986). This method was described in 1949 by Ludwig and Struthers, to detect gallstones and foreign bodies in living tissue. However they erroneously concluded from these studies, that the sound reflections were too erratic to be of practical value (Edler 1990).

Edler and Hertz (1954) used ultrasound to record the distance from the anterior thoracic wall to the posterior wall of the heart and were able to identify individuals with enlarged hearts. Ultrasound was reflected from the interfaces between the heart wall and blood, and the resultant echoes were displayed as vertical signals along the x axis of a cathode ray tube screen. The distance of the vertical signals along the x axis represented the distance between the transducer and the reflecting interface. The height of the vertical signal represented the intensity of the returning echo. This technique is termed "A-mode" (amplitude mode) echocardiography (Feigenbaum 1986). To record the movement of the heart walls, a continuous photographic recording was taken of the changes on the A-mode echocardiogram. The distance of the reflecting surfaces was displayed on the y axis, with time on the x axis. This technique is termed "M-mode" (motion mode) echocardiography (Feigenbaum 1986). The authors were now able to measure the

movements of the heart walls in normal and enlarged hearts, and demonstrated increased movement in a patient with aortic regurgitation. To improve their ability to interpret their recordings a synchronous ECG was recorded.

Two-dimensional real-time echocardiography was first introduced by Hertz in 1960 (Edler 1990). The ultrasound crystal was oscillated to and fro, to sweep the ultrasound beam through the heart (Hertz 1967). The display of the cathode ray tube was moved in a similar way, the reflected echos being displayed as changes in intensity of the electron beam. Unfortunately the objects investigated by this method had to be immersed in a water-bath, and therefore the clinical applications of the technique were limited. Modifications of the system, allowed in vivo images to be recorded of the mitral valve and other cardiac structures (Hertz 1967). Hertz was forced to discontinue his studies on two-dimensional echocardiography due to lack of research grants, as at this time diagnostic ultrasound was considered to be valueless (Edler 1990).

Ultrasound technology has advanced dramatically since these early studies (Wilde 1989), and recently the use of three-dimensional ultrasonography has been reported (Kisslo 1991).

CLINICAL INDICATIONS

Human medicine

In human medicine two-dimensional and M-mode echocardiography are used extensively to evaluate cardiac disorders, and has proved especially useful in the differentiation of functional or benign murmurs from those associated with valvular disease (Popp 1990a). The combined use of M-mode echocardiography with its rapid sampling rates and two-dimensional echocardiography with its greater spatial orientation, allows excellent visualisation of valve motion and shape (Feigenbaum 1986). Valvular stenosis can be easily identified (Scovil and Linhart 1982; Joyner and Montello 1982) and the severity quantified (Feigenbaum 1986; Carmo, Schapira and Harold 1990).

Valvular regurgitation can be inferred from two-dimensional and M-mode images, by identifying anatomical abnormalities, abnormal movement or associated pathology (Carmo, Shapira and Harold 1990). High frequency diastolic fluttering of the mitral valve is a reliable diagnostic feature of aortic regurgitation. This is caused by the regurgitant jet of aortic insufficiency striking the anterior mitral valve leaflet (Feigenbaum 1986; Carmo, Shapira and Harold 1990). Dilation of the left ventricle and increased left ventricular wall motion may also be evident in cases of aortic insufficiency (Feigenbaum 1986). However these findings are not specific and will occur in any condition causing volume overload of the ventricle, including mitral regurgitation (Carmo, Shapira and Harold 1990). Left atrial enlargement is often demonstrated in cases of mitral regurgitation, and can be used to assess severity (Gehl, Mintz, Kotler and Segal 1982). M-mode and two-dimensional images may aid diagnosis of the cause of mitral regurgitation. Systolic flutter of the mitral leaflet on M-mode images is indicative of chordal rupture (Meyer, Frank, Goldberg, Cheng 1977), and flail valve leaflets may be visualised by two-dimensional echocardiography in these cases (Feigenbaum 1986).

Echocardiography is considered to be the procedure of choice in the diagnosis of congenital cardiac lesions, including ventricular septal defects (ACC/AHA task force report 1990; Popp 1990b).

There has been an increase in the complexity of measurements derived from M-mode studies. Sahn, DeMaria, Kisslo and Weyman (1978) compiled a survey to determine the measurement criteria used by various echocardiographers and to identify those which resulted in the most reproducible measurements. This resulted in new recommendations for the standardisation of M-mode measurements. Normal M-mode values for various chamber and vessel dimensions have been reported (Feigenbaum 1986).

With increasing use of two-dimensional echocardiography, a committee was formed to standardise nomenclature and image orientation (Henry, DeMaria,

Gramiak, King, Kisslo, Popp, Sahn, Schiller, Tajik, Teichholz and Weyman 1980). Later Schnittger, Gordon, Fitzgerald and Popp (1983) described a standardised technique for intracardiac measurements from two-dimensional echocardiography which resulted in reproducible measurements.

Veterinary medicine

General medicine

Lindahl (1966) first reported the use of ultrasound as a diagnostic aid in veterinary science. Prior to this ultrasound had been used primarily for meat inspection applications (Lamb, Stowater and Pipers 1988). The first report of echocardiography in horses described the M-mode technique (Pipers and Hamlin 1977) using an unguided transducer. Various cardiac dimensions were measured in 25 normal sedated horses using a systematic M-mode examination similar to the standardised technique described in humans (Feigenbaum 1986). Other workers have since used this same technique to record various normal cardiac dimensions in foals (Lombard, Evans, Martin and Tehrani 1984; Stewart, Rose and Barko 1984) and unsedated horses (Lescure and Tamzali 1984; Paull, Wingfield, Bertone and Boon, 1987). Similar M-mode studies have been described in cats (Jacobs and Knight 1985; Pipers, Reef and Hamlin 1979), dogs (Boon, Wingfield and Miller 1983), pigs (Pipers, Hamlin and Muir 1978; Gwathmey, Nakao, Come and Abelmann 1989), calves (Amory, Jakovljevic and Lekeux 1991) and sheep (Moses and Ross 1987).

Equine medicine

M-mode echocardiography has proved useful in horses for the diagnosis of ventricular septal defects (Pipers, Hamlin and Reef 1979; Lombard, Scarratt and Buergelt 1983), valvular endocarditis (Pipers, Hamlin and Reef 1979; Bonagura and Pipers 1983), aortic insufficiency (Clark, Reef, Sweeney and Lichtensteiger 1987) pericardial effusion, mitral valve prolapse (Pipers, Hamlin and Reef 1979) and for assessing mitral valve motion in cases of atrial fibrillation (Wingfield, Miller, Voss, Bennett and Breukels 1980). M-mode echocardiography has also been used to

evaluate changes in cardiac function in horses following strenuous endurance competition (Bertone, Paull, Wingfield and Boon 1987).

O'Callaghan (1985) used measurements derived from M-mode studies to estimate heart weights in horses. This study showed that M-mode measurements of wall thickness and chamber dimensions did not correspond to post-mortem measurements. O'Callaghan concluded that the exsanguinated heart in rigor mortis was a poor control against which to compare in vivo measurements.

The diagnostic value of two-dimensional echocardiography in equine cardiology has been reported (Bonagura, Herring and Welker 1985; Rantanen, Byars, Hauser and Gaines 1984; Reef, Klumpp, Maxson and Sweeney 1990). Reef and Spencer (1987) described the two-dimensional and M-mode findings in 23 horses with aortic insufficiency. Intracardiac dimensions were compared with those from normal horses (Pipers and Hamlin 1977). The mean internal diameters of the left ventricle and the aortic root were increased. The left ventricular free wall thickness was decreased. The shortening fraction of the left ventricle, an index of systolic function (Bonagura, Herring and Welker 1985) was increased. The mean bodyweight of the normal horses was only 300 kg (Pipers and Hamlin 1977). This is lower than the horses studied by Reef and Spencer (1987). However, the mean ventricular diameters of the horses with aortic insufficiency were larger than values reported for larger normal horses (Lescure and Tamzali 1984). Two of the horses with aortic insufficiency also had mitral regurgitation, which could have resulted in dilation of the left ventricle (Bonagura, Herring and Welker 1985; Braunwald 1988). Gross dilation of the left ventricle has been demonstrated by two-dimensional and M-mode echocardiography in cases with severe mitral valve disease (Reef 1987; Dedrick, Reef, Sweeney and Morris 1988). Diastolic vibration of the mitral valve as described in humans with aortic insufficiency (Feigenbaum 1986) was seen in all of the horses with aortic insufficiency (Reef and Spencer 1987). This feature has also been reported by Pipers, Hamlin and Reef (1979). Thickening of the aortic valve cusps

was evident in eighteen of the 23 horses studied (Reef and Spencer 1987), with two horses showing lesions thought to be associated with vegetative endocarditis. The authors suggested that the thickened valve cusps evident by echocardiography corresponded to the fibrous band lesions and nodular lesions described previously at post-mortem (Bishop, Cole and Smetzer 1966; Else and Holmes 1972).

Abnormal valve motion has been demonstrated by M-mode and two-dimensional echocardiography in two foals with rupture of the mitral chordae tendineae. However, abnormal motion is not always detected in these cases (Reef 1987). Other forms of valvular dysfunction have been demonstrated by two-dimensional echocardiography. Reimmer, Reef and Sommer (1991) described pulmonic valve rupture in a horse with right sided heart failure. The authors described an increased right ventricular internal dimension during systole and diastole in this case, and a reduced left ventricular dimension in systole and diastole. Normal values were given for comparison with the cardiac dimensions reported, although the source of the normal values was not indicated. The normal values quoted in this study have also been reported by Reef (1990a).

Vegetative masses sited on the atrial wall (Collatos, Clark, Reef and Morris 1990), aortic valve (Hillyer, Mair and Holmes 1990) and mitral valves (Dedrick *et al.* 1988) have been demonstrated by two-dimensional echocardiography in horses with endocarditis.

The use of echocardiography to diagnose congenital cardiac abnormalities has been reviewed (Reef 1991b). Two-dimensional and M-mode echocardiography enabled a diagnosis of tricuspid atresia to be made pre-mortem in two foals with cardiac failure (Reef, Mann and Orsini 1987). In both cases the normal two-dimensional image of the tricuspid valve leaflets was replaced by a thick band of echoes. Both foals had ventricular septal defects which were visible on the two-dimensional images. Pipers, Reef and Wilson (1985) have previously reported the

use of two-dimensional and M-mode echocardiography in the diagnosis of ventricular septal defects in a foal and a two year old filly.

Two-dimensional echocardiography has aided the diagnosis (Freestone, Thomas, Carlson and Brumbaugh 1987; Voros, Felkai, Szilagyi and Papp 1991) and treatment (Voros *et al.* 1991) of pericardial effusion in horses.

Despite the extensive use of two-dimensional ultrasonography in clinical diagnosis in horses, standardised image planes and standard views have not been described in this species as they have in humans (Henry *et al.* 1980; Tajik, Seward, Hagler, Mair and Lie 1987) and dogs (Thomas 1984).

Carlsten (1987) used standardised intercostal locations to describe two-dimensional long-axis (longitudinal) and short-axis (cross-sectional) images from the left and right hemithorax of ten horses. Images could not be produced from two further sites, the thoracic inlet and a cranial abdominal site, which are commonly used for human echocardiography (Feigenbaum 1986). Ten cardiac structures were identified, and the image quality of these structures was assessed from each of the standardised locations. A scoring system was devised for image quality, and this was used to determine the optimal transducer location to view each structure. However, the different cardiac structures were not identified from the same intercostal location in all horses.

Stadler, D'Agostino and Deegan (1988) used the eight standardised transducer locations of Carlsten (1987) to obtain two-dimensional images of the heart in twenty warmblood horses with and without cardiac disease. The authors described the transducer rotation and beam angulation required to produce various images. However, the images were not standardised by reference to intracardiac landmarks.

Voros, Holmes and Gibbs (1990) used intracardiac landmarks to standardise nine image planes of the equine heart. Images were obtained from fifteen equine hearts formalised and suspended in a water tank. Five of the hearts had been studied in vivo using the transducer positions described by Carlsten (1987), prior to

euthanasia of the horse. During in vitro imaging, cardiac structures were located and identified using flexible probes. Measurements were made of various cardiac dimensions from four views. The hearts were then sliced along these image planes, photographs were taken, and comparative measurements were made from drawings obtained from the photographs. The authors demonstrated a good correlation between in vitro anatomical and echocardiographic data. However they did not assess the accuracy of the echocardiographic measurements. Differences in shape and size of the cardiac chambers and vessels were noted between in vivo and in vitro images. These differences were ascribed to over contraction of the exsanguinated dead heart. This was also suggested by O'Callaghan (1985), to explain differences between M-mode measurements recorded in life and anatomical measurements at post-mortem.

Voros, Holmes and Gibbs (1991) described the use of four of their standardised views to measure selected cardiac dimensions in 22 normal standing horses. Satisfactory images were obtained in eighteen of the 22 horses, but not in horses whose body weight was greater than 580kg. Measurements from this two-dimensional study were compared with the results of previous M-mode studies (Pipers and Hamlin 1977; Lescure and Tamzali 1984; O'Callaghan 1985). The two-dimensional measurements were found to be similar or slightly larger than previously described. It was suggested that this was due to variations in breed and body weight of horses in the different studies. Cardiac dimensions measured from M-mode studies differ significantly between different breeds of horses (Lescure and Tamzali 1984).

DOPPLER ECHOCARDIOGRAPHY

CHRISTIAN ANDREAS DOPPLER - A BRIEF BIOGRAPHY

Christian Doppler was born in Salzburg on November 29th 1803 (Eden 1985; Pasquale and Paulschock 1991) and not in 1805 as stated by White (1982) and Andrade (1959). He was christened Christian Andreas Doppler (Eden 1986) taking his middle name from the male saint's day closest to his birthday (Eden 1985). He is often incorrectly referred to as Christian Johann Doppler (Hatle and Angelsen 1982; Feigenbaum 1986; Edler 1990; Long 1990) or Johann Christian (White 1982; Goldberg *et al.* 1988). He usually signed his name simply as 'Doppler', and in formal documents added only his first name (Eden 1985).

Doppler was educated in mathematics and physics in Vienna, returning to Salzburg to complete his studies in science and philosophy (Eden 1986). He taught mathematics at Vienna "in a subordinate position" (Andrade 1959) until 1833, but unable to obtain a further teacher's position started work as a clerk in a cotton mill (Eden 1986). He was on the point of emigrating to America to find more suitable work when he was appointed Professor of Mathematics at the state secondary school at Prague, rejecting a second offer from Bern (Eden 1986).

Christian Doppler married Mathilda Sturm, a native of Salzburg in 1836 and together had five children. It is suggested by Eden (1986) that the obligations of marriage led Christian Doppler to take on the additional post of Supplementary Professor of Higher Mathematics at the technical Institute in Prague. It was during these years that he contracted the pulmonary tuberculosis from which he later died, "his by no means very strong physique could not bear the strain of so many long hours of lectures in small rooms, overfilled with students" (Eden 1986).

He became a full Professor of Mathematics and practical Geometry at Prague in 1841, where he remained until 1847 when he was appointed Professor of Mathematical Physics and Mechanics at the mining academy of Schemnitz (Andrade, 1959; Eden 1986). Doppler was now recognized as a man of distinction (Andrade

1959). He was given an Honorary Doctorate by the University of Prague, and was elected a member of the Academy of Sciences in Vienna and the Royal Bohemian Society of Sciences (Andrade 1959; Eden 1986). In 1850 he reached his academic goal (Andrade 1959; Eden 1986), by a decree of the Emperor Franz Josef I he was appointed to the Chair of Experimental Physics at the University of Vienna where he founded and directed an Institute of Physics. The esteem in which he was apparently held (Andrade 1959; Eden 1986) is contradicted by White (1982) who considers the paucity of knowledge about Doppler's personality is a reflection of the lack of regard felt for him by his colleagues. However this paucity of knowledge may reflect inadequate research by the author, as Eden (1985), reports a number of factual errors in the biography.

Christian Doppler died in San Giovanni in Venice in 1853 (Eden 1985), not in Vienna as reported by White (1982). The City of Venice provided a grave of honour and the physicists of Venice erected a memorial in the colonnades of the cemetery (Eden 1986). However, the author was unable to find the grave and no legible wording remains on the memorial tablet. It is suggested that Doppler's body may have been returned to Saltzburg or Vienna for burial (Eden 1985).

THE DOPPLER PRINCIPLE

Doppler echocardiography is an ultrasound technique which uses the Doppler principle to determine the direction and velocity of blood flow within the heart and great vessels. The Doppler principle was proposed by Christian Doppler in a paper read to the Royal Bohemian Society of Sciences in May 1842, and was published in 1843. Doppler proposed that when a wave source and observer are stationary, the frequency of the wave perceived by the observer is equal to the frequency of the emitted wave. However where an observer is moving towards a wave source the frequency and intensity of the wave increases for the observer, whereas if the observer is moving away from the wave source the perceived frequency and intensity of the wave decreases. A similar change in frequency (frequency shift) was proposed

in situations where there was movement of the wave source itself. Doppler calculated the velocity required of an observer, moving towards a sound source to distinguish a change in pitch of the sound. However the main aim of the publication was to apply his theory to the phenomena of light, to explain a series of nine astronomical observations.

The fallacy of Doppler's arguments in relation to his astronomical observations has been reviewed by a number of authors (Andrade 1959; White 1982; Eden 1986). However the principle he proposed and his theoretical observations on sound have been shown to be correct (Andrade 1959; Jonkman 1980; Eden 1986). The observations on sound were validated in 1845 by a Dutch scientist Buys Ballot (Jonkman 1980). Ballot was one of many critics who doubted Doppler's theory (White 1982). Aiming to test Doppler's calculations regarding sound, he placed two musicians playing the same note, one on a flat rail car pulled by a locomotive and one alongside the track. The note appeared half a tone higher when the train was moving towards the observer and half a tone lower when the train was moving away. Later experiments involving larger groups of musicians and more observers, showed that the Doppler effect could be clearly demonstrated and roughly measured (Jonkman 1980). However despite his findings Ballot still objected to Doppler's theory, which remained discredited for many years despite further verification of the acoustical Doppler effect (White 1982). In 1867 the British physicist Tyndall stated "the ingenuity of the theory is extreme but its correctness is more than doubtful" (Andrade 1959).

In spite of the criticism of his theory, Doppler appeared appreciative of the attention his paper had received, and remained confident in its worth. In a paper commenting on the results of the experiments of Buys Ballot he stated, "I still hold the trust, indeed stronger than ever before that in the course of time this theory will serve astronomers as a welcome help to probe the happenings of the universe at times when they feel deserted by all other methods. The not insignificant interest that has

already been shown in this theory fills me with joyful confidence and the danger has passed for it to be put to one side, untested and unnoticed, perhaps to sink into oblivion" (Eden 1986). This confident statement is in contrast to the suggestion of Andrade (1959) who proposes "the modest Austrian would be astonished to see the scope and importance of work which in his lifetime aroused but little attention".

APPLICATIONS IN CARDIOLOGY

The contribution of the Doppler effect to the study of the cardiovascular system is summarized by Pasquale and Paulschock (1991) and its history reviewed by Edler (1990).

Continuous wave and pulsed wave Doppler echocardiography

Satomura (1956) first reported the use of the Doppler effect to detect movements of the heart (Edler 1990). The frequency shift of a continuous sound beam was determined as it was reflected by the moving structures. The Doppler frequency shift from a continuous sound wave was later used to detect the flow of blood in a model (Franklin, Schlegel and Rushmer 1961). The Doppler frequency shift was found to be linearly related to the instantaneous blood flow velocity during both steady state and pulsatile flow. The transducer housing the transmitter and receiver crystal was then clamped around the aorta of an anaesthetised dog, and typical flow patterns were recorded. This equipment did not detect the direction of flow, and the authors expressed concerns that the sonic intensity required to produce detectable reflected sound waves may cause haemolysis of blood. This was not substantiated.

Light (1969a) described the use of a continuous sound beam to record the blood flow velocity in the human aorta. It was suggested that the instrument could be used to measure blood acceleration which was considered to be an index of myocardial strength, to identify high flow velocities in cases of aortic stenosis and by

integration of the velocity waveform, estimate cardiac output. The velocity of flow was determined from the Doppler frequency shift by the formula;

$$V = \frac{c \ \Delta F}{2F_0 \cos\theta}$$

F_0 = frequency of sound emitted by the transducer.

ΔF = Doppler frequency shift

V = velocity of the moving target.

c = speed of sound in tissue.

$\cos\theta$ = cosine of the angle of the ultrasound beam and blood flow.

It was suggested that when the angle between the ultrasound beam and the blood flow was less than 25°, absolute values of blood flow velocity could be calculated by assuming the cosine of the angle equalled unity. Other authors suggest this assumption should only be made at angles less than 20° (Goldberg *et al.* 1988). Assuming the cosine of angles less than 20° equals unity, will cause an underestimation of velocity by 6%, whereas this assumption at angles greater than 20° will cause a considerable reduction in velocity, as the value for the cosine falls rapidly. No experimental verification was given by Light (1969a) to show the accuracy of the velocity determinations. The angle between a continuous wave Doppler transducer and the aortic blood flow has been measured, from thoracic radiographs (Huntsman, Gams, Johnson and Fairbanks 1975). The minimum angles between the transducer and blood flow, in seven subjects, ranged from zero to eleven degrees (mean 6.3 degrees). The error associated with the assumption that the cosine of the angle equalled unity was calculated as less than 2%.

In 1969 Edler and Lindstrom used continuous wave echocardiography to examine blood flow in the left ventricular outflow tract and the mitral orifice (Edler 1990). These workers were able to differentiate from normal signals, those caused by aortic and mitral regurgitation, and mitral stenosis (Edler 1990).

Further development of these early continuous wave ultrasound systems enabled the direction of blood flow to be recorded (Light and Cross 1972) and accuracy to be improved by analysis of the full frequency spectrum of the received signal. This allowed linearity of response to be retained at different velocities (Light 1976). Early Doppler ultrasound equipment determined the Doppler frequency shift by zero crossing detectors. These detectors measured the time interval between zero crossing of the sine wave which related to the frequency of the wave. This method provided accurate information for a single sine wave but multiple simultaneous signals arriving back at the transducer caused error in frequency analysis, and nonlinearity (Goldberg *et al.* 1988). The frequency analysis device now most commonly utilized for Doppler echocardiography is Fast Fourier Transform. This is a digital method which allows simultaneous analysis of various frequency components and gives a linear response (Goldberg *et al.* 1988). With most equipment, the modal frequency is displayed in a dark shade of grey, whereas frequencies which occur less often are assigned a lighter shade of grey (Fisher, Sahn, Friedman, Larson, Valdes-Cruz, Horowitz, Goldberg and Allen 1983a).

The accuracy of the equipment described by Light and Cross (1972) to record flow velocities was evaluated by comparison with electromagnetic velocity meters located on a catheter tip (Sequeira, Light, Cross and Raftery 1976). The results of this study showed a good correlation between the two methods of measurement. However the correlation between measurements was shown to differ in individual patients and the velocities recorded by the electromagnetic velocity meters were lower than those recorded by the Doppler method.

One of the major goals of medical ultrasound research at this time, was the ability to quantify blood flow noninvasively (Brandestini 1978). To do this with Doppler echocardiography it is necessary to know the average velocity of flow across the vessel and to multiply this by the cross sectional area of the vessel at that point (Baker 1970). Continuous wave Doppler systems have no range resolution, flow

velocities being recorded from every moving target crossing the path of the ultrasound beam, therefore the velocity cannot be evaluated at a specific site (Hatle and Angelsen 1982). Baker (1970) described a noninvasive Doppler ultrasound system capable of interrogating blood flow velocity at a specific depth (range-gating) and from a specific sampling site (sample volume). A pulse of ultrasound was emitted, and after a time delay, corresponding to the depth at which the instrument was sampling, the backscattered signal was analysed. Placement of the sampling site was guided by the reflected pulses from intracardiac structures, which were amplified and displayed on an oscilloscope.

The use of pulsed ultrasound to achieve range specificity, imposes certain limitations. To avoid range ambiguity, the echoes from one pulse have to be received before the next pulse is transmitted. Thus to study deep arteries a low pulse repetition frequency is needed. To determine the Doppler frequency shift accurately, the sampling frequency must be at least twice the expected Doppler frequency shift (Baker 1970). Therefore to measure high velocities a high pulse repetition frequency is needed (Angelsen and Brubakk 1976). With pulsed Doppler technology the deeper the artery the lower the maximum velocity that can be measured. The maximum velocity that can be recorded at any given depth is termed the Nyquist limit which is equal to half the pulse repetition frequency (Feigenbaum 1986). If the Nyquist limit is exceeded, signal aliasing occurs and the velocity signal is displayed as though blood is flowing in the opposite direction (Goldberg *et al.* 1988).

Angelsen and Brubakk (1976) used a pulsed wave instrument to record blood flow velocities in the human aorta. The instrument was firstly validated in vitro, under conditions of pulsatile and steady flow. The ultrasound equipment accurately measured flow velocity, and by integration of the velocity curve, and multiplication by the area of flow, the stroke volume of the pump was accurately calculated. Due to the use of high pass filters, the ultrasound equipment overestimated the mean flow velocity by 4 centimetres per second. Clinical testing of the equipment was carried

out in eighteen subjects with normal aortic valves. The aortic velocities were recorded in the ascending aorta and aortic arch. The diameter of the vessel was calculated from echos from the posterior and anterior vessel walls when the transducer was held at 90°, that is when there was no detectable Doppler frequency shift. Stroke volume was calculated from the integral of the velocity waveform multiplied by the area of the vessel. Cardiac output was calculated by multiplication of the stroke volume by the heart rate. The calculated velocities in the eighteen subjects ranged from 0.34-0.68 metres per second, with a stroke volume from 1.7 to 3.5 litres per minute. The flow estimates were considered to be between 30-70% of those expected in normal subjects. Measurements of the aortic diameters were considered to be approximately correct. High pass filters caused over-estimation of velocity when validated in vitro. Therefore the under-estimation of velocity and flow in vivo was probably due to the large angle between the ultrasound beam and blood flow (page 27). This is contrary to the findings of Huntsmann *et al.* (1975). Angelsen and Brubakk (1976) concluded the Doppler method had limitations for absolute measurement of velocity and flow. However, they were able to obtain reproducible measurements from the same patients and suggested the method was useful for recording changes in flow and flow velocity. This is in agreement with the findings of Sequiera *et al.* (1976). These authors showed that stroke volume calculated by Doppler echocardiography was approximately proportional to that measured by indicator dye dilution, although major discrepancies were found between results in several subjects. They concluded that the technique was capable of providing relative measurements of velocity and flow, but were of the opinion that absolute values could be obtained in certain individuals. The differences between estimated stroke volumes in certain individuals was thought to be due to variation from a flat velocity profile leading to error in estimating the mean velocity and also error in estimation of the vessel cross-section. No mention was made of the possibility of error due to poor alignment of the ultrasound beam with flow. Brubakk, Angelsen and Hatle (1977)

demonstrated that continuous wave Doppler recorded maximum velocities accurately when aligned accurately with flow.

In 1978, Griffith and Henry described a pulsed wave Doppler instrument with the facility to record real-time two-dimensional images. In the two-dimensional imaging mode, the specific position from which Doppler information would be recorded was indicated by a highlighted line. The two-dimensional image was used to guide the Doppler sampling site into an area of interest, and to aid alignment of the ultrasound beam with blood flow. The instrument was then switched into the pulsed Doppler mode, and minor alterations in angulation were made until the highest audible frequency was obtained. It was suggested that the angle between the ultrasound beam and blood flow could be measured from the two-dimensional image, and this could then be used to correct the calculated flow velocity. However, two-dimensional images only allow alignment to be visualised in two planes. In addition, two-dimensional images were frozen during the final alignment of the transducer. Correction of the velocity calculation by this method can lead to gross over-estimation of flow velocities and should be avoided (Goldberg *et al.* 1988).

Diagnosis of valvular disease

Pulsed wave Doppler echocardiography has been used to diagnose the cause of cardiac murmurs (Johnson, Baker, Lute and Dodge 1973). These workers were able to identify, from the audible Doppler frequency shift, a pure sound in areas of normal blood flow and a harsh sound in areas of turbulent flow. In areas of normal blood flow the blood cells passed through the sampling site in the same direction, at approximately the same velocity, producing a Doppler shift with a narrow frequency bandwidth. In contrast, in areas of turbulent blood flow a wide frequency bandwidth was produced. Angiography of these patients confirmed the origin of the localised turbulent flow demonstrated by the pulsed Doppler technique. Turbulent flow was demonstrated in cases of mitral, pulmonary and aortic regurgitation, valvular stenosis, sub-aortic stenosis, and atrial septal defect. Pulsed wave Doppler echocardiography

was used on a further three patients in which tricuspid regurgitation and turbulent flow from a ventricular septal defect were diagnosed. The authors proposed that in addition to its application in the localization of cardiac murmurs, the pulsed Doppler technique could be used to measure jet areas as a means to estimate valve orifice area.

Brubakk, Angelsen and Hatle (1977) demonstrated the versatility of equipment combining both pulsed wave and continuous wave Doppler capabilities to diagnose valvular heart disease. A systematic method of recording flow velocities within the heart and great vessels using pulsed wave Doppler echocardiography was described. The mitral inflow during diastole was characterised by a double peak. The first peak was caused by the rapid inflow of blood in early diastole and the second by flow due to atrial contraction. The second peak was absent in a patient with atrial fibrillation. The signal from the tricuspid valve was similar to that from the mitral valve. The signals recorded from the pulmonary outflow were similar to those recorded from the aorta, although the pulmonary outflow waveform was more rounded than that obtained from the aorta. High flow velocities were detected in cases of valvular stenosis, which could be accurately recorded using the continuous wave technique. Mitral and aortic stenosis, and mitral, tricuspid and aortic regurgitation were diagnosed with this combined instrument.

Matsuo, Kitabatake, Hayashi, Asao, Terao, Senda, Hamanaka, Matsumoto, Nimuro and Abe (1977) described a combined pulsed wave Doppler and two-dimensional echocardiography system with the facility to display the range of velocities within the sampling site. Using a model to produce different types of flow, they demonstrated signals with a wide frequency bandwidth in areas of disturbed flow and signals with a narrow frequency bandwidth in areas of laminar flow. This difference in the Doppler signals from areas of disturbed and laminar flow had previously been detected by Johnson *et al.* (1973) from the audible Doppler signal. Matsuo *et al.* (1977) detected laminar flow patterns in the left ventricular outflow tract, aorta, right ventricular outflow tract, pulmonary artery and left ventricular

inflow tract of seventeen normal humans. Evaluation of a further 63 patients with heart disease revealed disturbed flow signals in the left atrium in cases of mitral regurgitation and in the left ventricular outflow tract in cases of aortic regurgitation.

The sensitivity of pulsed wave Doppler echocardiography to detect valvular regurgitation in humans has been compared with conventional invasive methods for mitral (Abbasi, Allen, DeCristofaro and Ungar 1980), aortic (Ciobanu, Abbasi, Allen, Hermer and Spellberg 1982), pulmonary and tricuspid regurgitation (Waggoner, Quinones, Young, Brandon, Shah, Verani and Miller 1981). Abbasi *et al.* (1980) reported a sensitivity of 92% and a specificity of 96% for the detection of mitral regurgitation in 47 patients. A higher sensitivity (96%) and specificity (100%) was reported for the detection of aortic insufficiency (Ciobanu *et al.* 1982) and a sensitivity of 94%, for tricuspid regurgitation (Waggoner *et al.* 1981). The lower sensitivity for the detection of mitral regurgitation may reflect the difficulty in locating eccentric jets (Abbasi, *et al.* 1980; Nishimura, Miller, Callahan, Benassi, Seward, and Tajik 1985). Regurgitant jets can easily be missed by pulsed wave Doppler echocardiography, when the degree of regurgitation is very mild, or cardiac output is low, when small low intensity regurgitant signals are produced (Ciobanu *et al.* 1982). Difficulty has been reported in differentiating signals of mitral stenosis from those of aortic regurgitation (Ciobanu *et al.* 1982), and of pulmonary venous flow from that of mitral regurgitation when cardiac output is high (Abbasi *et al.* 1980). In the study by Abbasi, *et al.* (1980), one patient who was wrongly diagnosed as having mild mitral regurgitation, had anaemia. In retrospect the authors suggested that it should have been possible to exclude mitral regurgitation in this subject as the abnormal signal, thought to be due to increased velocity flow from the pulmonary veins, could not be detected close to the mitral valve.

Waggoner *et al.* (1981) showed 100% sensitivity and 100% specificity using pulsed wave Doppler to detect tricuspid and pulmonary regurgitation in six dogs with thoracotomies, examined before and immediately after surgical disruption of the

valves. In this study contrast M-mode echocardiography was the invasive standard used for comparison.

Continuous wave and pulsed wave Doppler have been used to diagnose the cause of systolic murmurs which were clinically ill defined (Hoffmann and Burckhardt 1983). The study comprised 102 consecutive patients in which the diagnosis was later verified by cardiac catheterisation. The study aimed to differentiate patients with aortic stenosis from those with flow murmurs, to differentiate murmurs of mitral regurgitation from flow murmurs, to identify the presence of mitral regurgitation in patients with concurrent aortic stenosis and to differentiate mitral regurgitation from cases with ventricular septal defects. The diagnostic criterion of aortic stenosis by Doppler echocardiography was an aortic blood flow velocity of greater than 2.2 metres per second. Ventricular septal defects were diagnosed when a high velocity signal was detected flowing towards the left sternal border. Mitral regurgitation was detected as a disturbed signal flowing from left ventricle to left atrium during systole. A lower sensitivity and specificity was reported in this study (87% and 77% respectively) for the detection of mitral regurgitation compared to the study by Abbasi *et al.* (1980). Aortic stenosis was correctly diagnosed in nine out of ten cases (sensitivity 90%) and correctly ruled out in twelve cases with a functional flow murmur (specificity 100%). A ventricular septal defect was correctly identified in five patients, and excluded in three others (specificity 100%, sensitivity 100%). The overall diagnostic accuracy of the technique was 89%. The equipment used in this study allowed continuous wave and pulsed wave Doppler echocardiography but did not have the facility for two-dimensional echocardiography. The authors discussed the value of two-dimensional echocardiography for localization of the Doppler sample volume, but considered the ability to perform pulsed wave and continuous wave Doppler from the same transducer was more important for the accurate detection of high velocity flow. This is in agreement with Brubakk *et al.* (1977). Most equipment at this time combined

either continuous wave and pulsed wave Doppler echocardiography or pulsed wave Doppler echocardiography and two-dimensional imaging. Combined two-dimensional and pulsed wave Doppler transducers were heavy which made accurate alignment with flow difficult (Hoffmann and Burckhardt 1983). Modern Doppler ultrasound equipment allows continuous wave and pulsed wave Doppler echocardiography, and two-dimensional and M-mode imaging to be performed from a single small transducer (Goldberg *et al.* 1988). Some equipment also allows pulsed wave Doppler echocardiography to be used at a higher pulse repetition frequency (HPRF) for a given depth. In this mode a further pulse of ultrasound is emitted before the previous pulse has returned to the transducer. The increase in the pulse repetition frequency allows higher velocities to be recorded without aliasing (Nishimura *et al.* 1985), however, there is a loss of range specificity.

Quantification of valvular disease

The pressure gradient across a stenotic valve can be accurately calculated from the maximum blood flow velocity through the valve (Holen, Aaslid, Landmark and Simonsen 1976). The pressure gradient across the mitral valve was calculated using a modification of the Bernoulli equation, which can be simplified as

$$\text{Pressure gradient} = 4 \times \text{Maximum velocity}^2$$ (Goldberg *et al.* 1988)

The ability to estimate pressure gradients across the cardiac valves has not only enabled the diagnosis of valvular stenosis (Brubakk, Angelsen and Hatle 1977) but has allowed the severity of valvular stenosis and valvular regurgitation to be assessed (Masuyama, Kodama, Kitabatake, Nanto, Sato, Uematsu, Inoue and Kamada 1986; Beyer, Ramirez, Josephson and Shah 1987). In cases of severe aortic insufficiency the velocity of the regurgitant jet decreases during diastole (Beyer *et al.* 1987). However it has been shown that changes in ventricular compliance and peripheral vascular resistance also affect the shape of the aortic regurgitant velocity profile (Griffin, Flachskampf, Siu, Weyman and Thomas 1991).

The severity of valvular regurgitation can also be assessed by mapping the distribution of abnormal flow in the recipient chamber using pulsed wave Doppler echocardiography (Miyatake, Okamoto, Kinoshita, Ohta, Kozuka, Sakakibara and Nimura 1982; Liu and Louie 1989), guided either by M-mode (Abbasi *et al.* 1980) or two-dimensional echocardiography (Miyatake, Izumi, Okamoto, Kinoshita, Asonuma, Nakagawa, Yamamoto, Takamiya, Sakakibara and Nimura 1986).

In the study by Abbasi *et al.* (1980), the area of mitral regurgitation detected by pulsed wave Doppler echocardiography was graded on a 1-4 scale. Grade 1 (mild) mitral regurgitation consisted of systolic flow localized immediately posterior to the mitral valve. Mitral regurgitation was graded 4 (severe) when flow was detected diffusely all over the left atrium. This grading system showed a high correlation ($r = 0.88$) with gradings by left ventriculography. However a good correlation could be expected, as severity by angiography was also graded on the same scale. Cases described as mild, were considered so by both techniques although there was overlap of the other three grades, especially grades 2 and 3. This overlap is clinically important, as grade 1 and 2 mitral regurgitation is often well tolerated in human patients over long periods of time and rarely causes myocardial dysfunction. Grades 3 and 4 mitral regurgitation are indicative of more severe valvular regurgitation, with many patients becoming symptomatic and requiring surgical correction (Carabello 1992). However other patients can accommodate severe mitral regurgitation (especially chronic) without haemodynamic deterioration (Bolger, Eigler, Pfaff, Resser and Maurer 1988b; Appleton, Hatle, Nellessen, Schnittger and Popp 1990).

Liu and Louie (1989) described an alternative method of predicting the severity of mitral regurgitation by pulsed wave Doppler echocardiography. They measured the depth of penetration of the disturbed mitral regurgitation flow signal into the left atria, and divided this value by the left atrial length. Multiplication by 100 gave a mitral regurgitation ratio. Comparison with angiography revealed a correlation of $r = 0.70$, with overlap between grades 1 and 3. These results were

slightly poorer than those reported by Abbasi *et al.* (1980), however this may be due to the less favourable grouping system used for Doppler evaluation in this study. Re-analysis of the data by Liu and Louie (1989) having omitted cases with concurrent mitral inflow obstruction, resulted in an improved correlation ($r = 0.88$) with statistically significant separation between all angiographic grades, except for grades 1 and 2. The authors suggested that relative stenosis of the mitral orifice may result in a deeper and more distorted penetration of the mitral regurgitation jet into the left atrium rendering the disturbed signal unreliable as the sole predictor of severity. Failure to separate grades 1 and 2 mitral regurgitation is not considered to be of clinical relevance (Carabello 1992).

The severity of tricuspid (Miyatake *et al.* 1982) and aortic regurgitation (Ciobanu *et al.* 1982) have been assessed from the distance of penetration of the regurgitant jet relative to intracardiac structures. The severity of tricuspid regurgitation was further estimated from the area over which the regurgitant signal could be detected (Miyatake *et al.* 1982). The correlation with angiography for assessment of tricuspid regurgitation was $r = 0.75$ for jet length and $r = 0.74$ for jet area (overlap occurred between all grades). A significant correlation with only modest overlap between grades was reported by Ciobanu *et al.* (1982) for the assessment of aortic regurgitation. However the improvement in specificity of the grading system in this study may reflect reduced sensitivity of the grading system used (scale 1-3).

Wranne, Ask and Loyd (1985) have criticised the estimation of severity of valve regurgitation based on the relative length of the regurgitant jet. The distance a regurgitant jet travels, is related to the product of the mean flow velocity and the diameter of the orifice, and therefore cannot be used to quantify the volume of regurgitant flow. These authors suggest that the close correlation reported by many workers, between Doppler echocardiographic estimates of severity of valve regurgitation and angiography is not surprising as both techniques have similar

limitations. Angiography does not measure the volume of regurgitant flow. Severity of regurgitation is judged from the opacification of the left atrium by radiographic contrast medium injected into the ventricle (Carabello 1992). Severity is also commonly interpreted by the length and width of the regurgitant jet within the receiving chamber (Wranne, Ask and Loyd 1985). The degree of opacification will be affected by the amount and rate of injection of the contrast medium, rhythm disturbances, catheter position (Chen, Thomas, Anconina, Harrigan, Mueller, Picard, Levine and Weyman 1991), chamber size (Feigenbaum, 1988) and volume of forward flow (Bolger, Eigler and Maurer 1988a). Croft, Lipscomb, Mathis, Firth, Nicod, Tilton, Winniford and Hillis (1984) compared angiographic gradings of the severity of aortic insufficiency and mitral regurgitation with regurgitant flow volumes assessed by the Fick principle and indicator dilution. The results showed considerable overlap between grades and the volume of regurgitant flow varied markedly within grades, especially in people with large left ventricles. Aortic insufficiency grades 1 and 2 were not significantly different, nor were grades 3 and 4. In the assessment of mitral regurgitation, grade 1 was significantly different, but grades 2 and 3, and grades 3 and 4 were not significantly different. Angiography itself is recognised as being subjective and only semi-quantitative (Bolger, Eigler and Maurer 1988a) and subject to the same limitations inherent in assessing the severity of regurgitation by Doppler echocardiography (Wranne, Ask and Loyd 1985; Carabello 1992). It is accepted that the pulsed Doppler mapping technique allows only semi-quantification of the severity of regurgitation (Feigenbaum 1988). Several limitations of the pulsed Doppler technique have been discussed other than those relating to the length of the jet. Regurgitant jets are three-dimensional, and therefore pulsed wave Doppler mapping must be carried out in more than one imaging plane (Harold, Carmo, Schapira 1990). The size of the recipient chamber also influences the relative size of the regurgitant jet (Feigenbaum 1988). In spite of these limitations, Doppler echocardiography has been described as the only non-invasive technique capable of

detecting valvular regurgitation (Krayenbuehl and Jenni 1985). It has been suggested that Doppler echocardiography may be the most sensitive method of detecting the presence of tricuspid regurgitation and defining its severity (DePace, Ross, Iskandrian, Nestico, Kotler, Mintz, Segal, Hakki and Morganroth 1984). Williams, Walley and Ryan (1990) consider pulsed wave Doppler echocardiography to be the noninvasive standard for detecting and semi-quantifying tricuspid regurgitation. In a study by Curtius, Thyssen, Breuer and Loogen (1985) investigating tricuspid regurgitation only 77% of angiograms could be evaluated, whereas all patients were effectively assessed using pulsed wave Doppler echocardiography. Curtius *et al.* (1985) suggested that lack of specificity of the Doppler technique may reflect physiological regurgitation not detected by angiography. This is in contrast to the findings of Ciobanu *et al.* (1982) who suggested that in mild cases of aortic insufficiency the regurgitant jet could be difficult to locate by pulsed wave Doppler echocardiography.

Colour flow Doppler echocardiography

More recently an alternative method has been developed of displaying Doppler information (Edler 1990). Doppler colour flow mapping is a pulsed Doppler technique using multiple sample volumes (Goldberg 1989) to record Doppler information. The velocity of flow is colour coded and displayed on a two-dimensional (Goldberg *et al.* 1988; Feigenbaum 1988; Durell 1990), or M-mode image (Monaghan and Mills 1989; Mitchell 1990). Most current instruments code flow towards the transducer in the colour red and flow away from the transducer in a blue colour (Simpson and Camm 1990). Higher velocities are encoded in lighter shades of red or blue (Goldberg *et al.* 1988). In some equipment a 'variance' colour map is used to indicate variability in the received Doppler signals (Mitchell 1990). This variability is thought to be associated with turbulence (Monaghan and Mills 1989) and in most equipment is encoded in shades of green (Nishimura *et al.* 1985; Cooper, Nanda, Philpot and Fan 1989). The method by which variability in flow

velocities is detected and the arbitrary limits of variability at which a green signal is encoded varies between manufacturers (Goldberg *et al.* 1988). As there is no indication that flow encoded green is actually turbulent (Goldberg *et al.* 1988), many authors prefer to use the term disturbed flow to describe this flow signal (Mitchell 1990). Switzer, Yoganathan, Nanda, Woo, and Ridgway (1987) used streak photography to validate the flow patterns colour coded by an Irex-Aloka 880 Doppler colour flow instrument. They demonstrated disturbed flow in regions where the colour flow image predicted flow variance, whereas undisturbed laminar flow was encoded by a monochrome homogeneous colour.

As colour flow mapping is a form of pulsed Doppler echocardiography, it also has the limitation of signal aliasing (Monaghan and Mills 1989; Mitchell 1990). If flow velocity exceeds the Nyquist limit of the transducer, flow will be colour coded as though it is travelling in the opposite direction (Monaghan and Mills 1989). In areas of laminar flow this can be identified by the abrupt change in colour from a shade of red to a shade of blue (Cooper *et al.* 1989; Mitchell 1990). Aliasing is very easy to identify and draws the eye to areas of high velocity flow and possibly abnormal blood flow (Monaghan and Mills 1989). In areas of disturbed flow, or multidirectional flow, multiple aliasing or a mosaic pattern may occur (Cooper *et al.* 1989).

Colour flow Doppler echocardiography has been described as one of the most important advances in cardiac ultrasonography since the development of two-dimensional echocardiography (Simpson and Camm 1990). It allows rapid screening of cardiac chambers for abnormal flow (Cooper *et al.* 1989) and the detection of small eccentric jets, that are easily missed by continuous wave or pulsed wave Doppler echocardiography (Monaghan and Mills 1989). It also has the advantage of spatial resolution of regurgitant jets (Goldberg 1989). The spatial orientation of a regurgitant jet by colour flow Doppler, may aid the placement of a pulsed wave Doppler sample volume or continuous wave Doppler ultrasound for accurate velocity recording (Monaghan and Mills 1989). Colour flow Doppler echocardiography has been

proposed as a means to semi-quantify valve regurgitation (Omoto, Yokote, Takamoto, Kyo, Ueda, Asano, Namekawa, Kasai, Kondo and Koyano 1984). It has also been suggested that this technique is better suited for the assessment of valve regurgitation than pulsed wave Doppler echocardiography, due to the spatial appreciation of flow velocities relative to intracardiac structures (Simpson, Valdes-Cruz, Sahn, Murillo, Tamura and Chung 1989).

Diagnosis and quantification of valvular regurgitation

Mitral regurgitation

The diagnosis of mitral regurgitation using Doppler colour flow mapping, has been compared to that by ventriculography in 109 patients (Miyatake, Izumi, Okamoto, Kinoshita, Asonuma, Nakagawa, Yamamoto, Takamiya, Sakakibara and Nimura 1986). A sensitivity of 86% was reported, which was lower than described by Abbasi *et al.* (1980) although similar to that recorded by Hoffmann and Burckhardt (1983) using pulsed wave Doppler echocardiography. The specificity in the study by Miyatake *et al.* (1986) was 100% which was higher than that reported by previous workers (Abbasi *et al.* 1980; Hoffmann and Burckhardt 1983). The severity of valvular regurgitation was assessed by measuring the length and area of the regurgitant jet as previously reported for pulsed wave Doppler echocardiography (Miyatake *et al.* 1986). A good correlation was demonstrated between angiography and colour flow Doppler mapping of mitral regurgitant jet length ($r = 0.87$) and jet area ($r = 0.83$). However 53% of patients showed differences in grading between the two techniques and gradings showed considerable overlap. This would be expected in view of the limitations of angiography and pulsed wave Doppler echocardiography discussed previously. A more recent study of 147 patients by colour Doppler mapping and angiography revealed a higher sensitivity (100%) and specificity (100%) for the detection of mitral regurgitation (Helmcke, Nanda, Hsiung, Soto, Adey, Goyal, and Gatewood 1987). These workers measured the maximum length, width and area of the jet in three orthogonal planes for comparison with angiography (graded 1-3).

They found the best correlation with minimal overlap of angiographic grades, when the maximum or average jet area was divided by the left atrial area. The left atrial area was measured from the image plane in which the regurgitant jet was largest. The maximum jet dimensions in each plane showed a poor correlation and although this was improved by examination in two planes, there was considerable overlap with angiographic grades. Other workers have shown that jet area decreases as the volume and pressure of the recipient chamber increases. Therefore relating the regurgitant jet area to atrial size may significantly worsen the estimation of the severity of regurgitation compared to measurement of jet size alone (Maciel, Moises, Shandas, Simpson, Beltran, Valdes-Cruz and Sahn 1991). In contrast Gal, Shalev and Schmidt (1990) showed that the severity of mitral regurgitation was overestimated in patients with high left atrial pressures, which implies jet area was increased in these subjects. In patients with a large left atrium, the severity of mitral regurgitation was underestimated. When more than one image plane was studied, a good correlation ($r = 0.82$) was obtained between ventriculography and the maximum jet area of mitral regurgitation (Gal, Shalev and Schmidt 1990). Mild and severe mitral regurgitation can be distinguished by this method, but there is a limited correlation with regurgitant volume (Spain, Smith, Grayburn, Harlamert and DeMaria 1989).

A large number of variables influence jet size (see page 44), therefore relating jet area to left atrial size is unlikely to improve quantification of mitral regurgitation by Doppler colour flow mapping (Hoit, Jones, Eidbo, Elias and Sahn 1989).

Aortic regurgitation

Colour flow Doppler echocardiography has been used to assess the severity of aortic insufficiency (Perry, Helmcke, Nanda, Byard and Soto 1987). The maximal length and area of the regurgitant jet were poorly predictive of the angiographic grade of aortic insufficiency (Perry *et al.* 1987). A better prediction of angiographic grade was obtained when the width at the base of the jet or the area of the jet were related to the width or area of the left ventricular outflow tract. The former correctly classified

23 of 24 patients whilst the latter correctly classified 23 of 29 patients. The correlation of these two techniques with angiography was r = 0.93 and r = 0.91 respectively. In contrast to the work on mitral regurgitation, the method of measuring the cross-sectional area of the aortic regurgitant jet in relation to the cross-sectional area of the left ventricular outflow tract, has been shown to be a reliable means of semi-quantifying aortic insufficiency (Baumgartner, Kratzer, Helmreich and Kuhn 1988). Measurement of jet area from two image planes has been shown to correlate more closely with invasive techniques, r = 0.86, than measurement of jet area from a single image plane, r = 0.74 (Bouchard, Yock, Schiller, Blumlein, Botvinick, Greenberg, Cheitlin and Massie 1989).

The accuracy of Doppler colour flow mapping, in assessing the severity of aortic insufficiency, has been studied in dogs under different haemodynamic conditions (Louie, Krukenkamp, Hariman and Levitsky 1989). The severity of aortic insufficiency was determined in two orthogonal planes, from the area of the colour flow jet, expressed as a percentage of the left ventricular end diastolic area. The study showed a good correlation between the regurgitant fraction and the relative area of the regurgitant jet (r = 0.89). This is in contrast to human studies of mitral regurgitation, where regurgitant jet area, showed a poor correlation with regurgitant volume (Spain *et al.* 1989).

A significant relationship exists between the proximal jet width of aortic insufficiency and the regurgitant fraction over a range of pressures (Switzer *et al.* 1987). The maximal width, measured immediately below the aortic valve accurately differentiates defect size at pressures greater than 80mmHg. These findings support the work of Perry *et al.* (1987) and Baumgartner *et al.* (1988) on the correlation of colour flow Doppler findings with angiography, in cases of aortic insufficiency. Switzer *et al.* (1987) stressed the importance of obtaining the measurement of proximal jet area from a long-axis view of the jet which included its origin from the valve. Hoit *et al.* (1989) demonstrated that afterload induced increases in regurgitant

flow did not result in increased jet width at orifices of four and five millimetres, but did increase jet width across orifices of seven millimetres. This method may therefore be limited in predicting changes in regurgitant volume through smaller orifices. Pulsatility has been shown to affect the length of jets, but not the diameter at the origin of jets (Diebold, Delouche, Abergel, Delouche, Dumee and Peronneau 1991).

Jets of aortic regurgitation are considered to be more difficult to map than jets of mitral regurgitation (Bolger, Eigler and Maurer 1988a). The area of the regurgitant jet is influenced by the radial velocities of other flows entering the same chamber (Krabill, Sung, Tamura, Chung, Yoganathan and Sahn 1989). The jet of aortic insufficiency is mixed with the mitral inflow in the left ventricle. In addition, the left ventricular outflow tract is dynamic, being bordered by the mobile anterior mitral valve leaflet, whereas the left atrium is relatively static and symmetrical.

Tricuspid and pulmonary regurgitation

The severity of tricuspid and pulmonary regurgitation have been assessed using colour flow Doppler echocardiography (Cooper *et al.* 1989). There have been fewer studies to quantify tricuspid regurgitation in human medicine, compared to studies of other valves, due to the lack of a suitable standard for comparison (Switzer and Nanda 1985).

Factors influencing jet dimensions

Pressure gradient

The area of a regurgitant jet depicted by Doppler colour flow mapping is influenced by a large number of variables (Hoit *et al.* 1989). The greatest influence on jet area is the driving pressure, or pressure gradient, across the valve (Switzer *et al.* 1987; Krabill *et al.* 1989). The pressure gradient has been shown to influence jet dimensions to a greater extent than the regurgitant volume (Simpson *et al.* 1989). At constant pressure, a ten fold increase in regurgitant stroke volume, only corresponds to a two fold increase in colour jet area. However, Gal, Shalev and Schmidt (1990)

showed that measurement of the regurgitant jet area overestimated the severity of mitral regurgitation in patients with elevated left atrial pressures in which the transmitral pressure gradient would be reduced.

The effect of changes in driving pressure on jet size were not appreciated by Otsuji, Tei, Kisanuki, Natsugoe and Kawazoe (1987), who studied the effects of increasing and decreasing blood pressure and heart rate in 20 patients with mitral regurgitation. A positive correlation was observed between jet area and blood pressure. This was assumed to reflect acute changes in regurgitant volume, however there was no discussion on the direct effects on jet area caused by the increased driving pressure.

Due to the complex interaction of factors which affect jet size, it important to assess the haemodynamic status of the patient when quantifying the degree of regurgitation by colour flow mapping (Maciel *et al.* 1991). Gal, Shalev and Schmidt (1990) have shown that by correcting the estimated severity of mitral regurgitation, obtained by Doppler echocardiography, for known haemodynamic factors, the correlation with angiography is improved from $r = 0.83$ to $r = 0.91$. Patients with acute severe regurgitation may have smaller regurgitant jet areas for the same regurgitant volume, than patients with chronic regurgitation (Maciel *et al.* 1991). During acute regurgitation, there is an increase in pressure in the recipient chamber whilst the volume remains relatively constant. In chronic regurgitation the recipient chamber has time to expand, and so the pressure is only slightly increased.

Orifice size

When the pressure gradient across the aortic valve is constant, the spatial extent of an aortic regurgitant jet is proportional to the effective area of the regurgitant orifice (Louie *et al.* 1989). At a constant pressure gradient, an increase in regurgitant stroke volume caused by a change in orifice size, results in a smaller increase in colour jet area than is caused by a change in driving pressure (Simpson *et al.* 1989).

Identical regurgitant volumes, at identical flow rates, produce larger jet areas when injected through a small orifice compared to a larger one (Bolger *et al.* 1988b). Doppler colour flow mapping reflects the velocity profile of the fluid entering the chamber and that which it displaces. Higher velocity jets have greater kinetic energy and therefore displace more fluid (Bolger *et al.* 1988b).

A jet of a given length or width can originate from a large defect with a low pressure gradient or a small defect with a large pressure gradient (Switzer *et al.* 1987). The distribution of regurgitant jets imaged by colour flow mapping is related to the velocity of regurgitation and therefore to driving pressure rather than to orifice size or flow rates (Simpson *et al.* 1989; Krabill *et al.* 1989). Higher flow rates are required to maintain the same jet area with larger regurgitant orifices. Therefore the same colour area reflects a much higher regurgitant stroke volume for larger regurgitant orifices than for smaller ones. Higher flow rates are necessary at large regurgitant orifices to reach the minimal velocity for colour encoding (Krabill *et al.* 1989). A considerable decrease is found, in jet area, with large regurgitant orifices, despite identical flow (Simpson *et al.* 1989). The regurgitant fraction can not be predicted by jet width or length, but jet length (Switzer *et al.* 1987) and jet area (Maciel *et al.* 1991) can accurately predict flow velocity.

Switzer *et al.* (1987) demonstrated the sensitivity of the Doppler technique in identifying flow through small regurgitant orifices. Aortic insufficiency could be detected through an orifice as small as one millimetre in diameter, providing the cardiac output was greater than 1.5 litres per minute and the mean arterial pressure was greater than or equal to 75 mmHg.

Heart rate

Heart rate has been shown to influence the size of regurgitant jets by colour flow mapping. Tachycardia (heart rate greater than or equal to 110 beats per minute) compromised the sensitivity of colour flow mapping for the detection of aortic insufficiency (Switzer *et al.* 1987). Increased heart rate caused underestimation of jet

size in a model of mitral regurgitation when flow rate and orifice size were constant (Cape, Yoganathan and Levine 1991).

Cardiac output

Low cardiac output (less than or equal to 3.5 litres per minute) compromises the sensitivity of colour flow mapping for the detection of aortic insufficiency (Switzer *et al.* 1987). This has previously been reported for conventional pulsed wave Doppler echocardiography (Ciobanu *et al.* 1982).

Temporal changes in jet area

Jet area does not vary with increasing flow volumes, if the flow rate remains constant (Krabill *et al.* 1989). This is to be expected as Doppler colour flow mapping determines the jet area at one time and the duration of abnormal flow is not taken into account. Temporal variability of Doppler colour jet areas has been reported in patients with mitral and aortic stenosis (Smith, Kwan, Spain and DeMaria 1992). Planimetered jet areas were shown to vary markedly in individual patients throughout the regurgitant period. The point of maximum jet area and duration of maximum jet area also varied markedly between individuals. A large frame by frame variability was reported in regurgitant jet area. This may cause error in assessment of severity of regurgitation if low frame rates are used and short recordings are made. This variability in jet areas was also demonstrated by Switzer *et al.* (1987) who showed that colour variance persisted throughout diastole with small orifices, but was restricted to early diastole with large orifices. The temporal variability of Doppler colour flow jets represent a further limitation on the likely accuracy of a single two-dimensional area to accurately predict valve regurgitation.

Wall impingement

The width, length and area of regurgitant jets are not only dependent on haemodynamic factors and orifice size, but also on the effects of wall impingement. The dimensions of impinging wall jets and free jets have been compared using an in vitro flow model (Chen, Flachskampf, Anconina, Weyman and Thomas 1990). At matched flow rate and orifice size, the long-axis jet area of wall jets was significantly less than free jets. However for short-axis images the jet area of wall jets was significantly larger than free jets. This may explain the observations of Helmcke *et al.* (1987) who found that correlation of jet area of mitral regurgitation with angiographic grading was significantly improved when the maximum jet area was determined from three orthogonal image planes. For long-axis views, for identical jet areas, a wall jet is likely to represent more severe regurgitation than a free jet (Chen *et al.* 1991). Jet size is a function of the regurgitant volume and the fluid entrained from the receiving chamber. For free jets, size is also dependent on the momentum at the orifice (Cape, Yoganathan, Weyman and Levine 1991). Jets formed adjacent to walls, are deflected towards the wall and increase their area in this plane compared to free jets (Cape *et al.* 1991). Wall jets with their orifices at the level of the wall, entrain fluid and spread laterally over the surface of the wall, but the area in the vertical plane is reduced. Therefore the geometry of the jet also must be considered when assessing the severity of regurgitation (Cape *et al.* 1991). Chen *et al.* (1991) compared the correlation between maximum jet area and mitral regurgitant fraction in 30 patients with free jets of mitral regurgitation and fourteen patients with eccentric impinging wall jets of mitral regurgitation. Maximum jet area was found to correlate relatively well with the regurgitant fraction in cases with free mitral regurgitant jets ($r = 0.74$) but did not significantly correlate in cases with eccentric impinging wall jets ($r = 0.42$). However, although the authors used multiple image planes to determine the maximal jet area, short-axis views of the left atrium were not studied. Chen *et al.*

(1990) had previously shown that wall jets were significantly larger than free jets in the short-axis and significantly less than free jets in the long-axis planes.

Technical factors

The size of colour flow jets of valve regurgitation is also influenced by technical variables (Simpson and Sahn 1991). Increasing the amplification (gain) (Otsuji *et al.* 1987), decreasing the transducer frequency and decreasing the pulse repetition frequency significantly increases the measured colour flow areas of matched mitral regurgitation (Hoit *et al.* 1989). Other authors have demonstrated that jet areas also vary between different instruments (Sahn, Chung, Tamura, Meyer, Yoganathan, McMillan and Sung 1986). Some instruments produce consistently larger flow areas for the same degree of mitral regurgitation than other instruments. Frame update rate also affects the maximal colour flow jet area when heart rate increases (Cape, Yoganathan and Levine 1991). As heart rate increases, maximum jet area by colour flow mapping decreases. This decrease is much greater at lower frame rates. System gain, wall filter settings, pulse repetition frequency and frame rate also significantly influence the area of displayed variance (Utsunomiya, Ogawa, King, Sunada, Moore, Henry and Gardin 1990).

Further analysis of colour flow maps

Recently, more sophisticated computer analysis of colour flow Doppler regurgitant jets has been used to extract further information from which valvular regurgitation can be quantified (Bolger *et al.* 1988b; Simpson and Sahn 1991). Previously, colour flow maps merely demonstrated the presence or absence of flow and no use was made of the actual velocities within the jet (Thomas, Liu, Flachskampf, O'Shea, Davidoff and Weyman 1990). Power mode analysis, which is related to the number of moving targets within the beam, has been shown to be related to the volume of flow and is less sensitive to changes in transvalvular pressure gradient than conventional colour flow analysis. However, Parro, Philpot, Czuwala, Fan, Nanda, Cape, Yoganathan, Helmcke and Fujii (1991) have recently shown that

power mode is still velocity dependent and has a limited dependence on the concentration of moving targets. Bolger *et al.* (1988b) estimated jet kinetic energy by summing all the pixel velocities squared, within the jet. Early in vitro studies have shown a close linear relationship between estimated and known jet energy which may be useful in quantifying valvular regurgitation.

Other workers have demonstrated an area of flow acceleration proximal to a regurgitant orifice (Rodriguez, Anconina, Flachskampf, Weyman, Levine and Thomas 1992) and it has recently been shown in vitro, that regurgitant flow rate can be accurately estimated from this area of flow acceleration (Levine 1991). The advantage of this technique is that it does not appear to be affected by orifice shape or equipment factors such as gain, frame rate and transmit power (Utsunomiya, Ogawa, Doshi, Patel, Quan, Henry and Gardin 1991). Further work is needed to assess the accuracy of this method (Levine 1991).

Methods have been developed to quantify regurgitation based on the concept of conservation of momentum. Regurgitant flow rate through an orifice can be computed from the momentum of the jet distal to the regurgitant orifice, calculated by velocity analysis of colour flow maps (Thomas *et al.* 1990) or from pulsed wave Doppler studies (Cape, Skoufis, Weyman, Yoganathan and Levine 1989), and from the maximum velocity at the orifice. In vitro validation of the techniques have been promising (Cape *et al.* 1989; Thomas *et al.* 1990).

Estimation of the severity of regurgitation by the measurement of jets from colour flow maps does have limitations, but it is currently the best method available in routine practice (Levine 1991).

NORMAL FINDINGS IN HUMAN MEDICINE

Valvular regurgitation

Rahko (1989) reports that the widespread use of Doppler echocardiography has resulted in a "sudden epidemic" of valvular regurgitation. Doppler techniques are so sensitive in the detection of regurgitant flow, that physiological retrograde flow

disturbances are frequently detected in normal subjects (Yock, Naasz, Shnittger and Popp 1984). It appears that the incidence of normal valvular insufficiency is a function of the sensitivity of the Doppler equipment, the quality of the studies from the different population types and the criteria used to identify regurgitation. It is possible to identify, by colour flow mapping, both retrograde acceleration of flow through a valve, suggesting true regurgitation, and a low velocity closing signal as blood is pushed back by the coapting valve (Sahn and Maciel 1988). To avoid confusion between short regurgitant closure signals and more prolonged regurgitation, some workers only include signals which persist longer than a predetermined time, usually 100-200 milliseconds (Yoshida, Yoshikawa, Shakudo, Akasaka, Jyo, Takao, Shiratori, Koizumi, Okumachi, Kato and Fukaya 1988; Choong, Abascal, Weyman, Levine, Gentile, Thomas and Weyman 1989).

The reported prevalence of right sided valve regurgitation in normal subjects has been reviewed (Wittlich, Erbel, Drexler, Mohr-Kahaly, Brennecke and Meyer 1988). Tricuspid and pulmonary regurgitation is detected by Doppler echocardiography in 0% - 96% of normal humans. The incidence of mitral regurgitation ranges between 19% and 45% (Kostucki, Vandenbossche, Friart and Englert 1986; Yoshida *et al.* 1988; Choong *et al.* 1989; Berger, Hecht, Tosh and Lingam 1989). The reported prevalence of aortic insufficiency varies significantly between authors. Kostucki *et al.* (1986) reported a prevalence of 33% using pulsed wave Doppler echocardiography. A clear audio signal of aortic regurgitation was not obtained in this study and a disturbed signal could only be recorded when the sample volume was placed close to the valve leaflets. The regurgitant velocities were low. In contrast, Yoshida *et al.* (1988) failed to identify signals of aortic regurgitation in a larger group of healthy volunteers. They suggested that aortic regurgitation was not a normal finding in healthy patients and that the flow pattern reported by Kostucki *et al.* (1986) was artifactual. Other authors have reported a low incidence of aortic

regurgitation (1% - 3%) detected close to the valve (Wittlich *et al.* 1988; Berger *et al.* 1989; Choong *et al.* 1989).

Wittlich *et al.* (1988) stressed the need to differentiate between regurgitant signals of brief duration, associated with valve closure and regurgitant signals of longer duration. These workers reported a higher incidence of regurgitation associated with valve closure, than longer regurgitant signals, at all valves with the exception of the pulmonary valve. The incidence of regurgitation, associated with valve closure, at the aortic valve was 32% which is similar to that reported by Kostucki *et al.* (1986). The suspicion that the regurgitant signal reported by Kostucki *et al.* (1986) was associated with valve closure is supported by the fact that it was always detected in early diastole. The lower incidence of a closure signal at the pulmonary valve reported by Wittlich *et al.* (1988) is supported by the findings of Kostucki *et al.* (1986). The latter authors showed that the regurgitant flow pattern of pulmonary insufficiency was never detected in early diastole despite it covering up to 81% of diastolic period.

The area of the regurgitant jets in normal subjects are significantly smaller than those occurring in patients with organic valve disease (Yoshida *et al.* 1988). Choong *et al.* (1989) graded the severity of valvular regurgitation in normal subjects (grade 1 - 4), based on the maximum distance of the jet from the valve leaflets. For cases of mitral regurgitation, 98% showed either a trace of regurgitation at the point of leaflet closure, or regurgitation limited to the proximal one quarter of the atria (grade 1). No cases had either grade 3 or grade 4 mitral regurgitation (that is the jet did not extend into the distal half of the atrium). A similar distribution was found for regurgitant flow at the tricuspid valve. Berger *et al.* (1989) never detected regurgitant signals further than one centimetre away from the valve leaflets. This was also reported for pulmonary regurgitation (Takao, Miyatake, Izumi, Kinoshita, Sakakibara and Nimura 1985). It has been suggested that jet dimensions could be used to differentiate physiological and pathological regurgitation (Takao *et al.* 1985).

However, other workers demonstrated jet lengths up to 2.3 centimetres in normal subjects, with most jets exceeding one centimetre (Wittlich *et al.* 1988).

The regurgitant signals detected in normal subjects are always of low intensity with poorly defined spectral envelopes (Berger *et al.* 1989; Wittlich, Erbel, Siemer, Mohr-Kahaly, Drexler and Meyer 1990). The intensity of the Doppler signal is related to the severity of regurgitation, low intensity signals indicating a low regurgitant volume (Hatle and Angelsen 1982).

Older patients are reported to have an increased prevalence of mitral regurgitation (Yoshida *et al.* 1988; Berger *et al.* 1989; Choong *et al.* 1989). It has been suggested that this is the result of progressive degeneration of the valves on the high pressure side of the heart. However these workers failed to demonstrate an increased incidence of aortic insufficiency with age, with the exception of the study by Choong *et al.* (1989). In contrast a study by Akasaka, Yoshikawa, Yoshida, Okumachi, Koizumi, Shiratori, Takao, Shakudo and Kato (1987) demonstrated an increased prevalence of valvular regurgitation at all valves with increasing age, especially at the aortic valve. Yoshida *et al.* (1988) found both tricuspid and pulmonary regurgitation to be less prevalent in older subjects. This may reflect difficulty in obtaining quality images in older patients. Other authors have shown an increased incidence of tricuspid regurgitation with age (Choong *et al.* 1989) or no relation with age (Berger *et al.* 1989).

The prevalence of mitral and tricuspid regurgitation is higher in athletes than in sedentary people (Douglas, Berman, O'Toole, Hiller, Douglas and Reichek 1989). The increased incidence of tricuspid regurgitation in athletes was also reported by Pollak, McMillan, Knopff, Wharff, Yoganathan and Felner (1988), who showed a significantly higher incidence of tricuspid and pulmonary regurgitation in female athletes compared to sedentary females. However a similar increase was not detected for mitral regurgitation.

Transoesophageal colour flow Doppler echocardiography is more sensitive in the detection of mitral and tricuspid regurgitation when compared to transthoracic Doppler techniques. The placement of the transducer within the oesophagus ensures no interposition of chest wall or lung tissue, and the location of the transducer is very close to the heart valves (Wittlich *et al.* 1990). Studies of normal subjects by transoesophageal Doppler echocardiography revealed that all subjects had a regurgitant flow pattern at the mitral and tricuspid valve (Wittlich *et al.* 1990). In 68% of subjects a regurgitant signal of very short duration was found at the aortic valve immediately after valve closure, but regurgitation of longer duration was not detected. The authors concluded that normal atrioventricular valves show some regurgitation.

Diastolic mitral regurgitation has been detected by pulsed wave Doppler echocardiography in humans with atrioventricular block (Panadis, Ross, Munley, Nestico and Mintz 1986). Colour M-mode studies have shown that diastolic mitral regurgitation is more likely the longer the PR interval (Covalesky, Ross, Chandrasekaran and Mintz 1989). Studies of 2:1 atrioventricular block have demonstrated that mitral regurgitation occurred when atrial contraction was not followed by contraction of the ventricle (Williams, O'Donovan, Vandenberg, Sturm and Wood 1968).

In view of the high incidence of valvular regurgitation detected by Doppler echocardiography in normal subjects, Rahko (1989) investigated the relationship between valve regurgitation detected by Doppler echocardiography and the audibility of corresponding regurgitant murmurs in a group of patients with various cardiac abnormalities. A murmur corresponding to the regurgitation detected by Doppler echocardiography was heard in 56% of patients with mitral regurgitation, 61% of patients with aortic regurgitation, 28% of patients with tricuspid regurgitation and 15% of patients with pulmonary regurgitation. A significant correlation was observed between audibility and the severity of valvular regurgitation assessed by pulsed wave

Doppler mapping of the area of regurgitant flow. Audibility ranged from 10-40% for mild regurgitation and 86-100% for severe regurgitation.

Flow velocities

Reference values have been established using pulsed wave Doppler echocardiography in normal humans (Hatle and Angelsen 1982; Wilson, Goldberg, Dickinson and Scott 1985). Flow velocities have been recorded from the mitral and tricuspid inflow (Nishimura, Abel, Hatle and Tajik 1989b; Pye, Pringle and Cobbe 1991) and the aortic and pulmonary outflow (Gardin, Davidson, Rohan, Butman, Knoll, Garcia, Dubria, Gardin and Henry 1987) . The velocity waveforms from the mitral and tricuspid inflow show a similar biphasic shape. The first waveform (E) represents the rapid filling phase of the ventricle, and the second waveform (A) corresponds to the flow due to atrial contraction.

Gardin, Burn, Childs and Henry (1984a) demonstrated significant differences between the flow profiles recorded from the aorta and main pulmonary artery. The peak velocity and average acceleration were greater in the aorta than the pulmonary artery. The blood being accelerated two to three times more rapidly into the ascending aorta than into the pulmonary artery. The time to peak velocity (acceleration time) and the ejection time were longer for the pulmonary outflow than for the aortic outflow. Measurements of systolic time intervals are clinically useful in cases of aortic insufficiency and mitral regurgitation (Lewis, Rittgers, Forrester and Boudoulas, 1977). Left ventricular ejection time increases in patients with aortic insufficiency (Weissler, Peeler, Roehil and Durham 1961) and decreases with mitral regurgitation (Lewis *et al.* 1977). The pre-ejection period and ventricular ejection time have been successfully measured noninvasively from Doppler waveforms recorded from the aorta and pulmonary artery (Sequeira *et al.* 1976; Koito and Spodick 1989). Doppler recordings from the aorta and pulmonary artery are discussed more fully in Part 2 of this thesis.

The peak velocity of rapid filling (E) and atrial contraction (A) is higher for the mitral inflow than for the tricuspid inflow (Nishimura *et al.* 1989b; Pye, Pringle and Cobbe 1991), with the E peak normally higher than the A peak. The higher E velocity at the mitral valve may reflect better alignment with mitral inflow than tricuspid inflow (Pye, Pringle and Cobbe 1991) or may be due to the larger tricuspid valve area. Zoghbi, Habib and Quinones (1990) suggest that the difference in the peak velocities of the E wave for the left and right ventricles are greater than could be accounted for by the difference in valve area. These authors suggest that the difference in peak velocities represents a lower early filling rate of the right ventricle compared to the left ventricle. Zoghbi, Habib and Quinones (1990) consider that the longer acceleration time for the right ventricle supports this suggestion. The mitral E wave has a steeper deceleration slope and shorter deceleration time than the tricuspid E wave (Pye, Pringle and Cobbe 1991). Marked changes in tricuspid inflow velocities occur during inspiration (Nishimura *et al.* 1989b; Zoghbi, Habib and Quinones 1990). The E and A waves are significantly greater during inspiration and the deceleration time significantly shorter. Smaller but significant changes also occur at the mitral valve during inspiration (Zoghbi, Habib and Quinones 1990), the E and A signal being significantly smaller during inspiration than during expiration.

The velocity of the E and A peak and the E/A ratio have been associated with the diastolic properties of the ventricle (Goldberg *et al.* 1988). However, although Doppler E and A values are considered to give information on diastolic function, they do not represent pure indices of diastolic function, as they have been shown to vary with preload and afterload (Plotnick and Vogel 1989).

Diastolic filling is very complex (Devereux 1989; Levine and Thomas 1989; Nishimura *et al.* 1989b; Gottdiener 1991). Doppler inflow measurements cannot be correlated to a single haemodynamic variable, as many interrelated factors affect the ventricle. The velocity curves represent the overall result of these factors (Nishimura *et al.* 1989a; Gottdiener 1991).

The E signal is dependent on the active relaxation of the ventricle and therefore on the rate of decrease of ventricular pressure (Devereux 1989). The E signal is also dependent on the left atrial pressure at mitral valve opening (preload), and on the rate of passive filling (Devereux 1989). A decrease in preload causing a decrease in E peak velocity (Nishimura *et al.* 1989a).

The A signal is dependent on the left ventricular compliance (Devereux 1989), the left ventricular pressure before atrial contraction (Levine and Thomas 1989) and atrial systolic function (Devereux 1989). Atrial systolic function is dependent on the intrinsic properties of the atrial muscle and the left atrial volume before atrial contraction, atrial preload (Devereux 1989). If atrial preload is decreased the peak A velocity will decrease (Nishimura *et al.* 1989a).

Devereux (1989) suggests that if compliance or relaxation are affected such that either the E or A signal is reduced, a reciprocal increase in the other occurs, so that stroke volume remains constant. If however relaxation and compliance are both affected this does not occur.

The deceleration time reflects the relationship between the left atrial and left ventricular pressures (Devereux 1989; Nishimura *et al.* 1989a). This pressure relationship is determined by the peak velocity of the rapid filling wave and the viscoelastic force of the left ventricle. A reduced peak E velocity results in a significantly longer deceleration time, unless the filling pressure and cardiac output is very low, when deceleration time is reduced (Nishimura *et al.* 1989a). An increase in preload increases the peak E velocity and causes a decrease in the deceleration time (Nishimura *et al.* 1989a).

The effect of afterload on mitral inflow velocities depends on the left atrial pressure (Nishimura *et al.* 1989a). If afterload increases and filling pressures are low peak E velocity will decrease. However, if filling pressures are sufficient, an increase in afterload does not result in a decrease in the E velocity, although a significant decrease in deceleration time will occur. Increases in afterload cause a decrease in

the rate of relaxation (Nishimura *et al.* 1989a). As inertial and viscous forces are present during ventricular filling, the modified Bernouilli equation cannot be applied to ventricular filling (Gottdiener 1991). However, at maximum flow rate, acceleration and inertia are zero and therefore pressure gradient is related to flow (Nishimura *et al.* 1989a).

Factors influencing flow velocities

Sampling site

A pulsed wave Doppler study from the aortic arch has demonstrated that the velocity profile varies with the location of the sampling site (Peronneau, Bugnon, Bournat, Xhaard and Hinglais 1973). The velocity profile at the inlet of a vessel is normally flat, and acceleration of flow also causes flattening of the velocity profile (Hatle and Angelsen 1982). Therefore the ascending aorta should demonstrate a flat velocity profile. Seed and Wood (1971) in a study in dogs, showed this to be true in early systole, but the velocity profile became skewed in late systole, with a peaking of the velocity waveform near the anterior vessel wall. However, they concluded that a centre-line velocity measurement gave a very good approximation of the cross-sectional mean velocity, even with skewed profiles.

The effects of sampling site on pulsed wave Doppler velocity recordings have been studied in vitro, using a model of the pulmonary artery (Gardin, Sung, Yoganathan, Ball, McMillan and Henry 1988b). There was no significant difference in recordings taken at the valve tips, and two centimetres distal to the valve tips when the sample volume was positioned in the centre of the vessel. However, placement of the sample volume close to the vessel wall resulted in notching of the velocity profile, increased dispersion of the Doppler spectrum, decreased maximum flow velocities and decreased acceleration. In contrast, other workers studying similar sampling sites in vivo, have demonstrated an increase in the maximum velocity as the sample volume was moved from the right ventricular outflow tract into the pulmonary artery and when positioned against the vessel wall (Panadis, Ross and Mintz 1986). The

acceleration time, and the acceleration time divided by the right ventricular ejection time, decreased. These authors also demonstrated increased spectral dispersal in recordings made close to the vessel wall. This was evident as an increase in variance in Doppler colour flow recordings (Panadis, Ross and Mintz 1986). Velocity and acceleration time, recorded at the centre of the vessel, increased significantly with increases in stroke volume, whereas stroke volume showed a weak correlation with velocity when recordings were made close to the vessel wall and no significant correlation with acceleration time.

In view of these findings, measurements of blood outflow velocities by Doppler echocardiography should be recorded from the central portion of the vessel (Goldberg *et al.* 1988), within two centimetres of the valve tips (Gardin *et al.* 1988b).

Doppler measurements also differ when recordings are taken from different image planes. Lighty, Gargiulo, Kronzon and Politzer (1986) showed differences between 7% and 48% in pulmonary artery maximum velocity measurements recorded from different planes. This probably reflects improved alignment of the transducer with blood flow in certain views.

Sample volume location also influences ventricular filling velocities recorded by pulsed wave Doppler echocardiography (Jaffe, Dewhurst, Otto and Pearlman 1991). Mean E velocity (velocity of blood flow during passive filling) and mean A velocity (velocity of flow due to atrial contraction) are significantly higher when measured at the valve tips, than when measured at the valve annulus (Gardin, Dabestani, Takenaka, Rohan, Knoll, Russell and Henry 1986; Nishimura, Abel, Hatle, Holmes, Housmans, Ritman and Tajik 1989a; Jaffe *et al.* 1991). The continuity equation states, for a closed system, flow in one region equals flow in a second region (Gardin *et al.* 1986). As the cross-sectional area at the valve tips is smaller than that of the annulus, for flow to remain constant, the velocity of flow at the valve tips must be increased. Gardin *et al.* (1986) studied the effects of different image planes on Doppler measurements of mitral inflow. No significant differences were found

between measurements recorded from an apical 4-chambered view or from a 2-chambered view. However, both these views allow a longitudinal alignment with the mitral inflow.

Patient age

Doppler measurements from the aorta (peak velocity, acceleration, integral of the velocity waveform) have been shown in humans, to decrease with increasing age (Gardin *et al.* 1987). This has been thought to be due to increases in aortic root diameter with age (Gardin *et al.* 1987; Innes, Simon, Murphy and Guz 1988). The aortic ejection time (corrected for heart rate) increased and aortic acceleration time did not change significantly with age (Gardin *et al.* 1987). No relationship was demonstrated between age and pulmonary artery measurements. Blood pressure, gender and body surface area did not significantly influence Doppler measurements from the aorta.

The peak E velocity has been shown to decrease significantly with age both for the mitral inflow (Pye, Pringle and Cobbe 1991; Kitzman, Sheikh, Beere, Philips and Higginbotham 1991; Benjamin, Levy, Anderson, Wolf, Plehn, Evans, Comai, Fuller and Sutton 1992) and for the tricuspid inflow (Zoghbi, Habib and Quinones 1990). The peak A velocity has shown a significant positive correlation with age (Zoghbi, Habib and Quinones 1990; Pye, Pringle and Cobbe 1991; Kitzman *et al.* 1991; Benjamin *et al.* 1992). Kitzman *et al.* (1991) demonstrated that the changes associated with age were not related to differences in left ventricular mass, or to differences in preload or afterload.

Heart rate

Heart rate has a significant effect on Doppler measurements of aortic and pulmonary outflow, although contrasting results have been reported. Harrison, Clifton, Sublett and DeMaria (1989) showed a decrease in aortic peak velocity, velocity time integral and peak acceleration with increasing heart rate. However the

changes in heart rate were induced by electrical pacing, which also resulted in a decrease in preload. This may have affected peak acceleration and peak velocity via changes in stroke volume. Invasive indices of myocardial contractility (dp/dt) increase with increasing heart rate only when preload is maintained (Schaefer, Taylor, Lee, Niggemann, Levine, Popma, Mitchell and Hillis 1988). If preload is not maintained, dp/dt does not increase or is reduced. During exercise, the aortic peak acceleration increases linearly with increases in heart rate (Mehta, Boyle, Bennett, Gilmour, Noble, Mills and Pugh 1988). The latter authors concluded that the increase in acceleration with increasing heart rate is due to a major inotropic effect which is absent during pacing alone. The aortic peak velocity increased with increasing heart rate during exercise, then reached a plateau as heart rate increased further (Mehta *et al.* 1988). In the same study the velocity time integral was shown to increase initially, due to an augmentation of preload, then decrease as heart rate increased further.

Studies on pulmonary artery blood flow showed that acceleration time, ejection time and pre-ejection period (PEP) decrease as heart rate increases, but the pre-ejection period divided by the ejection time was independent of changes in heart rate (Gardin, Sato, Rohan, Shu, Allfie, Gardin and Henry 1988a).

Changes in heart rate also affect the E and A velocity waveforms (Zoghbi, Habib and Quinones 1990; Harrison, Clifton, Pennell, and DeMaria 1991; Benjamin *et al.* 1992). Harrison *et al.* (1991) recorded mitral inflow velocities with pulsed wave Doppler at resting heart rates and during electrical pacing at a higher rate. There was no significant difference in the E peak velocity at elevated heart rates. However Zoghbi, Habib and Quinones (1990) and Benjamin *et al.* (1992) demonstrated a weak negative correlation between E peak velocity and heart rate. A significant decrease in E peak velocity with increased heart rate was also demonstrated by Johannessen, Cerqueira, Veith and Stratton (1991) following atropine administration to normal humans. This study investigated a much larger increase in heart rate than the previous reports, diastolic filling period being decreased by 60%. Harrison *et al.*

(1991) demonstrated a linear relationship between peak A velocity and heart rate. Zoghbi, Habib and Quinones (1990) and Benjamin *et al.* (1992) also demonstrated a strong positive correlation between A peak velocity and heart rate, possibly due to the loss of diastolic filling time causing increased preload of the left atrium prior to contraction (Benjamin *et al.* 1992). Following the administration of atropine (to increase heart rate), a significant increase in A peak velocity was reported (Johannessen *et al.* 1991). This increase in A peak velocity was not related to systolic function, preload, blood pressure, or plasma catecholamines, but was dependent on the decrease in the diastolic filling period (Johannessen *et al.* 1991). This finding supports the suggestions of Benjamin *et al.* (1992). Following administration of adrenaline to normal volunteers, the increased heart rate is associated with a significant increase in E and A peak velocity (Johannessen *et al.* 1991). The reduction in the diastolic period following adrenaline administration was smaller than following atropine administration. However, unlike the peak A signal, the peak E signal was not significantly correlated to the diastolic filling period. The peak E and A velocity were however significantly correlated to Doppler and M-mode indices of ventricular systolic function.

Effects of valvular disease on ventricular inflow velocities

Aortic regurgitation

Changes occur in mitral and tricuspid inflow velocities secondary to valvular disease (Oh, Hatle, Sinak, Seward and Tajik, 1989; Marcus, Neumann, Borow and Lang 1990). Oh *et al.* (1989) demonstrated a significant increase in the mitral E wave in cases with severe aortic insufficiency. The authors suggested that the increase in left ventricular end diastolic pressure caused a decrease in filling associated with atrial contraction. This impairment in left atrial emptying at end diastole results in an increase in left atrial volume and pressure causing an increase in the mitral E velocity. However the authors failed to demonstrate a significant decrease in the Doppler A velocity in their study, although it tended to be lower in the patient group. The E/A

ratio was found to be significantly higher than normal in the patients with severe aortic insufficiency, and the deceleration time of the mitral E wave was shown to be significantly reduced. A decrease in the deceleration time of the E wave in cases of aortic insufficiency has also been reported by other workers (Levine and Thomas 1989; Nishimura *et al.* 1989b; Marcus *et al.* 1990). In contrast to the findings of Oh *et al.* (1989), Rahko, Whitesell and Nellis (1989) reported a significant decrease in the mitral E wave velocity during acute graded aortic insufficiency in dogs with thoracotomies. The reduction in the E wave velocity was not associated with changes in filling pressures. Levine and Thomas (1989) reported a decrease in mitral peak E velocity in human patients with aortic insufficiency. This has also been reported by Marcus *et al.* (1990), however the patient in this study was 69 years old, and it has been shown that peak E velocities decrease with age (Pye, Pringle and Cobbe 1991). The high atrial pressure should increase the A wave velocity but this is offset by the elevated end diastolic ventricular pressures and therefore the A velocity is blunted.

Myreng, Smiseth and Risoe (1990) have demonstrated that an increase in left ventricular end diastolic pressure will decrease the peak A velocity, despite an increased atrial preload. The authors concluded that if aortic insufficiency is sufficiently severe, the limits of ventricular preload will be exceeded, left ventricular afterload will rise and stroke volume will fall. This would result in an increased end systolic volume and cause an increased left ventricular pressure at the onset of diastole. A high early diastolic left ventricular pressure will result in a reduction in the left atrial left ventricular pressure gradient and therefore the E peak will be reduced. The authors concluded that in severe aortic insufficiency the peak E wave may not be high especially in cases with diminished left ventricular performance. However, although the peak E velocity in this study was lower than those reported in the aortic insufficiency group by Oh *et al.* (1989), it was higher than the values reported by Oniki, Hashimoto, Shimizu, Iwakami, Kato, Aerbajinai, Kishi, Yajima and Numano (1992) for normal subjects of similar age.

Oniki *et al.* (1992) failed to show a significant increase in the mitral peak E flow velocity in patients with aortic insufficiency. However the peak E flow velocity, measured at the level of the mitral annulus, was significantly decreased. This was considered to indicate restriction to flow through the mitral annulus. The peak flow velocity at the valve tips was considered to represent the transvalvular pressure gradient at the onset of systole. It is suggested that the difference in the peak E velocity measured at the valve annulus and valve tips would indicate the flow restriction present in cases of aortic insufficiency and was therefore a valuable new haemodynamic index of its severity. In severe cases of aortic insufficiency, an increase in preload causes an increase in left ventricular outflow velocities (Goldberg *et al.* 1988).

Tricuspid regurgitation

Severe tricuspid regurgitation alters the left ventricular inflow velocities (Louie, Bieniarz, Moore, Levitsky, 1990). Patients with severe tricuspid regurgitation and right ventricular volume overload show a significant decrease in the mitral peak A velocity. The velocity time integral of the mitral A waveform is also significantly reduced, indicating a reduction in the atrial contribution to left ventricular filling. It was suggested that this reduction was caused by compression of the left ventricle by both the overloaded right ventricle in late diastole and by the reduced preload of the left atrium due to compression by the right atrium. In severe tricuspid regurgitation an increase in preload causes an increase in right ventricular inflow velocities (Goldberg *et al.* 1988).

Mitral regurgitation

Left ventricular inflow velocities increase in severe mitral regurgitation (Appleton *et al.* 1990) due to increased preload of the left ventricle (Goldberg *et al.* 1988). However where cardiac output is low, lower peak velocities have been

reported (Appleton, Hatle and Popp 1990). Increases in mitral inflow velocities were not demonstrated in mild mitral regurgitation.

Differentiation of the cause of increased flow velocities

The increased transvalvular flow velocities observed in cases of valvular regurgitation can be distinguished from those caused by valvular stenosis, by measuring the flow velocity proximal (V1) and distal (V2) to the valve (Goldberg *et al.* 1988). In cases of increased flow velocity due to valvular regurgitation, V1 and V2 will increase due to increased flow through the valve. In cases of valvular stenosis, where flow through the valve is not increased, V1 will be normal, but V2 will increase demonstrating an increased pressure gradient across the valve (Goldberg *et al.* 1988).

DOPPLER ECHOCARDIOGRAPHY IN VETERINARY MEDICINE

Canine medicine

Much of the validation of Doppler techniques has been performed in dogs (Stein, Sabbah, Albert and Snyder 1987; Nishimura *et al.* 1989a). Recently, the transducer orientation for optimal Doppler studies in dogs has been described (Darke, Bonagura and Miller 1993). Reference values for Doppler flow variables have been described in a group of mixed breed clinically healthy dogs (Brown, Knight and King 1991).

Equine medicine

Pulsed wave and continuous wave Doppler echocardiography have been used to diagnose valvular regurgitation (Marr, Love, Pirie and Northridge 1990) and congenital cardiac defects in horses (Reef 1988a and b). Reimmer, Reef and Sommer (1991) used pulsed wave Doppler echocardiography to map the right atrium of a horse in right sided heart failure to assess the severity of tricuspid regurgitation. This method was also used to identify pulmonary regurgitation and mild mitral regurgitation in the same horse. Maximum jet velocities were measured using

continuous wave Doppler echocardiography. Dedrick *et al.* (1988) used Doppler echocardiography to aid diagnosis in a horse presented with a grade 3/5 systolic murmur. A jet of mitral regurgitation was detected in the left atrium. The progression of this case was monitored periodically using Doppler echocardiography. A larger regurgitant jet was detected five months later associated with a louder grade 4/5 murmur, audible over a larger area of the thorax. Reef (1988a) suggests that pulsed wave Doppler can be used to semiquantify mitral and tricuspid regurgitation based on the distance the jet travels into the atria, and the intensity of the signal. Mild regurgitation is described when the jet extends only a small distance into the atria, moderate if the jet extends halfway or more into the atria and severe if a very intense signal extends to the base of the atria. Reef (1988b) has also described the use of pulsed and continuous wave Doppler echocardiography to identify and quantify aortic and pulmonary regurgitation in horses. Abnormal diastolic flow velocities were described in the left ventricular outflow tract in horses with and without diastolic murmurs. Despite the use of pulsed wave Doppler echocardiography to identify valvular regurgitation in horses, there has only been one reported study to evaluate pulsed wave Doppler echocardiography in normal horses (Reef, Lalezari, De Boo, Van Der Belt, Spencer and Dik 1989), and no studies to investigate horses with valvular disease.

Reef *et al.* (1989) recorded blood flow velocities in 30 normal Standardbred horses, from the right and left ventricular inflow, the right and left ventricular outflow tracts, the aorta and pulmonary artery. Ventricular inflow velocities were recorded by placing the sample volume on the atrial side of the valve. This may result in lower velocity measurements than are obtained from the valve tips. The ventricular inflow and outflow patterns appeared similar to those described for humans. The mitral valve peak E and A velocities were higher than the tricuspid inflow velocities, which tended to vary with inspiration. No significant difference was recorded in peak velocity between the aortic and pulmonary outflow, although the acceleration time

was shorter for aortic flow. The angles between the ultrasound beam and blood flow were assessed from the two-dimensional image. The mean angles used in this study varied between 40.86° and 67° for the different sampling sites. Data were excluded from horses in which the angle between the ultrasound beam and blood flow exceeded 75°. The authors corrected the Doppler calculation using the estimated angle. The large angles obtained in this study could cause considerable error in the estimation of flow velocities (Goldberg *et al.* 1988).

There have been no studies to evaluate the efficacy of colour flow Doppler echocardiography in horses. Reef (1990b) describes the use of Doppler colour flow mapping in horses referred for cardiac evaluation. The author suggests that the limitations of the technique in horses relate to the depth of imaging and the angle of interrogation of the valves. Better alignment is described for the right sided valves than the left sided valves where the angle of interrogation tends to be perpendicular to flow. In the authors' experience, mild tricuspid regurgitation is the most common finding in horses referred for cardiac examination, the regurgitant jet occupying approximately 20% to 25% of the right atrium. Insignificant tricuspid regurgitation on the right side of the valve leaflets is also described. However, this report does not indicate the prevalence of this flow pattern, or whether a simultaneous murmur can be auscultated. Mitral regurgitation is detected less commonly. The author considers this is related to the problem of imaging depth (parts of the atria cannot be studied). It is not indicated if the horses in which mitral regurgitation cannot be detected have a murmur suggestive of this condition. Pulmonary insufficiency is described most infrequently. This report advises the use of pulsed wave Doppler echocardiography to delineate the size of the jet, if poor alignment with flow results in a poor colour flow image. However as accurate alignment with flow is important for both techniques, this finding may indicate lack of sensitivity of the colour flow equipment.

Despite the difficulties in aligning with intracardiac, aortic and pulmonary artery blood flow in horses (Reef *et al.* 1989), Reef (1991a) considers that the use of

Doppler echocardiography to semiquantify valvular regurgitation and intracardiac shunts, has revolutionised equine cardiology. No information is available, however, concerning the accuracy of the technique or the incidence of valvular regurgitation in normal horses.

Doppler echocardiography has become an established technique for the noninvasive investigation of cardiac disease in humans, and has substantially improved the understanding of blood flow in the normal and diseased heart (Simpson and Camm 1990). Although Doppler echocardiography has been used to aid diagnosis of heart disease in horses, there has been limited evaluation of the technique in this species.

AIMS OF PART 1 OF THIS WORK

1) To standardise suitable images for two-dimensional, M-mode and Doppler echocardiography in horses.

2) To determine normal values for selected cardiac dimensions in Thoroughbred and Thoroughbred cross horses and to compare these values with those from horses with cardiac murmurs.

3) To record colour flow Doppler patterns in normal Thoroughbred and Thoroughbred cross horses and to observe variations in horses with cardiac murmurs.

4) To establish reference values for flow velocities from the aortic and pulmonary outflow and the mitral and tricuspid inflow in normal Thoroughbred and Thoroughbred cross horses. To compare these values with those from horses with cardiac murmurs.

CHAPTER 2. MATERIALS AND METHODS

HORSES

CONTROL GROUP

The control group consisted of 40 Thoroughbred or Thoroughbred cross horses (three stallions, twenty one geldings and sixteen mares). The horses were aged from two to seventeen years and weighed between 428kg and 648kg (mean 513kg). The group was selected randomly from clinically normal horses referred to the R.(D).S.V.S. for non cardiac and non medical disorders, and from clinically normal horses from riding and racing establishments. The horses had no history or evidence of cardiac dysfunction on clinical and electrocardiographic (ECG) examination. Horses with the following functional murmurs were included in the control group. These murmurs, described in Table 1, were present in 57.5% of the control horses.

Table 1. Description of murmurs designated as functional. S2 = Second heart sound. S3 = Third heart sound. S4 = Fourth heart sound. PMI = Point of maximum intensity.

TYPE	TIMING	PMI	% OF CONTROL GROUP
Ejection	Early/mid systole	Left hemithorax Aortic valve area	35
Presystolic	audible between S4 and S1	Right or left hemithorax	22.5
Early diastolic	audible between S2 and S3	Right or left hemithorax	42.5

The clinical details of the control horses are given in Appendix 1.

HORSES WITH CARDIAC MURMURS

These horses were Thoroughbred or Thoroughbred cross horses from the same sources as the control group. This group also included horses which were specifically referred to the R.(D).S.V.S. for the evaluation of cardiac murmurs. The horses with cardiac murmurs were divided into groups based on the point of maximum intensity (PMI) of the murmur and its timing within the cardiac cycle. The murmurs were graded in intensity based on the system described by Levine and Harvey (1949). The grading system is described in Table 2.

Table 2. System used to grade cardiac murmurs.

GRADE	DESCRIPTION	THRILL
Grade 1	The softest audible murmur only heard after minutes of careful auscultation.	-
Grade 2	A faint murmur heard almost immediately.	-
Grade 3	A murmur of moderate intensity.	-
Grade 4	A loud murmur not accompanied by a palpable thrill.	-
Grade 5	A loud murmur that was inaudible when the stethoscope was just lifted from the thoracic wall.	+
Grade 6.	A loud murmur which remained audible when the stethoscope was just lifted from the thoracic wall.	+

Group 1 comprised horses with a systolic murmur audible over the left hemithorax with the PMI over the mitral valve area. The mitral valve area was defined as that area where the apical impulse was palpable on the left thoracic wall (Bonagura 1990). This type of murmur is thought to be indicative of mitral

regurgitation (Glazier 1984; Reef 1989; Bonagura 1990). This group contained eight horses. Two horses had a grade 2 murmur, three horses had a grade 3 murmur, two horses had a grade 4 murmur and one horse had a grade 5 murmur (Table 3).

Group 2 comprised horses with a systolic murmur audible over the right hemithorax, with the PMI over the tricuspid valve area. The tricuspid valve area was located over the right hemithorax slightly cranial to the corresponding mitral area (Glendinning 1972; Reef 1989; Bonagura 1990). At this point the first heart sound was clearly audible. This group did not include horses in which the murmur radiated ventrally over the sternal border. Group 2 contained eight horses with murmurs of the same intensity as the horses in group 1 (Table 3). The type of murmur in this group was thought to be indicative of tricuspid regurgitation (Glazier 1984; Reef 1989; Bonagura 1990).

In order to compare a group of horses with low intensity murmurs to a group with high intensity murmurs, two further groups of horses with a systolic murmur over the tricuspid valve area were studied. These were subdivided as follows: group 3 contained seven horses with a grade 2 murmur and one horse with a grade 1 murmur. Two of these horses were obtained from group 2. Group 4 contained eight horses, 3 with a grade 3 murmur, 3 with a grade 4 murmur and 2 with a grade 5 murmur (Table 3). Six of these horses were obtained from group 2.

Group 5 comprised horses with a decrescendo diastolic murmur with the PMI over the left hemithorax at the level of the aortic valve. The aortic valve area was identified as the point where the second heart sound could be heard most clearly. It was located dorsal and cranial to the mitral valve area. Horses in which the murmur could also be heard over the right hemithorax were included in this group. This type of murmur is thought to be indicative of aortic regurgitation (Smetzer, Bishop and Smith 1966). Group 5 consisted of eight horses. Three horses had a grade 2 murmur, three had a grade 3 murmur, one had a grade 4 murmur and one had a grade 5 murmur (Table 3).

Table 3. Number of horses and intensity of murmurs in groups 1 - 5.

GROUP	INTENSITY OF MURMUR					
	1	**2**	**3**	**4**	**5**	**6**
Group 1 (Mitral)	0	2	3	2	1	0
Group 2 (Tricuspid)	0	2	3	2	1	0
Group 3 (Tricuspid)	1	7	0	0	0	0
Group 4 (Tricuspid)	0	0	3	3	2	0
Group 5 (Aortic)	0	3	3	1	1	0

To allow the three types of cardiac murmur (aortic, mitral and tricuspid) to be studied in isolation, horses with more than one murmur were excluded from the study, with the exception of horses which had co-existing functional murmurs.

The clinical details of the horses in groups 1 to 5 are given in Appendix 2.

ECHOCARDIOGRAPHY

Echocardiographic studies were performed using a Vingmed CFM 700 ultrasound system with a 2.25 MHz transducer. The transducer was a mechanically steered, six ringed annular phased array, with dynamic focussing. The equipment enabled two-dimensional imaging, M-mode imaging, colour flow mapping and spectral Doppler (continuous wave and pulsed wave Doppler) studies to be performed by the same transducer. All studies were recorded on videotape (Maxell E-180 EX) using a Panasonic AG 6200 video recorder. Hard copy photographs were obtained directly from the digital output using a freeze frame camera (Polaroid). An ECG recording obtained simultaneously during echocardiography was displayed below the ultrasound image on the monitor (Figure 1). The ECG electrodes consisted of flat tin plates which were placed underneath an elastic circingle positioned immediately

behind the withers. The negative electrode was placed dorsal to the right of the midline and the positive electrode was placed ventral to the left of the midline. The earth electrode was positioned on the right side of the horse approximately midway up the thoracic wall.

TWO-DIMENSIONAL IMAGING

During two-dimensional imaging the sector angle of the Vingmed CFM700 could be varied from 45° to 90°. Increasing the sector angle above 70° doubled the number of beams from which the sector was constructed, from 64 to 128. This resulted in a decrease in the frame up-date rate (frame rate). In this study the sector angle was maintained between 80° and 90°. The imaging depth of the equipment also influenced the frame rate. Alteration of the sector angle and imaging depth changed the frame rate from between nineteen to 55 frames per second. Imaging at the maximum depth of 24 centimetres with a sector angle of 80° to 90° resulted in the lowest frame rate of nineteen frames per second. The frame rate was displayed on the right of the monitor below the sector (Figure 1). A frame change indicator displayed below the ECG indicated the timing of the frame rate changes from the far right of the screen (Figure 1).

The quality of the two-dimensional image was adjusted to obtain good definition of intracardiac structures and endocardial surfaces. Low amplitude signals were rejected (reject) and the remaining signals were amplified so that deeper structures could be seen clearly. The signal amplification (gain) was varied at five specified levels over the image sector. The image quality was further adjusted by changing the relationship between the signal amplitude and the grey scale displayed on the two-dimensional image (compression). These three controls (reject, compression and gain) were pre-set to obtain a good image in a Thoroughbred type horse. Minor adjustments in these settings were then made to obtain optimal image quality for each individual horse. All controls were set to the lowest level which produced a good quality image.

M-MODE IMAGING.

M-mode images are constructed from a single imaging beam using rapid sampling rates (Feigenbaum 1986). The reject, compression and gain of the M-mode image was adjusted as for the two-dimensional image. The horizontal sweep speed of the M-mode trace could also be adjusted. This was set at four seconds, which represented a sweep speed of 14.5 centimetres per second.

COLOUR FLOW DOPPLER ECHOCARDIOGRAPHY

For colour flow mapping, the equipment constructed first the two-dimensional image, then overlaid the image with colour flow Doppler information. As each element on the screen could only display a grey scale image or a colour coded image at any one time, the gain or signal amplitude of the two-dimensional image was reduced during colour flow mapping. The colour flow map used in this work was an enhanced "red towards" "blue away" rainbow map. Flow towards the transducer was coded in red, with the faster velocities coded in a lighter shades of red. Flow away from the transducer was coded blue, with the highest velocities coded in the lightest shades of blue. Where velocities exceeded the Nyquist limit, aliasing occurred (see page 29) and the blood was coded as flowing in the opposite direction. In areas of highly aliased flow a green colour was added, to depict the wide bandwidth and intensity of the aliased signal (Angelsen, Kristoffersen, Torp, Linker and Skjaerpe 1989). This green coding allowed areas of disturbed flow to be clearly distinguished from areas of normal flow.

During colour flow imaging the maximum depth of the two-dimensional image was twenty centimetres. At this depth colour flow information could be obtained up to a depth of eighteen centimetres. The pulse repetition frequency which determined the Nyquist limit or the speed at which aliasing occurred (see page 29) was inversely related to the depth of the colour flow map.

The angle of the colour flow sector could be varied between 20° and 70°. The greater the angle of the flow sector the lower the frame rate. The frame rate of the colour flow image was also influenced by the level of the quality setting and the low velocity reject. The quality setting determined the rotational velocity of the transducer which inversely altered the number of ultrasound pulses generated per image beam. The more pulses generated the greater the information collected at each location on the colour flow map. The higher the quality setting the slower the transducer moved and the slower the frame rate. The low velocity reject controlled the filtration of low frequency information from the returning Doppler signal. This filtered out strong signals from slow moving structures, such as the cardiac walls. The sector angle, depth, quality and low velocity reject settings used in these experiments were set to achieve a Nyquist limit of 0.7 metres per second (m/s) with a frame rate of between 9 and 12 frames per second. The frame rate was displayed on the monitor, along with a frame display indicator as described for two-dimensional imaging (Figure 1).

The gain or amplification of the colour signal was set at the level which just failed to produce colour artifacts within the sector image. The colour settings were kept constant for all horses imaged.

Colour Doppler information could also be overlaid on an M-mode image. The colour M-mode is not limited by frame update rate and could therefore be used for more accurate timing of colour flow events.

SPECTRAL DOPPLER ECHOCARDIOGRAPHY

The annular phased array transducer was used in the high pulsed repetition frequency mode (HPRF), at an emitted frequency of 2MHz. The quality of the Doppler display could be varied in a number of ways. The level of grey scale that was used to display each velocity could be adjusted (compression), signals below a selected intensity could be rejected and the remaining signals could be amplified. These three controls (compression, reject and gain) were adjusted so that the Doppler

waveforms could be clearly seen without the appearance of artifacts. The settings remained constant for the investigation of all inflow and outflow velocities. The level at which the reject and compression were set was displayed in the top left corner of the monitor (Figure 1). The horizontal sweep speed of the spectral display was four seconds unless otherwise stated. A low velocity reject filter was used as described previously for colour flow Doppler studies. This allowed low velocity, high intensity signals to be rejected. The low velocity reject filters could be varied to filter signals from 0.05 to 0.5 metres per second. During the collection of inflow and outflow data these filters were set as low as possible.

The velocity scale of the spectral display could be varied, and the baseline positioned at any level within the display. The maximum velocity scale in the HPRF mode was twelve metres per second. During HPRF Doppler the velocity of flow was interrogated at a specific point. The sampling site or sample volume was positioned in an area of interest by guidance from the colour flow image. Once the sampling site was located in the area of choice, the colour flow image was frozen, and the transducer was then dedicated to the collection of Doppler information. The length of the sample volume was variable between 0.8 and 22.5 millimetres. The size of the sample volume remained constant at approximately 2.5 millimetres during the recording of all inflow and outflow velocities. The number of sample volumes available for velocity analysis, increased as the velocity scale was increased. At the minimum velocity scale of 1.5 m/s only one sample volume was available. The velocity scale was set to the lowest level that still allowed measurement of the maximum flow velocity.

Accurate alignment with blood flow was assumed when the audible signal was clear and the spectral envelope was complete. The audible signal was transmitted via headphones to the operator to avoid disturbing the horses.

MEASUREMENTS

Measurements of cardiac dimensions and blood flow variables were made from videotape play-back, using computer software inbuilt in the Vingmed CFM700. Prior to measuring, each video frame was calibrated by inserting a digital code into the equipment computer. Calibration of the videotape prompted the display of specific measurement packages for each type of study. In the two-dimensional imaging mode electronic calipers were activated to measure linear distances to the nearest 0.07 centimetres. In the M-mode imaging mode, the electronic calipers were used to determine the horizontal time scale, thus allowing measurement of time intervals from the ECG. These calipers measured to an accuracy of 0.01 seconds. Specific M-mode study menus enabled a group of measurements to be made at fixed points on the ECG. These menus were used to compare systolic and diastolic measurements. Diastolic measurements were taken at the onset of the QRS complex. Systolic measurements were taken at the point of maximum excursion of the interventricular septum. This follows the recommendations of the American Society of Echocardiography for M-mode measurements (Sahn *et al.* 1978). In the two-dimensional imaging mode diastolic recordings were taken from the frame closest to the onset of the QRS complex. To avoid inaccuracy in the timing of measurements, where possible all intracardiac measurements were taken from M-mode images.

M-mode measurements were made using the leading edge method (Feigenbaum 1986). The measurements were recorded from the leading edge of the first endocardial surface to the leading edge of the second surface. In this way the thickness of the first endocardial surface was included in the measurement. This method was also used for two-dimensional measurements when tissue interfaces were perpendicular to the axial beam. The leading edge - trailing edge method, measuring from the leading edge of the first endocardium to the trailing edge of the second includes the thickness of both endocardial surfaces (Wyatt, Haendchen, Meerbaum

and Corday, 1983). This method was used for two-dimensional measurements when tissue interfaces were aligned parallel to the axial beam.

Linear dimensions from the colour flow image were measured to the nearest 0.06 centimetre. Area measurements from the colour flow image were recorded by manual planimetry and were measured to the nearest 0.01 centimetre^2.

In the spectral Doppler mode, activation of an electronic caliper allowed the velocity of blood flow to be measured in metres per second at any point on the trace. A second caliper was available, which allowed a second velocity to be recorded. The use of a second caliper enabled measurements to be made between the two calipers. This was used to record change in time (dt), in seconds, on the horizontal axis and change in velocity (dv), in metres per second, on the vertical axis. A combination of these two measurements enabled the change in velocity by time (dv/dt) to be calculated. This value was given in metres per second^2. An electronic tracker-ball was used to trace the outline of spectral Doppler signals manually. From this trace, the area under the velocity waveform (velocity time integral, VTI) was calculated. By tracing from the onset of one spectral envelope to the onset of the next an estimation of the heart rate was obtained. The specific measurements recorded during each study are detailed in the relevant chapter.

STATISTICAL ANALYSIS OF RESULTS.

Statistical analyses were performed using Minitab statistical software located on an Akhter LC386-25 personal computer. Box and whisker plots and normal probability plots were performed on all data to assess the pattern of distribution (Ryan, Joiner and Ryan, 1985). Normally distributed data was analysed using parametric techniques and non normal data was analysed using non parametric techniques (Snedecor and Cochran 1980).

CHAPTER 3. ESTABLISHMENT OF A STANDARDISED IMAGING TECHNIQUE USING TWO-DIMENSIONAL ECHOCARDIOGRAPHY.

INTRODUCTION

The aim of these experiments was to establish a series of standardised ultrasound images in a group of normal horses and describe the techniques required to obtain them. Two-dimensional ultrasound images can be recorded in multiple planes (Tajik *et al.* 1987), and can be used to provide spatial information by which M-mode and Doppler studies can be guided (Feigenbaum 1986). They also form the background on which Doppler colour flow maps are displayed. Standardised ultrasound images have not been described for the horse despite the widespread use of ultrasound in clinical diagnosis in this species (page 21). If the results of different workers are to be compared accurately it is important to standardise image planes and to detail standard views.

METHODS

Fourteen clinically normal horses were imaged in order to develop the standard imaging planes. Eighteen standard views were defined to evaluate cardiac structures and to perform M-mode and Doppler studies (Tables 4 and 5). The views were standardised by relating the position of the axial beam relative to various intracardiac landmarks.

Ultrasound images were recorded from the right and left sides of the thorax. The hair coat was clipped and acoustic coupling was achieved using ultrasound gel[a]. The horse was positioned with the appropriate foreleg slightly abducted so that the olecranon process was free of the thoracic wall. This could be achieved by standing the horse alongside the operator and pushing the hindquarters away slightly. In some horses it was necessary to place the foreleg slightly cranial and lateral.

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a Euro Diffusion Medicale, Rosney, St. Bois, France.

The transducer location, sector rotation and axial (central) beam angulation required to obtain each view were recorded. The location of the transducer describes the position of the transducer on the body wall relative to a long-axis reference image. Imaging commenced from the right hemithorax using a long-axis view of the ventricular inlets as a reference image (Table 4, Figure 2a). This image was obtained by placing the transducer at the fourth or fifth intercostal space just dorsal to the olecranon process. The sector edge was rotated dorsally (0^{o}) and the axial beam was then adjusted to image the ventricular inlets and the interventricular septum (IVS). The image was considered acceptable when the axial beam crossed the left ventricle (LV) at the level of the chordae tendineae and the IVS was orientated almost horizontally across the sector. The reference image from the left hemithorax was obtained by placing the transducer caudal to the olecranon process and dorsal to the left apical impulse with the sector edge rotated dorsally (0^{o}). This generated a long-axis view of the left atrium and ventricle optimised to view the mitral valve and left ventricular inlet. (Table 5, Figure 5a). The axial beam was adjusted to cross the LV at the level of the chordae tendineae. Once again the IVS was orientated almost horizontally across the sector. Thus the location of the transducer required to obtain the reference images provided the reference point from which the other standardised views could be obtained. Placement of the transducer away from these points was described as cranial, caudal, dorsal or ventral.

The sector rotation of the transducer was defined as "+" when rotated in a clockwise direction (as viewed from the handle) and "-" when rotated in an anti-clockwise direction. Zero was defined as "no rotation" i.e. when the index mark was in the dorsal 12 o'clock position. The approximate sector rotation required for each view was assessed visually.

Angulation was achieved by pivoting the transducer about a fixed location on the body wall in either a cranial, caudal, dorsal or ventral direction. The angulation of the transducer was defined as "0" when the transducer was held perpendicular to the

body wall. An angulation of "+3" corresponded to the maximum angulation that could be achieved between the transducer and the body wall without loss of contact at the body surface. The maximum angulation achieved with the Vingmed 2.25 MHz transducer was approximately 65° from the perpendicular position. Transducer angulation between these limits was assessed as "+1" (slight) or "+2" (moderate).

The nomenclature and standards used in this study followed the proposals of Henry *et al.* (1980) for human echocardiography. Long-axis images were displayed with the apex to the left of the screen and the atria to the right. The index mark of the transducer indicating the edge of the imaging plane corresponded to the edge which appeared on the right of the image display. The transducer was held with the index mark under the thumb of the left hand when imaging the right hemithorax and the right hand when imaging the left hemithorax. Sagittal images are referred to as "long-axis", coronal images as "short-axis", views obtained with the transducer in a ventral thoracic location as "apical" and the term "angled view" is used to describe images in which some structures are visualised in long-axis and others in short-axis (Weyman 1982).

Table 4. Standardised images obtained from the right hemithorax.

Right parasternal long-axis views.

View	Structures imaged	Location of axial beam	Comments	Fig
Reference view Ventricular inlets.	RA, RV, TV, IVS, LA, LV, MV, LVW.	RV, IVS, CT of MV.	The LVOT is not included in this view. The IVS crosses the axial beam at right angles. The LA and LV size is maximised.	2a
Tipped view LV inlet/outlet.	RA, RV, TV, LV, IVS, Ao, AV, LVOT CT of MV. LA in systole.	RV, CT of TV. In diastole: Junction of Ao root and MV annulus. In systole: AV.	The long- axis of the heart crosses the axial beam at an angle of 40^{o} to 60^{o}. The cardiac apex is tipped dorsally towards the transducer.	2b
Long-axis aorta.	LVOT, Ao, AV, RA, RV, TV, LV, LA, CT of MV.	CT of TV or valve tips. In diastole: LVOT and CT of MV. In systole: AV, TV.	The long-axis of the aorta is perpendicular to the axial beam.	2c
Dorsal location RV inlet.	RA, TV, RV, Ao, (RVOT or LVOT).	TV, Ao.	From this view it is possible to sweep through a wide range of rotations and cranial and dorsal angulations. The aorta will appear in short-axis, long-axis or obliquely depending on the rotation of the transducer.	2d
Apical view Ventricular inlets.	RA, TV, RV, IVS, LV, MV, LA.	RV, TV, RA.	The long-axis of the heart is displayed as vertically as possible.	2e

Table 4 (continued). Standardised images obtained from the right hemithorax.

Right parasternal short-axis views.

View	Structures imaged	Location of axial beam	Comments	Fig
Papillary muscle level.	PMs, RV, RVW, IVS, LV, LVW.	Junction of the IVS and LV lateral wall.	This is a true short-axis view with papillary muscles evenly displayed.	3a
Chordal level.	RV, RVW, IVS, LV, LVW, CT.	Junction of the IVS and LV lateral wall.	This level is immediately above the PM level and immediately below the MV level. The LV lumen is as large as possible without imaging the MV The view is a true short-axis of the LV.	3b
Mitral valve level.	TV, IVS, MV.	TV and lateral edge of anterior mitral valve leaflet.	The MV leaflets are visualised in systole and diastole.	3c
Aortic valve level.	Ao, TV, RA, RV RVOT, LA.	TV, Ao.	The axial beam bisects the aorta. The valve leaflets are obvious during diastole.	3d
Pulmonary artery level.	Ao, SV crest, PA, coronary artery, RVOT.	SV crest in systole. Ao in diastole.	The SV crest and Ao move in and out of the axial beam during the cardiac cycle.	3e
Right parasternal angled view.				
Dorsal location RVOT.	PA, PV, RVOT, Ao, TV, RV, SV crest, coronary artery.	RV inflow tract. SV crest in diastole. RVOT and PA in systole.	The SV crest and PA move in and out of the beam as the heart contracts and relaxes The RVOT, PV and PA are in long-axis.	4

Table 5. Standardised images obtained from the left hemithorax.

View	Structures imaged	Location of axial beam	Comments	Fig
Left parasternal long-axis views.				
Reference view LV inlet.	LA, LV, CT of MV, RV, RA, TV.	CT of MV, IVS, RV.	The long-axis of the heart crosses the axial beam at right angles.	5a
Apical view LV inlet.	LV, LA, MV, RV, RA.	LV, MV.	The long-axis of the heart is displayed as vertically as possible.	5b
5-chambered.	LV, LVOT, AV, RV, TV, RA.	LV, AV, Ao.	The aortic root and AV are in long-axis.	5c
Left parasternal short-axis views.				
LV chordal level.	RV, IVS, LV.	LV. Junction of the LV wall and IVS.	This is a true short-axis view of the LV. The LV lumen is as large as possible without including the MV. The posterior PM and the LVOT are not included in this view.	6a
RV wall.	RVW, RV, IVS, LV.	IVS, RVW.	The LV is in short axis.	6b
Left parasternal angled views.				
RV inlet / outlet.	TV, RA, PA.	RV, TV, RA,	The PV and PA are in long-axis.	7a
LVOT / Aorta.	AV, LVOT, Ao.	LV, LVOT, AV, Ao.	The LVOT and AV are in long-axis.	7b

RESULTS

The eighteen standardised images were obtained in all horses. The transducer location, sector rotation and axial beam angulation required to obtain each image are detailed in Appendix 3 and 4. These findings are summarised in Table 6.

In most horses it was necessary to angle the transducer slightly caudally and dorsally to achieve the reference view from the right hemithorax (Figure 2a). Minimal dorsal angulation was also required to obtain the left parasternal reference view (Figure 5a). Ventral angulation was only required for three of the eighteen standardised views; the right parasternal short axis view at the papillary muscle level (Figure 3a) and chordal level (Figure 3b), and the left parasternal short-axis view of the right ventricular wall (Figure 6b). The latter view was the only image that required ventral angulation in all horses. Slight cranial and dorsal angulation was required in most horses to obtain images of the aorta from both the left and right parasternal long-axis views (Figures 2c and 5c). In the right parasternal short-axis views, as the transducer was adjusted, to produce images from the papillary muscle level to the pulmonary artery level (Figures 3a to 3e), increasing dorsal and cranial angulation was required. For apical views (Figures 2e and 5b) maximal dorsal angulation was required in most horses. The dorsal location, right parasternal long-axis view of the right ventricular inlet (Figure 2d) was obtained with the full range of cranial and dorsal angulation.

It was not necessary to rotate the transducer in any horse to obtain the reference images. Clockwise (positive) rotation was required to image the aorta in the right parasternal long-axis views (Figures 2b and 2c), whilst anti-clockwise (negative) rotation was necessary to image the aorta from the left parasternal long axis. Anti-clockwise (negative) rotation was required to obtain all of the short-axis images from the right hemithorax. The degree of negative rotation required, progressively decreased as the transducer was adjusted to image from the papillary muscle level to the pulmonary artery level (Figures 3a to 3e).

Table 6.

Summary of the transducer orientation required to obtain each standardised view and the potential uses. CR = a cranial angulation of + or ++. CD = a caudal angulation of + or ++. D = a dorsal angulation of + or ++. V = a ventral angulation of + or ++. * = angulation of ++ or +++. ( ) = angulation of 0 to +.

View	Transducer Orientation (Location, Rotation, CR/CD D/V Angulation).	Comments	Typical Uses
Right parasternal long-axis views.			
Reference view. Ventricular inlets.	Standard 0^{o} (CD) (D)	-	Imaging, Colour Flow Mapping (Ventricular Septal Defect, MR, TR)
Tipped view. LV inlet/outlet.	Standard/Ventral $+30^{o}$ (CR) D	-	Imaging, Colour Flow Mapping (Ventricular Septal Defect, Aortic insufficiency, MR, TR)
Long-axis aorta.	Standard/Dorsal $+30^{o}$ (CR) (D)	Can alter rotation up to 45^{o} also CR angulation to image more aorta in long-axis.	Imaging, Colour Flow Mapping (Ventricular Septal Defect, Aortic insufficiency)
Dorsal location RV inlet.	Dorsal 0^{o} to $+90^{o}$ (CR) to CR* (D) to D*	Can alter rotation, cranial and dorsal angulation through complete range to image RA	Imaging, colour Flow Mapping, Spectral Doppler (RV inflow, TR)
Apical view. Ventricular inlets.	Ventral 0^{o} (CR) D*	Can alter rotation up to 45^{o} also can add CR angulation to image more RA	Imaging, Colour Flow Mapping Spectral Doppler, (RV inflow)

Table 6 (Continued).

View	Transducer Orientation (Location, Rotation, CR/CD D/V Angulation).	Comments	Typical Uses
Right parasternal short-axis views.			
Papillary muscle level.	Standard/Ventral -90° (CD) (D)	Adjust rotation through -80° to -90° to obtain true short-axis	Imaging
Chordal level.	Standard -90° (CD) (D)	Adjust rotation through -80° to -90° to obtain true short-axis	Imaging, LV measurements (M-Mode and 2-D)
Mitral valve level.	Standard -80° (CR) (CD) D	Adjust rotation through -60° to -80° to obtain true short-axis	Imaging, M-Mode (MV motion), Colour Flow Mapping (MR)
Aortic valve level.	Standard -30° CR D	Adjust rotation through -20° to -60° to obtain true short-axis	Imaging, M-Mode (aortic diameter, AV motion), Colour Flow Mapping (AI)
Pulmonary artery level.	Standard -20° to -30° CR* D	Adjust rotation through -20° to -60° to obtain true short-axis	Imaging, Spectral Doppler, Colour Flow Mapping, (RV inflow, PI)
Right parasternal angled view.			
Dorsal location RVOT.	Cranial/Dorsal -30° CR D	-	Imaging, Spectral Doppler, Colour Flow Mapping (RV inflow, PI, TR, Pulmonary outflow, Sub Pulmonic VSD)

Table 6 (Continued).

View	Transducer Orientation (Location, Rotation, CR/CD D/V Angulation).	Comments	Typical Uses
Left parasternal long-axis views.			
Reference view. LV inlet.	Standard 0^{o} (CD) (D)	-	Imaging, Colour Flow Mapping (Ventricular Septal Defect, MR)
Apical view. LV inlet	Standard/Caudal/Ventral 0^{o} (CR) (CD) D*	Can alter rotation up to -40^{o} to image more LA	Imaging, Colour Flow Mapping, Spectral Doppler (LV inflow, MR)
5-chambered.	Standard/Ventral 0^{o} to -30^{o} CR D	Slide transducer to caudal edge of intercostal space and angle cranially	Imaging, Colour Flow Mapping, Spectral Doppler (Aortic outflow, AI)
Left parasternal short-axis views.			
LV Chordal level.	Standard $+90^{o}$ (CR) (D)	In a small number of cases caudal angulation is necessary. Adjust rotation from $+30^{o}$ to $+90^{o}$ to obtain true short-axis.	Imaging. LV measurements (M-Mode and 2-D)
Right ventricular wall.	Cranial $+90^{o}$ (CR) (CD) (V)	In a small number of cases caudal angulation is necessary. Adjust rotation from $+30^{o}$ to $+90^{o}$ to obtain true short-axis	RV wall measurement (M-mode)
Left parasternal angled views.			
RV inlet / outlet.	Cranial/Ventral -45^{o} (CD) D	Position as cranially and ventrally as possible. Try to angle caudally and dorsally.	Imaging, Spectral Doppler, Colour Flow Mapping (RV inflow, TR, PI)
LVOT / Aorta.	Standard/Ventral $+30^{o}$ (CR) D*	Some cases may require (CD) or CR angulation to obtain the LVOT and Aorta in long-axis.	Imaging, Colour Flow Mapping, Spectral Doppler (Aortic outflow, AI)

Two right parasternal long-axis views of the right ventricular inlet (Figures 2d and 2e) were obtained from both dorsal and apical locations using a wide range of transducer rotation.

DISCUSSION

The standardised images were recorded with a narrow range of transducer location, rotation and angulation, with the following exceptions. Two, right parasternal, views of the right ventricular inlet (Figure 2d and 2e) could be obtained from a wide range of sector rotations and cranial/caudal angulations. This was due to a lack of precision in fully defining these views. It was possible to alter the rotation and cranial angulation to view a large area of the right atrium yet still maintain the available intracardiac landmarks within the axial beam. A wide range of sector rotation could also be used to image the right ventricular outflow tract from the right parasternal dorsal location (Figure 4), and the parasternal short-axis views at the aortic (Figure 3d) and pulmonary valve levels (Figure 3e). As these views were specifically defined, the variation in sector rotation is probably either a reflection of differences between subjects or in the initial placement of the transducer.

This systematic method of standardisation of equine echocardiographic images differs from those of prior reports. Carlsten (1987) used standardised intercostal locations to obtain long-axis and short-axis images from the left and right hemithoraces. The disadvantage of using standard transducer placement, rather than standard views, is that there is considerable variation between individual horses. In some horses an initial cranial placement of the transducer was required to obtain the reference views; whereas in other horses, these were obtained from more caudal positions. Once the reference views were achieved in each horse it was possible to relate all subsequent views to these original transducer placements.

Voros, Holmes and Gibbs (1990) used intracardiac landmarks to standardise nine specific image planes of the equine heart. Anatomical validation of intracardiac

structures was achieved using in vitro techniques. The exact positioning of the transducer required to obtain specific views was not addressed

The concept of recording transducer rotation and beam angulation was considered by Stadler *et al.* (1988) in their work using the method of Carlsten (1987) to obtain two-dimensional images of the equine heart. The approximate transducer rotation and angulation required to achieve each image were detailed, although the images were not standardised by describing relationship of the axial beam to the intracardiac landmarks. When the images depicted in this study appeared similar to the standardised views of the present study, the results of transducer rotation and angulation were in general agreement. However, comparison of the transducer orientations required to obtain cranial views from the left and right hemithorax was difficult as the resultant images were different.

Six of the standardised views in the present study were obtained from six of the image planes standardised by Voros, Holmes and Gibbs (1990). However, although the same image plane was used for the short-axis views from the left hemithorax, the transducer rotation was opposite. This resulted in a reverse orientation of the left and right ventricle, with the right ventricle appearing to the left of the screen. This orientation also differed from that used by Carlsten (1987) and Stadler *et al.* (1988). The positive rotation was chosen in the present study, so that the hand was always supinated to obtain short axis views, whether imaging from the right or the left side of the thorax.

The orientation of the short-axis views from the right hemithorax in the present study were the same as described by Voros Holmes and Gibbs (1990) and Stadler *et al.* (1988), but differed from the images depicted by Carlsten (1986).

The long-axis plane from the left hemithorax described by Voros, Holmes and Gibbs (1990) differed from the long-axis reference view described in the present study, as the image plane did not transect the IVS. The short-axis plane through the mitral valve from the left hemithorax (Voros, Holmes and Gibbs 1990) was not

included in the present study although it may be possible to standardise a view at this level. A standardised short-axis view of the aortic and pulmonary valve from the left hemithorax was also not described in the present study. The standardised plane described by Voros, Holmes and Gibbs (1990) at this level could not be truly standardised as the pulmonary and aortic valves lie in different planes (Voros, Holmes and Gibbs 1990).

The position of the axial beam in relation to intracardiac structures was used to identify the location and angulation of the transducer. It was therefore possible to determine the transducer position required to obtain a subsequent view from any given image. The reference image can be used to illustrate this point. If the axial beam is cranial to the aorta, more caudal placement or angulation of the transducer is required to obtain the reference image. If the left ventricular outflow tract appears in the reference image then either excessive positive rotation or cranial angulation of the transducer must be present. If the septum is not orientated perpendicular to the axial beam, ventral transducer placement with dorsal beam angulation must be present. When the transducer is held perpendicularly to the thoracic wall, with the sector angulation at 0^{o}, ventral placement of the transducer results in the axial beam crossing the reference image towards the apex of the heart. A dorsal location of the transducer results in the axial beam crossing the image at the level of the atrioventricular valves or atria. Whilst the method of estimating the angulation and rotation in this study was subjective, this standardised technique of imaging the equine heart has proved to be repeatable.

The nomenclature and standards used in this study followed the proposals of Henry *et al.* (1980) for human echocardiography. However, due to the lateral compression of the thorax in the horse, the transducer placement required to obtain the parasternal views in this species could be more accurately described as left and right intercostal. This has also been reported in canine echocardiography (Thomas 1984). Whilst accepting these observations, the human nomenclature was adopted for

this work, as the images obtained from the intercostal locations in the horse were similar to the images obtained from the parasternal location in man.

Correct positioning of the horse was essential to obtain good images. The more cranial transducer placements were poorly tolerated by most horses and advancement of the appropriate forelimb was necessary to obtain these views. No advantage was achieved by holding the leg up as this merely flexed the leg at the elbow and produced more caudal placement of the triceps muscle mass. A metal stand was used by Stadler *et al.* (1988) in fourteen horses to extend the foreleg sufficiently to allow cranial transducer placement. In the remaining six horses sufficient cranial placement was achieved by merely extending the foreleg cranially and placing it on the ground. Broad chested animals and overweight animals produced images of poorer quality. Images obtained from the left side were sometimes of poorer quality than those obtained from the right. This is in agreement with Stadler *et al.* (1988) but is in contrast to the observations of Carlsten (1987).

Summary

This study demonstrates that it is possible to obtain standardised echocardiographic views in the horse. The adoption of a standardised method of examining the heart will facilitate the comparison of results from different workers. The method has advantages over standardised transducer positions as it will allow more accurate comparisons to be made.

CHAPTER 4. MEASUREMENT OF INTRACARDIAC DIMENSIONS FROM STANDARDISED TWO-DIMENSIONAL AND M-MODE IMAGES IN NORMAL HORSES AND HORSES WITH SPECIFIC CARDIAC MURMURS

INTRODUCTION

In Chapter 3 eighteen standardised ultrasound images were established which were considered to be of potential value in the investigation of the equine heart. In the following experiments these images were used to guide M-mode studies from which various cardiac dimensions were measured. Measurements were also made from one two-dimensional image. Ultrasound images were recorded from the group of normal horses and from the horses with valvular disease described in Chapter 2. The clinical details of these horses are given in Appendices 1 and 2.

METHODS

M-mode images were recorded from the right and left hemithoraces, using two-dimensional images to guide placement of the M-mode cursor. Selected intracardiac measurements were made from each M-mode image by the leading edge method (page 77).

The following two-dimensional images were used to guide the placement of the M-mode cursor.

1. The right parasternal short-axis view at the chordal level (Figure 3b).

The M-mode cursor was placed across the left ventricle so that the interventricular septum and left ventricular wall were intersected at right angles. In some horses the image of the left ventricle had to be placed at the far right of the sector display to ensure that the ventricle was bisected (Figure 8). This was achieved by angling the transducer cranially. The following measurements were taken from the M-mode image:

Left ventricular internal diameter in systole (LVIDs) and diastole (LVIDd).

Left ventricular wall thickness in systole (LVWs) and diastole (LVWd).

Interventricular septal thickness in systole (IVSs) and diastole (IVSd).

Right ventricular internal diameter in systole (RVIDs) and diastole (RVIDd).

Left ventricular measurements were used to calculate the fractional shortening using the following equation:

$$FS = \frac{LVIDd - LVIDs}{LVIDd} \times 100$$

The ejection fraction (EF) was calculated using the equation:

$$EF = \frac{LVIDd^3 - LVIDs^3}{LVIDd^3}$$

2. The right parasternal short-axis view at the aortic valve level (Figure 3d).

The cursor was positioned to bisect the aortic valve annulus. The M-mode image was considered acceptable if one of the valve cusps could be seen clearly during systole and diastole (Figure 9). The diameter of the annulus was measured in diastole (Aod).

3. The left parasternal short-axis view at the chordal level (Figure 6a).

The cursor was positioned to cross the ventricular free wall and the junction of the left ventricular wall and interventricular septum, at right angles, thus dividing the left ventricle in half (Figure 10). The posterior papillary muscle and the left ventricular outflow tract were not included in this image. The left ventricular internal diameter was measured from the M-mode image in systole (LLVIDs) and diastole (LLVIDd). The fractional shortening was calculated from these measurements (LFS) as previously described for measurements obtained from the right hemithorax.

4. The left parasternal short-axis view of the right ventricular wall (Figure 6b).

The M-mode cursor crossed the right ventricular wall perpendicularly (Figure 11). The thickness of the right ventricular wall was measured in systole (RVWs) and diastole (RVWd).

The thickness of the intraventricular septum (LIVSd) and left ventricular wall (LLVWd) were also measured from a two dimensional image recorded from the left hemithorax. The measurements were made in diastole from a short-axis view at the

chordal level by the 'leading trailing' method (page 78). Measurements were made from the image frame constructed closest to the onset of the QRS complex.

Each dimension was measured from five consecutive cardiac cycles. The maximum, minimum and median measurements were then used for analysis. In horses with second degree atrioventricular block, the post block beat was not measured. All recordings were made at resting heart rates. Six horses were imaged on three consecutive days to assess the repeatability of these selected cardiac measurements.

Statistical analysis of results

Descriptive statistics were determined for the maximum, minimum and median values of each dimension, from 40 normal horses. These included the range, mean, standard deviation and coefficient of variation. Correlation was used to determine any relationship between measurements and bodyweight, and between measurements and age. Differences between measurements obtained from the right and left hemithorax were compared by a paired and unpaired Student's t-test, this included measurements of LVIDs and LLVIDs; LVIDd and LLVIDd; FS and LFS; and LIVSd and IVSd. Repeatability for each dimension was assessed by a Wilcoxon Rank test. The values obtained from the normal horses and from the five groups of horses with cardiac murmurs were compared by a Mann-Whitney test.

RESULTS

Summary statistics of measurements from forty normal horses are given in Table 7. Four cardiac dimensions had a coefficient of variation (cv) greater than 15%. These were the right ventricular internal diameter in systole (RVIDs Max, Med, Min) and diastole (RVIDd Max, Med, Min) where the cv was greater than 20% and the right ventricular wall thickness in systole (LRVWs Med, Min) and diastole (LRVWd Max) where the cv was greater than 15% but less than 16%. The limitation of imaging depth when scanning from the right hemithorax prevented measurement of the left ventricular wall thickness in systole (LVWs) in twenty four horses, and the

left ventricular wall thickness in diastole (LVWd) in thirty five horses. This also prevented measurement of the left ventricular internal dimension in diastole (LVIDd) in six horses, and the diameter of the aortic annulus (Aod) in one horse. The horse (C12) in which the Aod could not be measured was the largest horse studied (648kg). The six horses (C10, C13, C22, C23, C26, C31) in which the LVIDd could not be measured were all heavier than 520kg but were not the six heaviest horses studied. Poor resolution of near field structures made it impossible to measure the RVIDs in two horses and the RVIDd in one horse. Measurement of the LIVSd, LLVWd, LRVWs and LRVWd was attempted in only twenty nine horses.

The correlation coefficients and p values describing the correlation of median dimensions with bodyweight and age, are given in Table 8. Correlation data for all measurements is given in Appendix 5. No linear correlation was found between the cardiac measurements and bodyweight except for the following dimensions; LVWs Med, Min, IVS Max, Med, Min, RVIDd Min, FS Max and LIVSd Min. There was no correlation between cardiac measurements and age, except for FS Max.

The LVIDd was significantly larger when measured from the left hemithorax, than when measured from the right hemithorax (Table 9). No significant differences were found between the LVIDs, the IVSd, the FS and EF when measured from the right and left parasternal short-axis views (Table 9). The results of the repeatability study for median values are given in Table 10. The results for all values are given in Appendix 6. There was no significant difference in the values obtained from six horses when imaged on consecutive days except for the IVSd Max and Med and the RVIDs Med. The IVSd Max and Med were significantly different between days 1 and 2 but not between days 1 and 3 or days 2 and 3. The RVIDs Med was significantly different between days 1 and 2 and between days 1 and 3.

The median, maximum and minimum values for all dimensions of normal horses and horses with cardiac murmurs are given in Appendix 7. There was no significant difference between the normal horses (group C) and the horses with

cardiac murmurs (groups 1-5) when analysed using a Mann-Whitney test, except for the following dimensions. The LVIDs Max and the LVIDd Min were significantly greater in group 3 (horses with a low grade murmur over the tricuspid valve) than in group C (normal group). The LVIDd Max, Med, Min, Aod Max and LEF Max, Med were greater in group 5 (horses with a diastolic murmur over the aortic valve) than in group C (normal group). Box and whisker plots displaying the range, interquartile range and mean of the LVIDd Med measurements of all groups, are shown in Figure 12. The IVSd Min, RVIDs Max, RVIDs Min and LLVIDs Med were greater in group 1 (horses with a systolic murmur over the mitral valve) than in group C (normal group). Figure 13 shows box and whisker plots of LLVIDs Med measurements of all groups. The RVIDs Min was significantly greater, and the LRVWd Max was significantly less in group 2 (horses with a systolic murmur over the tricuspid valve) than in group C (normal group). The LRVWd Max was significantly less in group 3 (horses with a low grade systolic murmur over the tricuspid valve) than in group C (normal group). Box and whisker plots displaying the mean, range and interquartile range of FS Med measurements from all groups are shown in Figure 14. There were no significant differences between groups for FS measurements.

Table 7. Summary statistics of measurements from a group of normal horses.

N= Number of horses. MIN = Minimum value. MAX = Maximum value. SD = Standard Deviation. CV = Coefficient of variation SD/Mean. * = values where CV > 15%. All measurements are given in centimetres, except FS and LFS which are expressed as a percentage, and EF and LEF which are expressed as a fraction.

Dimension	N	MEAN	MAX	MIN	SD	CV
LVIDs Max	40	7.861	9.23	6.62	0.681	8.66
LVIDs Med	40	7.577	9.15	6.18	0.733	9.67
LVIDs Min	40	7.269	9.06	6.01	0.735	10.12
LVIDd Max	34	12.152	13.85	10.37	0.686	5.64
LVIDd Med	34	11.870	13.50	10.02	0.706	5.95
LVIDd Min	34	11.614	13.15	9.67	0.724	6.23
LVWs Max	16	3.994	4.88	3.74	0.299	7.48
LVWs Med	16	3.709	4.62	3.31	0.330	8.90
LVWs Min	16	3.427	4.09	2.96	0.331	9.66
LVWd Max	5	2.282	2.70	1.92	0.283	12.40
LVWd Med	5	2.162	2.53	1.92	0.226	10.45
LVWd Min	5	1.916	2.09	1.74	0.149	7.79
IVSs Max	40	4.613	5.31	3.92	0.428	9.28
IVSs Med	40	4.413	5.23	3.75	0.410	9.28
IVSs Min	40	4.213	5.14	3.31	0.414	9.83
IVSd Max	40	3.204	3.92	2.44	0.366	11.43
IVSd Med	40	3.042	3.66	2.35	0.366	12.02
IVSd Min	40	2.865	3.57	2.18	0.381	13.31
RVIDs Max	38	3.351	4.97	1.31	0.872	**26.02** *
RVIDs Med	38	2.985	4.88	1.05	0.943	**31.59** *
RVIDs Min	38	2.570	4.88	0.87	0.893	**34.75** *
RVIDd Max	39	4.338	6.18	2.26	0.895	**20.63** *
RVIDd Med	39	3.937	5.92	2.09	0.851	**21.61** *
RVIDd Min	39	3.638	5.40	1.83	0.775	**21.30** *
EF Max	34	0.773	0.85	0.66	0.044	5.69
EF Med	34	0.744	0.82	0.64	0.049	6.59
EF Min	34	0.714	0.81	0.63	0.049	6.86

Dimension	N	MEAN	MAX	MIN	SD	CV
FS Max	34	38.9	46	29	4.09	10.51
FS Med	34	36.4	44	28	4.04	11.10
FS Min	34	33.9	42	28	3.80	11.21
Aod Max	39	8.532	9.50	7.23	0.593	6.96
Aod Med	39	8.305	9.41	6.71	0.625	7.53
Aod Min	39	8.117	9.32	6.62	0.593	7.30
LIVSd Max	29	3.134	3.49	2.70	0.214	6.82
LIVSd Med	29	2.968	3.36	2.49	0.207	6.97
LIVSd Min	29	2.754	3.11	2.00	0.292	10.61
LLVWd Max	29	2.557	3.25	1.96	0.270	10.57
LLVWd Med	29	2.355	2.91	1.68	0.248	10.55
LLVWd Min	29	2.151	2.76	1.61	0.207	7.52
LRVWd Max	29	1.485	1.92	1.13	0.223	**15.03** *
LRVWd Med	29	1.337	1.83	1.13	0.184	13.74
LRVWd Min	29	1.209	1.48	0.96	0.144	11.88
LRVWs Max	29	2.572	3.53	1.83	0.376	14.62
LRVWs Med	29	2.361	3.31	1.74	0.355	**15.05** *
LRVWs Min	29	2.148	2.87	1.48	0.339	**15.78** *
LLVIDs Max	40	8.031	9.76	6.18	0.761	9.48
LLVIDs Med	40	7.753	9.49	6.10	0.730	9.42
LLVIDs Min	40	7.458	9.49	6.01	0.724	9.71
LLVIDd Max	40	12.736	14.46	10.80	0.800	6.28
LLVIDd Med	40	12.455	14.20	10.54	0.782	6.28
LLVIDd Min	40	12.202	13.76	10.10	0.757	6.20
LEF Max	40	0.782	0.85	0.65	0.039	5.00
LEF Med	40	0.757	0.84	0.62	0.044	5.81
LEF Min	40	0.731	0.82	0.62	0.043	5.95
LFS Max	40	39.6	46	29	3.48	8.80
LFS Med	40	37.4	45	27	3.72	9.94
LFS Min	40	35.1	43	27	3.48	9.91

Table 8. Probability values for correlation of median two-dimensional and M-mode measurements against bodyweight (kg) and age (years). Values are considered significant (*) when $p < 0.05$.

Dimension	Correlation vs bodyweight		Correlation vs age	
	r	p	r	p
LVIDs Med	-0.099	0.544	-0.274	0.087
LVIDd Med	0.003	0.984	-0.199	0.258
LVWs Med	**0.513**	**0.042** *	0.007	0.979
IVSs Med	**0.394**	**0.012** *	0.011	0.944
IVSd Med	0.183	0.258	0.162	0.317
RVIDs Med	0.176	0.290	0.090	0.592
RVIDd Med	0.255	0.177	0.049	0.767
EF Med	0.272	0.120	0.233	0.185
FS Med	0.293	0.093	0.206	0.242
Aod Med	0.067	0.687	-0.008	0.629
LIVSd Med	0.166	0.390	0.082	0.673
LLVWd Med	-0.065	0.736	0.069	0.721
LRVWd Med	-0.269	0.159	-0.215	0.263
LRVWs Med	-0.116	0.551	-0.023	0.905
LLVIDs Med	0.087	0.593	-0.232	0.149
LLVIDd Med	0.238	0.139	-0.256	0.110
LEF Med	0.116	0.477	0.106	0.514
LFS Med	0.119	0.463	0.125	0.440

Table 9. Comparison of measurements taken from the right and left hemithoraces using a Student's t-test and paired t-test. Differences were considered significant (*) when $p<0.05$.

Measurement from Right hemithorax	Measurement from Left hemithorax	Paired t-test	Unpaired t-test
LVIDs Max	**LLVIDs max**	0.11	0.30
LVIDs Med	**LLVIDs med**	0.11	0.29
LVIDs Min	**LLVIDs min**	0.094	0.25
LVIDd Max	**LLVIDd max**	**0.0000 ***	**0.0012 ***
LVIDd Med	**LLVIDd med**	**0.0000 ***	**0.0012 ***
LVIDd Min	**LLVIDd min**	**0.0000 ***	**0.0011 ***
IVSd Max	**LIVSd max**	0.74	0.33
IVSd Med	**LIVSd med**	0.59	0.28
IVSd Min	**LIVSd min**	0.34	0.17
FS Max	**LFS max**	0.26	0.44
FS Med	**LFS med**	0.110	0.24
FS Min	**LFS min**	0.079	0.14
EF Max	**LEF max**	0.18	0.33
EF Med	**LEF med**	0.12	0.23
EF Min	**LEF min**	0.081	0.13

Table 10. Results of Wilcoxon tests performed on median measurements, obtained from six horses on three consecutive days. * indicates a significant difference ($p<0.05$) between the measurements obtained on the different days.

Dimension	Day 1 vs Day 2		Day 2 vs Day 3	Day 1 vs Day 3	
LVIDs Med	0.584		0.059	0.059	
LVIDd Med	0.584		0.855	1.000	
LVWs Med			Insufficient Data		
LVWd Med			Insufficient Data		
IVSs Med	0.142		0.593	0.138	
IVSd Med	**0.036**	*	0.590	0.106	
RVIDs Med	**0.036**	*	1.000	**0.036**	*
RVIDd Med	0.675		0.402	0.787	
EF Med	1.000		1.000	1.000	
FS Med	0.855		1.000	0.787	
Aod Med	0.675		1.000	0.345	
LIVSd Med	1.000		0.584	0.345	
LLVWd Med	0.345		1.000	0.584	
LRVWd Med	0.584		1.000	0.142	
LRVWs Med	0.345		1.000	0.201	
LLVIDs Med	0.059		0.402	0.675	
LLVIDd Med	0.463		0.059	0.402	
LEF Med	0.075		0.855	0.418	
LFS Med	0.075		0.855	0.500	

DISCUSSION

Measurement technique

The recommendations of the American Society of Echocardiography were followed for all M-mode measurements (Sahn *et al.* 1978). The 'leading edge method' has been shown to be more accurate than the 'leading trailing' or the 'trailing leading' method for measurement of myocardial thickness (Wyatt *et al.* 1983). The 'leading edge trailing edge' method was used for tissue interfaces which were parallel to the axial beam, such as the IVS when measured from a left parasternal short-axis view of the right ventricular wall. Although this may theoretically lead to over-estimation of the true width of the structure (Wyatt *et al.* 1983), it was difficult to determine the width of the endocardium due to the poor lateral resolution of ultrasound equipment.

Measurements were taken from M-mode studies where possible rather than the two-dimensional image, as the timing of M-mode measurements is not limited by frame update rates. This allows more accurate timing of measurements within the cardiac cycle. The guided M-mode technique enables the M-mode cursor to be placed accurately. Incorrect angulation of the M-mode cursor on the two-dimensional image can be appreciated and corrected. It was possible to achieve a true minor axis of the left ventricle in all horses using this technique. The M-mode studies were taken from the short-axis view, as the cursor could be placed accurately across the maximum diameter of the chamber. In this view it was also easy to ensure that the M-mode cursor was not placed obliquely across the septum and ventricular walls. It was helpful firstly to orientate the cursor in the long-axis view, ensuring the cursor was at the chordal level and crossed the long-axis perpendicularly, before turning to the short-axis view. Previous workers following the M-mode techniques described in human medicine (Feigenbaum 1986) used long-axis views of the heart to record M-mode images (Lescure and Tamzali 1984; Stewart, Rose and Barko 1984; Lombard *et al.* 1984; Pipers and Hamlin 1977).

Measurement of the right ventricle

The thickness of the right ventricular wall was difficult to measure from the right parasternal short-axis view, due to poor resolution of near field structures. This poor resolution was due to transducer artifacts and poor focussing of the transducer in the near field (Feigenbaum 1986). The right ventricular wall could be clearly imaged from the left parasternal short-axis view (Figure 5b) and measured from the derived M-mode study (Figure 11). The coefficient of variation for the systolic and diastolic measurements was just greater than 15% in three of the six right ventricular wall measurements. This large coefficient of variation may be due to difficulty in aligning the M-mode cursor perpendicularly to the endocardial surface, or may be due to variability in the thickness of the right ventricular wall (Dixon, Nicholls, Mc.Pherson, Lawson, Thomson, Pirie and Breeze 1982). A higher coefficient of variation was found in the measurements of the RVID, both in systole and diastole. This is probably due to the non circular geometry of the RV. The other cardiac dimensions showed limited variation, and are likely to be of greater value in the assessment of cardiovascular dysfunction.

Measurement of the atria

No attempt was made to measure the atria. The entire atrium could not be imaged in any view. In the right and left parasternal long-axis views echo dropout in the near field and lung interference laterally prevented the measurement of the major or minor diameter of the atria. Measuring the atria at the level of the atrioventricular valves would give a measure of the valve annulus rather than a true atrial diameter. Measurement of the atria from a two-dimensional image was attempted by Voros, Holmes and Gibbs (1991). These authors reported difficulty in measuring a true left atrial diameter due to problems in identifying the dorsal border of the left atrium. This problem was more notable in horses weighing more than 500kg. To overcome this, the authors obtained images of the ventricles and atria separately, to bring the measurable structures into view. This would imply that the left atrial diameter was

not measured from a standardised plane. The authors also reported problems in measuring the atrial diameter at end systole, due to difficulty in identifying the dorsal border of the left atrium at its maximum diameter. Despite these difficulties, atrial dimensions were reported for all eighteen horses in which satisfactory two-dimensional images were obtained.

The ideal method of measuring both atria would be from an apical 4-chambered plane. This plane lies mutually perpendicular to the long-axis and short-axis planes (Henry *et al.* 1980). Schnittger *et al.* (1983) have shown in man that the minor axis of the left atrium measured from the left parasternal long-axis view is equal to that obtained from the left parasternal short-axis view at the level of the aorta and only 0.9% larger than that obtained from the apical 4-chambered plane. The left parasternal long-axis view in man corresponds to the right parasternal long-axis view of the aorta in the horse (Figure 2c). Unfortunately the depth limitations of the ultrasound equipment made it impossible to image the far wall of the left atrium in horses, and the measurement could not be made. This was also true for the left parasternal short-axis view. More recent equipment has a maximum imaging depth of 30cm (Vingmed CFM 750, CFM 800). When imaging at this depth, accurate measurement of the left atrium may be possible. With older unguided M-mode equipment, imaging depth was greater than in most modern ultrasound machines and therefore measurement of the left atria was possible in earlier studies from an M-mode recorded from a right parasternal long-axis view (Pipers 1978; Lescure and Tamzali 1984). The apical 4-chambered plane is used to measure right atrial dimensions in man. This could not be achieved in adult horses due to the width of the sternum, although this plane can be obtained in small foals. In adult horses the apical long-axis views (Figures 2e and 5b) allow visualisation of the RA, RV, LA and LV. However, these views are inferior to the 4-chambered plane for measurement of the atria as the atria are not truly bisected. It would be beneficial to record a range of atrial dimensions in normal horses for comparison with horses with regurgitation of

the atrioventricular valves. Atrioventricular valve regurgitation is a common finding in horses and atrial enlargement is a recognised sequela (Miller and Holmes 1985; Holmes 1987).

Comparison of results with previous publications

Table 11 compares the results of this study with those of previous workers. The results given for the study by Lescure and Tamzali (1984) are related to the group of twenty English Thoroughbreds. The results of this study are in agreement with those of Lescure and Tamzali (1984) except for the measurement of the Aod, LVWs, IVSs, FS and LVIDs. Lescure and Tamzali (1984) used the 'trailing edge leading edge' method for measuring the Aod. This will result in smaller values than would be obtained with the 'leading edge leading edge' method. The greater FS and smaller LVIDs in the study by Lescure and Tamzali (1984) may reflect an increased ventricular contractility in these horses. This difference in contractility may be due to differences in heart rate of horses in the two studies. Increases in heart rate are associated with decreased parasympathetic and increased sympathetic nerve activity which causes an increase in contractility. An increase in heart rate alone will also lead to an increase in contractility due to an increase in intracellular calcium ions (Levick 1991). The measurements of Lescure and Tamzali were made at heart rates of thirty to seventy beats per minute whereas the present study was performed at resting heart rates which varied from thirty two to forty eight beats per minute. This increase in contractility in the study of Lescure and Tamzali may also explain the larger systolic dimensions of the IVS and LVW.

The measurements recorded in the present study are greater than those obtained by the unguided M-Mode studies of O'Callaghan (1985) and Pipers and Hamlin (1977) (Table 11). This may be attributed to the larger bodyweights of horses in this study or more accurate placement of the M-mode cursor using two-dimensional guidance.

Table 11. Comparison of results from present study with those of previous workers. SD = Standard Deviation.
Lescure (M-mode) 1984 = Lescure and Tamzali (1984). Pipers (M-mode) 1977 = Pipers and Hamlin (1977). Voros (2D) 1991 = Voros, Holmes and Gibbs 1991.

	Present (M-mode) mean (SD)	**Lescure (M-mode) (1984)** mean (SD)	**O'Callaghan (1985)** mean	**Pipers (M-mode) (1977)** mean (SD)	**Voros (2-D) 1991** mean (SD)
RVIDs	2.98 (0.94)	-	-	-	4.7 (0.61)
RVIDd	3.94 (0.85)	3.88 (0.75)	-	-	5.9 (0.61)
LVIDs	7.58 (0.73)	6.31 (1.13)	6.8	5.7 (0.23)	-
LVIDd	11.87 (0.71)	11.28 (1.18)	9.8	9.3 (0.30)	-
LVWs	3.71 (0.33)	4.71 (0.47)	-	-	-
IVSs	4.41 (0.41)	5.47 (0.41)	3.6	-	4.7 (0.48)
IVSd	3.04 (0.37)	3.27 (0.43)	2.4	-	3.8 (0.27)
FS	36.4 (4.04)	44.19 (6.00)	-	38.6 (1.6)	-
Aod	8.30 (0.62)	7.43 (0.62)	-	7.7	7.8 (0.4)
LLVIDs	7.75 (0.73)	-	-	-	7.3 (0.76)
LLVIDd	12.45 (0.78)	-	-	-	11.3 (1.37)
LFS	37.4 (3.72)	-	-	-	35.3 (3.9)
BWT	513kg			300kg	482kg

The average weight of the horses in the study by Pipers and Hamlin (1977) was 300kg and the horses imaged by O'Callaghan (1985) ranged from Welsh ponies to adult Thoroughbreds.

Lescure and Tamzali (1984) compared cardiac dimensions in different breeds of horses. They found that the English Thoroughbred had a significantly larger LVID when compared to a group of other breed types. The English Thoroughbred also had a significantly larger IVSd and LVWd than the French Saddlebred, whereas the French Saddlebred had a significantly larger aorta. Breed differences may explain some of the discrepancies between the results of various workers.

The measurements obtained by Voros, Holmes and Gibbs (1991) from two-dimensional images were similar to those obtained from the M-mode images in this study, although in the previous study, measurements were not obtained from the four heaviest horses (580kg - 650kg). The RVIDd was larger and had a much smaller coefficient of variation than the present study or the study by Lescure and Tamzali (1984) (cv = 19.4). The RVIDs was also larger in the two-dimensional study. The standardised short-axis view (Voros, Holmes and Gibbs 1991) was obtained from the chordal level and the RVID was measured from the two-dimensional image along a line perpendicular to a reference line across the left ventricular chamber. It is possible that the RVID was measured at a wider point by this technique than by the guided M-mode method. In the guided M-mode method positioning of the left ventricle to the right of the sector to obtain a true minor axis of the left ventricle resulted in the right ventricle being bisected closer to the junction of the right ventricular wall and septum, rather than the centre of the ventricle. This results in a smaller dimension than would be achieved if the right ventricle was measured in the middle. The measurement of the left ventricular internal diameter from the left hemithorax was smaller in the study by Voros, Holmes and Gibbs (1991) than in the present study. In both studies the measurement was made at the chordal level, although in the study by Voros, Holmes and Gibbs (1991) the measurement was made

from a long-axis view whereas in the present study the measurement was made from a short-axis view. In the short-axis view the measurement was seen to be made across the widest part of the ventricle whereas in the long-axis view, the IVS was not included in the image, and therefore it is possible that the image plane did not truly bisect the ventricle. In human echocardiography LVID measurements from the left hemithorax are made from a long-axis view, but the view used is similar to the right parasternal long-axis aorta (Figure 2c). The IVS is included in the image and the section across the ventricle is standardised by the presence of the LVOT and aorta. Schnittger *et al.* (1983) showed that the left ventricular diameter measured from the parasternal long-axis view was 1.9% smaller than obtained from the parasternal short-axis view.

Voros, Holmes and Gibbs (1991) were unable to measure the heart in four horses whose bodyweight was greater than 580kg, due to the absence of cardiac structures in the image plane. The authors were limited to a maximum penetration of 20cm with the 3.5MHz transducer. Lack of imaging depth prevented measurement of the Aod in ten horses. In the present study the transducer frequency was 2.25MHz with a maximum imaging depth of 24 cm. This allowed the aortic diameter to be measured in all but one horse.

In the present study there was no correlation between bodyweight and most cardiac dimensions; however this was only evaluated in Thoroughbred and Thoroughbred cross horses whose bodyweights ranged from 428 to 648kg. The LVWs Med, Min, IVS Max, Min, Med, RVID Min, FS Max and LIVSd Min did show a correlation with bodyweight. As only the IVS showed a correlation for all three measurements (Max, Med, Min) it is possible that the correlation obtained from the other values was significant due to chance. A correlation between IVS thickness and bodyweight was recorded by Voros, Holmes and Gibbs (1991), in addition these workers demonstrated a correlation between bodyweight and the aortic diameter at end systole. The aortic diameter at end systole was not recorded in the present study.

The lack of correlation of the other values with bodyweight is in agreement with the findings of Lescure and Tamzali (1984) and Voros, Holmes and Gibbs (1991). However Lombard *et al.* (1984) and Stewart, Rose and Barko (1984) have shown a correlation between echocardiographic variables and bodyweight in the growing pony foal. This appears to be contrary to the findings in the adult horse. If horses with a wider range of bodyweights had been studied, a correlation may have been seen. O'Grady, Bonagura, Powers and Herring (1986) reported a positive correlation between two-dimensional echocardiographic measurements and bodyweight in dogs. However this study comprised dogs with a large range of bodyweights (4.5kg to 30kg).

Repeatability study

The repeatability study in this work was a single observer repeatability and did not test for differences that might be obtained between different examiners. The results show a close agreement between measurements from different days. Only four of fifty one measurements showed significant differences between days. The IVS Max and Med were significantly different between days one and two but not between the other days. The RVIDs was significantly different between days one and two and days one and three. As RVID measurements had a larger coefficient of variation, it is more likely that variation may occur in these measurements from day to day. Measurements from the right ventricle may vary with respiration, therefore five consecutive cardiac cycles were measured to encompass a full respiratory cycle. However it is possible that variation occurred in the timing of measurements, within the respiratory cycle, between different days.

Comparison of measurements from the left and right hemithoraces

There was no significant difference between cardiac dimensions when measured from the left and right hemithorax, except for the LVIDd. This dimension was significantly larger when measured from an M-mode taken from the left

hemithorax than from an M-mode recorded from the right hemithorax. When recording an M-mode of the left ventricle from the right hemithorax, the cursor bisects the IVS and crosses the left ventricular free wall half way between, but dorsal to, the two papillary muscles. When recording an M-mode of the left ventricle from the left hemithorax, the cursor crosses the left ventricular free wall immediately above the posterior papillary muscle and intersects the IVS at its junction with the LV free wall. Thus, measurements of the left ventricular internal diameter are not made between the same points when measured from the left and right hemithoraces. Differences between measurements may reflect non-circular geometry of the ventricle. It is also possible that measurements from the left hemithorax, although still made at the chordal level, are recorded closer to the mitral valve, and are therefore larger than measurements made from the right hemithorax.

Comparison of measurements from control horses and horses with cardiac murmurs

Mitral regurgitation

Severe mitral regurgitation leads to compensatory dilation of the left ventricle due to the increased volume of blood flowing into it during diastole (Miller and Holmes 1985). Dilation of the left ventricle, assessed by echocardiography, has been reported in foals with severe mitral regurgitation caused by rupture of the chordae tendineae (Reef 1987). In the present study no significant differences were found in the diameter of the left ventricle during systole (LVIDs) or diastole (LVIDd), between group 1 horses (horses suspected of having mitral regurgitation) and the control group (group C) when measured from the right hemithorax. A significant difference however was noted in the median left ventricular dimension during systole (LLVIDs Med) between the horses in group 1 and group C when measured from the left hemithorax (Figure 13).

Horses with mitral regurgitation may be expected to show increased contraction of the left ventricle due to the increased preload of the ventricle, and the

decreased afterload. The afterload will be decreased, as part of the stroke volume of the ventricle will be ejected into the left atrium which will offer a low resistance to flow. Decreased afterload and increased preload of the ventricle will result in an increase in the fractional shortening (FS) of the ventricle (Bonagura 1985; Feigenbaum 1986). However in this study no significant difference was found between group 1 horses and the control group for EF or FS measured from the right or the left hemithoraces. Significant differences were found between the control group and group 1 horses for the RVIDs Max and Min and also the IVSd Min. The RVIDs and IVSd being significantly larger in the horses with murmurs of suspected mitral origin.

The inability to detect enlargement of the left ventricle or an increased FS and EF in group 1 horses with suspected mitral valve dysfunction may reflect the lack of severity of the mitral regurgitation in these cases. Group 1 comprised two horses with a grade 2 murmur and three horses with a grade 3 murmur. Therefore five of the eight horses had low grade murmurs which were audible over a small area of the thorax, and may have represented non-significant valvular disease (Holmes 1977; Brown 1985). The murmurs auscultated may not have been associated with mitral valve disease, the murmur being misdiagnosed due to the difficulty in determining the origin of systolic murmurs in horses (Glendinning 1972; Miller and Holmes 1985). Two-dimensional and M-mode echocardiography may be an insensitive technique for detecting minor changes in cardiac dimensions due to the variation between normal horses and also due to the lack of sensitivity of two-dimensional and M-mode measurements in assessing volume changes of the ventricle (Elkayam, Gardin, Berkley, Hughes and Henry 1983b).

Tricuspid regurgitation

There was no difference in the right ventricular diameter or the right ventricular wall thickness, in horses in groups 2, 3 and 4, when compared to the normal group (group C), with the following exceptions. Group 2 horses had a

significantly larger RVIDs Min, and a significantly smaller LRVWd Max compared to the control group. The LRVWd Max of the group 4 horses (horses considered to have more severe tricuspid regurgitation) was also significantly smaller than the control group. Group 3 horses (horses considered to have mild tricuspid insufficiency) showed no differences in the measurements of RVID or in the thickness of the LRVW when compared to the control group.

Tricuspid regurgitation may cause compensatory dilation and hypertrophy of the right ventricle, followed by thinning and failure of the ventricular wall. However excision of the tricuspid valve is well tolerated in human patients and experimental animals, providing the right ventricular systolic pressure remains normal. Dilation of the ventricle in these cases may not occur for several months or years. It is possible that tricuspid regurgitation is also well tolerated in horses, with dilation of the right ventricle only occurring in the more severe long-standing cases. If an increase in the intensity of a murmur is indicative of increased severity of valvular regurgitation, it would be expected that Group 4 horses would show more significant changes than the other 2 groups (groups 2 and 3). Although groups 2 and 4 did show significant differences in RVIDs and LRVWd when compared to the control group, these differences were not present for all measurements (Max, Med, Min) and the measurements fell within the range of the normal group. The possibility that these values were significant only by chance, cannot be ignored. Group 3 horses were found to have a significantly larger LVIDs Max compared to the control group, although the range of measurements fell within the range of the control group.

Aortic insufficiency

Horses with aortic insufficiency would be expected to have compensatory dilation of the left ventricle caused by increased flow of blood into the ventricle during diastole (Braunwald 1988). Reef and Spencer (1987) have demonstrated dilation of the left ventricle by echocardiography in horses with aortic insufficiency. In the present study, the Aod Max and the LVIDd Max, Med, Min, measured from the

right hemithorax were significantly larger for group 5 horses when compared to the control group (Figure 12). However the LLVIDd measurements were not significantly different from the control group when measured from the left hemithorax, although the maximum dimensions of these measurements were greater than the normal range established from the control group (Appendix 7).

The FS Max, Med, Min, measured from the right hemithorax, although not significantly different from the control group, had an upper limit which exceeded that established as normal from the control group. However the horse with the grade 5 murmur in this group, and therefore possibly the most severe valvular regurgitation, was excluded from the calculation of FS. In this horse the left ventricular free wall did not fit onto the screen when imaged from the right hemithorax and therefore the LVID could not be measured. The EF was significantly larger for group 5 horses, than for the control group. The differences between group 5 and the control group for the LFS max, med, and min measurements approached statistical significance, 0.052, 0.056 and 0.052 respectively. The upper limit of these measurements exceeded the range established as normal from the control group (Figure 14).

The lack of statistical significance of the left sided measurements may be due to the low number of horses (7) for comparison to the normal group. It may also be due to the number of horses in group 5 with low grade murmurs. Five horses having a murmur of grade 3 intensity or less. The increased number of significant differences between group 5 and the controls compared to group 1 and the controls may indicate more accurate diagnosis of the cause of the diastolic murmurs compared to the systolic murmurs. Alternatively lower grade murmurs of aortic insufficiency may result in more compensatory dilation of the left ventricle than do similar graded murmurs of mitral insufficiency. For the same volume of regurgitant flow, jets of mitral regurgition may have more kinetic energy and therefore may produce louder murmurs than jets of aortic insufficiency.

Measurements outwith the control range

Although statistical differences were not found between many of the groups and the control horses, the following observations were noted. The upper limit for the LVID measurements of group 5 exceeded those of the control group. All other groups were within the upper limit established from the control group. Analysis of the individual horses in the group showed that for the LVID systolic measurements (Max, Med, Min), the horse with the grade 5 murmur was the horse whose LVID measurement fell outwith the normal range. For the diastolic measurements, the left ventricle of the horse with the grade 5 murmur could not be measured because the image of the far wall of the ventricle did not fit on the screen and it was the LVID measurement in the horse with the grade 4 murmur, that was outwith the upper limit of the normal range.

The upper limit for IVS measurements in horses from group 5 and group 1 exceeded these of the control group, except for the IVSd Min of group 5. In all other groups the upper limit was within the range established by the control group. This may indicate increased contractility of the septum associated with increased preload in horses with aortic and mitral insufficiency. Had horses with more severe valvular dysfunction been studied significant differences may have been seen. Analysis of the individual horses revealed that this may be true for the group 5 horses, as it was the IVS measurement of the horse with the grade 5 murmur that fell outwith the normal range. However in group 1, it was found that the IVS measurement of the horse with the lowest grade murmur was outwith both the upper and lower limit of the normal range.

The upper limit of the RVIDs Max, Med and RVIDd Max in horses with tricuspid insufficiency (Groups 2, 3, 4) exceeded those of the control group. The upper limit of RVID measurements for groups 1 and 5 fell within the range established from the control group. However analysis of the individual horses showed that it was horses with the lowest grade murmurs, grades 2 and 3, which

exceeded the normal range except in group 4. In group 4 the upper limit of the RVIDs Max, established by the control group was exceeded by a horse with a grade 5 murmur.

The upper limit for the measurement of RVIDd Med and Min of group 2 and group 3 (horses with murmurs suggestive of mild tricuspid regurgitation) exceeded those of the control group. The upper limit of these measurements in groups 1, 5 and 4 (horses with murmurs indicative of more severe tricuspid insufficiency), fell within the range established by the control group. Analysis of individual horses from groups 2 and 3 showed that it was the same horse with a grade 2 murmur in both groups which exceeded the upper limit of the normal range.

These results may represent dilation of the right ventricle in the horses with tricuspid regurgitation. Dilation of the ventricle may occur in horses with more long-standing tricuspid regurgitation, rather than in those horses suspected of having more severe tricuspid regurgitation. The time of onset of the tricuspid regurgitation in these horses was not known. The upper limit of all other measurements for all groups fell within the range established as normal by the control group except for the Aod Max (group 2 and group 3), Aod Med (group 3) and LRVWd Min (group 3).

The minimum value of the IVSs Max, Med and Min of group 1 horses was below the range of the control group. Other minimum values sporadically fell outwith the normal range. These were IVSs Max, Med, (group 2) IVSd Med group 5, IVSd Min (groups 2 and 3), RVIDd Med, Min (Group 5), LRVWd Med groups 2 and 3, LRVWd Min group 1 and LRVWs Med, Min group 5. All other measurements were within the range established as normal by the control group.

Summary

This study shows that M-mode measurements may be useful in determining the severity of aortic regurgitation in horses. However, M-mode measurements appear not to be sufficiently sensitive to identify horses with mild aortic insufficiency, or to differentiate the cause of systolic murmurs. Although significant differences

were evident between groups 1 to 5 and the control groups, measurements showed considerable overlap with the normal group and therefore are unlikely to be of clinical value. Also, it would be expected that 5% of the measurements would be significant by chance. These results show that 5.8% of the measurements were significant, and therefore the element of chance cannot be ignored.

Most of the horses in groups 1-5 had no evidence of cardiovascular disease other than the presence of a cardiac murmur, and in many cases the murmur was of very low intensity. It is possible that many of these horses did not have functional overload of the cardiac chambers and therefore showed no evidence of chamber dilation. Had horses with more severe cardiovascular dysfunction been studied, more obvious differences may have been observed between groups. Horses with more severe valvular disease were examined, however these horses had regurgitation of more than one valve and therefore were excluded from this work. M-mode and two-dimensional measurements may be of limited value in the detection of mild chamber dilation, as the diameter of a chamber may only change as a cube root of the change in volume, as suggested in humans (Elkayam *et al.* 1983b). This technique therefore may be of limited value due to its lack of sensitivity.

CHAPTER 5. COLOUR FLOW DOPPLER STUDIES FROM STANDARDISED ULTRASOUND IMAGES IN NORMAL HORSES AND HORSES WITH SPECIFIC CARDIAC MURMURS.

INTRODUCTION

In Chapter 4, various cardiac dimensions were measured from the standardised ultrasound images established in Chapter 3. Although significant differences in selected measurements were found between groups, these measurements could not be used to identify horses from any one group. In human medicine, Doppler colour flow mapping has been shown to be a more sensitive technique for identifying the cause of cardiac murmurs (Monaghan and Mills 1989). This chapter describes Doppler colour flow studies from selected images established in Chapter 3, in the group of normal horses and the horses with cardiac murmurs.

METHODS

Colour flow studies were recorded, as described in Chapter 2, from the following standardised views;

1) Right parasternal long-axis views (Figures 2).

   a) Reference view. Ventricular inlets (Figure 2a).

   b) Tipped view. LV inlet / outlet (Figure 2b).

   c) Long-axis aorta (Figure 2c).

   d) Dorsal location RV inlet (Figure 2d).

   e) Apical view. Ventricular inlets (Figure 2e).

2) Right parasternal short-axis view.

   Pulmonary artery level (Figure 3e).

3) Right parasternal angled view.

   Dorsal location right ventricular outflow tract (Figure 4).

4) Left parasternal long axis views (Figures 5).

   a) Reference view. LV inlet (Figure 5a).

   b) Apical view. LV inlet (Figure 5b).

c) 5-chambered view (Figure 5c).

The groups of horses studied were as in Chapter 3. The colour flow patterns within the chambers were analysed in a qualitative manner. The predominant flow patterns were described at selected intracardiac landmarks during specific phases in the cardiac cycle. Systole was divided into two phases. Systole 1 corresponded to the first half of the ejection period and systole 2 the second half. Diastole was divided into three phases. Early diastole, extending from the end of systole to the end of the rapid filling phase of the ventricle. Mid-diastole (diastasis) representing the period immediately following the rapid filling phase of the ventricle up to the inscription of the P wave of the ECG. Presystole is the period immediately prior to the atrial contraction and ending during the first third of the QRS complex of the ECG.

The timing of events within the cardiac cycle was determined from a frame change indicator which displayed the exact duration of each colour frame relative to the ECG (Figure 1). When more accurate timing of events was required the ECG was compared to a colour M-mode or spectral Doppler recording taken from the area of interest. At least ten cardiac cycles were analysed frame by frame from videotape recordings of each study.

Discrete high velocity signals, or jets were recorded at the level of the heart valves. The width at the level of the valve, the length and area of the three largest signals were measured, and the average value was used for analysis. When horses showed a closure pattern and a regurgitant jet, the area of the jet was measured. The duration of the regurgitant flow was estimated from the number of colour frames in which it persisted. The duration could not be measured directly from the two-dimensional colour flow image using the software on the Vingmed CFM700. However the distance moved by the ECG and the simultaneously recorded frame rate indicator whilst the jet was displayed, could be measured. This enabled comparison of the duration of the signal and duration of systole. The duration of systole was classified as the period from the onset of the QRS complex to the end of the T wave

of the ECG. The onset time of the signal was determined by measuring the distance moved by the frame rate indicator, between the onset of the QRS complex and the onset of the signal. Time was then calculated from the frame rate and the distance moved by the frame indicator in one second.

The same measurements were made from discrete signals or jets recorded from the groups of horses with cardiac murmurs. Signals were only measured at the valve suspected of being the site of origin of the murmur. Therefore signals occurring at the tricuspid valve were measured in groups 2, 3 and 4, signals from the aortic valve in group 5, and signals from the mitral valve in group 1.

Statistical analysis of results

Descriptive statistics were determined for the length, width, area, duration relative to the duration of electrical systole, and time of onset of any discrete signal or jet. The measurements obtained from the horses with cardiac murmurs were compared to measurements from the same valve from the normal group, using a Mann-Whitney test. Differences in age between groups were also determined using a Mann-Whitney test.

RESULTS

Normal horses

The flow patterns observed in the 40 normal Thoroughbred and Thoroughbred cross horses are detailed in Tables 12 and 13. Most horses showed no flow signals in the right atrium during the first half of systole, from the right parasternal long-axis views. In the second part of systole a red flow signal appeared in the right atrium (Figure 15). During early diastole, an intense red signal was recorded in the right atrium, right ventricle and across the open tricuspid valve (Figure 16). This signal ended abruptly. During mid diastole no flow, or very small weak flow signals were recorded. Following the P wave of the ECG an intense red signal was again recorded in the right atrium, right ventricle and across the tricuspid valve (Figure 17). In some horses this red inflow signal could not be detected. The timing of these flow signals

is demonstrated in the colour M-mode (Figure 18) recorded from a line through the right ventricle, tricuspid valve and right atrium.

In the right parasternal short-axis and angled views, blue flow was detected during systole in the right ventricular outflow tract and pulmonary artery. This flow signal, blue at the onset of systole, changed to blue with aliasing in the second half of systole, and finally returned to a blue colour in late systole. Signal aliasing occurred at the level of the supraventricular crest (Figure 19). During diastole, no flow signal was observed in the right ventricular outflow tract. However, just above the outflow tract a blue signal was seen on the left of the sector. This was immediately followed by a much larger red signal to the right of the blue pattern. The blue pattern subsequently disappeared (Figure 20). In some horses the blue and red pattern appeared simultaneously. In late diastole, following the P wave, a red signal was evident on the ventricular side of the tricuspid valve dorsal to the supraventricular crest.

Recordings from the left parasternal reference and apical views, showed predominantly no flow throughout systole. In early diastole, a red signal was seen in the left atrium, the left ventricle and across the mitral valve annulus. In some horses, this red flow signal showed aliasing in its central core (Figure 21). During mid diastole no flow was recorded in most horses, although a few showed small areas of low velocity colour. After the P wave, a red signal was seen in the left ventricle and crossing the mitral valve annulus.

A large blue regurgitant flow signal was detected at the atrioventricular valves and atria of horses with second degree atrioventricular block. The regurgitant flow pattern occurred after the non-conducted P wave (Figure 22) and in some cases persisted until the following atrial contraction.

During systole, a blue flow signal was seen in the left ventricular outflow tract and aorta in the 5-chambered view. This blue flow signal was aliased in most horses (Figure 23). During diastole a red pattern was seen at the ventricular side of the

outflow tract. This signal changed to a blue colour in the second part of early diastole. In some horses an initial blue colour was seen, which changed to a blue/red colour.

The following additional flow patterns were noted at the cardiac valves. Specific details of these flow patterns (width, length, area, time of onset and duration) are given in Table 14.

Tricuspid valve

Thirty one of the forty normal horses (77.5%) showed a high velocity signal at the tricuspid valve either immediately following the onset of the QRS complex (twenty nine horses) or immediately prior to the onset of the QRS complex (two horses). The flow pattern was present for one frame only in twenty seven horses (67.5%), and for more than one frame in four horses (10%). The signal was recorded on the right atrial side of the tricuspid valve either as a blue regurgitant jet, or an aliased red/blue closure signal (Figure 24). The closure signal was present in twenty two horses (55%) whereas 9 horses (22.5%) showed a closure noise and a discrete blue jet. There was no significant age difference between the horses showing a high velocity signal at the tricuspid valve and those showing no signal ($p = 0.38$).

Seventeen horses (42.5%) showed a blue regurgitant flow pattern at the tricuspid valve immediately following the T wave (Figure 25).

Mitral valve

Twenty seven of the forty normal horses (67.5%) showed a high velocity signal around the mitral valve. This signal occurred with or immediately after the onset of the QRS complex (twenty one horses) or immediately prior to the onset of the QRS complex (two horses). The flow signal was recorded on the left atrial side of the valve either as a distinct blue jet or as a closure pattern around the valve. The closure pattern was recorded in 9 horses (22.5%) whereas a distinct jet was present in 18 horses (45%). Five horses had a closure pattern and a distinct jet. In these horses

the closure pattern (Figure 26) was larger than the subsequent jet (Figure 27), and lasted for only one colour frame. The onset time of the signal was not measured in 4 horses due to technical problems with the ECG. There was no significant age difference between the horses showing a high velocity signal at the mitral valve and those showing no signal ($p = 0.16$).

Four horses (10%) showed a blue signal after the T wave. This signal was not measured. In ten horses (25%), a high velocity pattern occurred after the early diastolic filling pattern, as the mitral valve leaflets were drawn together.

Pulmonary valve

Sixteen horses (40%) showed a high velocity closure signal at the pulmonary valve immediately following the T wave of the ECG. The signal was red or blue or both, but always had a green or white core, indicative of disturbed flow or a velocity greater than 0.7 metres/second. In one horse this signal was present throughout diastole. There was no significant age difference between the group of horses showing a high velocity signal at the pulmonary valve and the group of horses showing no such signal ($p = 0.82$).

Twelve of the forty normal horses (30%) showed a red flow signal on the ventricular side of the pulmonary valve after the P wave (Figure 28). This signal was only measured in two horses, as in ten of the horses the signal was either too narrow to be measured accurately, or was too weak to be clearly delineated.

Aortic valve

Nineteen horses (47.5%) showed a red flow signal at the aortic valve immediately following the T wave. In eleven horses (27.5%) the signal was only present for one frame and appeared to be a closure noise. In two horses the signal persisted throughout diastole. In four horses in which the signal persisted for more than one frame it was too weak or too narrow to be measured. There was no

significant age difference between the group of horses showing a red flow signal at the aortic valve and the horses not showing a red signal at this valve ($p = 0.60$).

A red signal was seen on the ventricular side of the valve immediately following the P wave in eight horses.

Horses with cardiac murmurs

Tricuspid valve

All horses in groups 2, 3 and 4 showed a blue regurgitant signal at the tricuspid valve during systole (Figure 29) which persisted for a significantly greater proportion of systole, than the signals occurring at the tricuspid valve in normal horses (Table 15). There was no significant difference in the relative duration of the regurgitant signal between groups 3 (horses with a low grade tricuspid murmur) and 4 (horses with a high grade tricuspid murmur). The regurgitant signal in groups 2, 3 and 4, was most often recorded from the right parasternal angled view (Figure 4), the right parasternal short-axis view pulmonary artery level (Figure 3e) and the right parasternal long-axis view, long-axis aorta (Figure 2c). In some horses a signal was recorded in these views, but not from the other right parasternal long-axis views. Horses in group 2 and group 4 had a significantly longer signal, of larger area, than the control group. However there was no significant difference in jet length or area between the group 3 horses (horses with low grade tricuspid murmurs) and the control group. No significant difference was detected in the width of the signals between groups. There was no significant age difference between the group of horses with low grade murmurs of tricuspid regurgitation and the horses in the control group with a high velocity signal at the tricuspid valve ($p = 0.34$).

The regurgitant signal occurred significantly later in group 4 horses (horses with high grade tricuspid murmurs) than the control group. None of the other groups showed a significant difference in the onset time of the regurgitant signal. The group of horses with high grade murmurs of tricuspid regurgitation were significantly older (median age 7.5 years) than the horses in the control group which had a high velocity

signal at the tricuspid valve (median age 4 years, p = 0.047). There was no significant age difference between the groups of horses with low grade and high grade murmurs of tricuspid regurgitation (p = 0.3351).

Mitral valve

Horses in group 1, showed a blue regurgitant signal at the mitral valve during systole (Figure 30). Occasionally the regurgitant jet flowed towards the transducer and therefore was coded red (Figure 31). There was no significant difference in the length, width, area and time of onset of the signals in the group 1 horses when compared to the normal group (Table 15). However, the duration of signals relative to the duration of systole, was significantly longer in group 1 horses than in the control group. There was no significant age difference between the group of horses with murmurs indicative of mitral regurgitation and the horses in the control group with a high velocity signal at the mitral valve (p = 0.16).

Aortic valve

Horses in group 5 showed a red regurgitant signal at the aortic valve during diastole. This could be recorded from the right (Figure 32) and left hemithoraces (Figure 33). In group 5 the regurgitant signals were present throughout diastole in all horses. The width of the regurgitant signals in the horses in group 5 was not significantly different from horses in the control group; however the area and length of the signals was significantly larger (Table 15). Horses with aortic insufficiency were significantly older (median age 12.5 years) than the horses in the control group (median age 6 years) which showed a high velocity signal at the aortic valve.

Table 12. Flow patterns observed from the right hemithorax in 40 normal Thoroughbred and Thoroughbred cross horses. The figures in brackets indicate the number of horses in which the flow pattern was observed. The predominant flow patterns are printed boldly. Details of higher velocity flow signals recorded at the valves are described in the footnotes and detailed in Table 14. LVB = Low velocity blue. LVR = Low velocity red. LVB/R = Low velocity red and blue. Al = aliased. R/B = red and blue. B/R = blue and red.

Location	Syst 1	Syst 2	E	M	L
RA	**No Flow (22)** Red (18)	**Red (36)** No Flow (4)	**Red (40)**	**No Flow (29)** LVB (4) LVB/R (4) LVR (3)	**Red (32)** No Flow (8)
TV	**No Flow (35)** Blue (5)	**No Flow (40)**[a]	**Red (40)**	**No Flow (26)** LVB (13) LVR (1)	**Red (32)**[b] No Flow (6) Red Al (2)
RVInlet (LAx)	**No Flow (39)** Red (1)	**No Flow (40)**	**Red (36)** Red Al (2) R/B (2)	**No Flow (28)** LVB (8) LVR (2) LVR/B (2)	**Red (31)** R/B (3) No Flow (4) Red Al (1) LVB (1)
RVInlet (SAx)	**No Flow (21)** Blue (19)[c]	**No Flow (23)** Blue (5) Blue Al (2)	**Red (30)**[d] B/R (8) Blue (2)	**No Flow (25)** Red (5) Blue (3) LVB (3) R/B (3) Red Al (1)	**Red (32)**[e] B/R (4) No Flow (3) Red Al (1)
RVOutlet	**Blue (36)** Blue Al (4)	**Blue Al (40)**[f]	**No Flow (26)** Blue (6)[g] LVB (4) Red (4)	**No Flow (40)**	**No Flow (35)** LVB (2) Red (2) R/B (1)
PV	**Blue (20)** No Flow (15) Blue Al (4) R/B (1)	**Blue (21)** No Flow (16) Blue Al (3)	**No Flow (39)**[h] Red (1)	**No Flow (40)**	**No Flow (37)** Red (2) Blue (1)

[a] 17 horses showed blue signals at or immediately following the T wave of the ECG.

[b] 31 horses showed blue/red 'closure' signals at or immediately following the onset of the QRS complex.

[c] In the second half of systole 1, the flow pattern in nine of the nineteen horses showing a blue colour, changed to a blue pattern with aliasing.

[d] 27 horses showed a blue flow pattern immediately prior to this red flow pattern.

[e] Twelve horses showed a blue flow pattern prior to the red flow pattern.

[f] All the horses showed a blue pattern without aliasing in the second half of systole 2.

[g] In all horses showing a blue pattern, this became red in the second part of early diastole. The colour in two horses changed to blue with aliasing before becoming red.

[h] 16 horses showed a red 'closure' pattern immediately after the T wave of the ECG.

Table 13. Flow patterns observed from the left hemithorax in the 40 normal Thoroughbred and Thoroughbred cross horses. The figures in brackets indicate the number of horses in which the flow pattern was observed. The predominant flow patterns are printed boldly. Details of higher velocity flow signals recorded at the valves are described in the footnotes and detailed in Table 14. LVB = Low velocity blue. LVR = Low velocity red. LVB/R = Low velocity red and blue. Al = aliased. R/B = red and blue. B/R = blue and red.

Location	Syst 1	Syst 2	E	M	L
LA	**No Flow (38)** LVB (2)	**No Flow (33)** Red (5)[a] Blue (2)	**Red (39)** Red Al (1)	**No Flow (34)** Red (4) LVB (2)	**No Flow (21)** Red (19)
MV	**No Flow (40)**	**No Flow (40)**	**Red (37)** Red Al (3)	**No Flow (31)** LVB (5) Red (3) Red Al (1)	**Red (32)** No Flow (5) Red Al (1) LVB (1) R/B (1)
LVInlet	**No Flow (40)**	**No Flow (40)**	**Red (32)** Red Al (8)	**No Flow (22)** Red (12) Red Al (2) LVR/B (2) LVB (2)	**Red (28)** No Flow (11) Red Al (1)
LVOutlet	**Blue Al (29)** Blue (11)	**Blue Al (34)** Blue (6)	**Red (30)[b]** Blue (6)[c] No Flow (4)	**No Flow (33)** LVB (4) Red (2) B/R (1)	**No Flow (33)** LVB (2) Red (3) LVB/R (2)
Aov	**Blue Al (29)** Blue (11)	**Blue Al (34)** Blue (6)	**No Flow (36)[d]** Red (4)	**No Flow (39)** LVR (1)	**No Flow (36)** LVB (3) Red (1)

[a] Two horses showing a red flow pattern developed a blue flow pattern in the second half of systole 2.

[b] In six of the 30 horses showing red flow pattern, this changed to a blue flow pattern in the second part of early diastole.

[c] Three horses showing a blue flow pattern changed to a blue/red pattern in the second part of early diastole.

[d] Fifteen horses showed a red closure pattern immediately after the T wave of the ECG.

[e] Eight horses showed a red pattern immediately before the onset of the QRS complex

Table 14. Details of colour flow signals observed at the heart valves in a group of 40 normal Thoroughbred and Thoroughbred cross horses. N = number of horses in which a signal was detected. Max = maximum value. Min = minimum value. Q-onset = time from the onset of the QRS signal to the onset of the colour flow signal. Dur/Q-T = duration of the colour flow signal divided by the Q-T interval. Time measurements are in seconds. Linear measurements are in centimetres. Area measurements are in square centimetres.

Tricuspid Valve. Pre systole / Systole.

	GROUP	N	MEDIAN	MAX	MIN
Length	C	31	3.250	5.77	1.08
Width	C	31	1.346	3.89	0.53
Area	C	31	3.630	10.44	0.52
Q-Onset	C	31	0.0453	0.113	-0.040
Dur/Q-T	C	31	0.2174	0.540	0.170

Tricuspid Valve. Late systole.

	GROUP	N	MEDIAN	MAX	MIN
Length	C	17	2.710	4.50	0.62
Width	C	17	0.763	1.54	0.30
Area	C	17	2.080	5.26	0.13
Q-Onset	C	16	0.4000	0.50	0.27
Dur/Q-T	C	16	0.2775	0.812	0.155

Mitral Valve. Pre systole / Systole.

	GROUP	N	MEDIAN	MAX	MIN
Length	C	27	3.180	9.84	2.02
Width	C	27	1.373	2.22	0.57
Area	C	27	4.260	23.82	1.57
Q-Onset	C	23	0.0610	0.196	-0.072
Dur/Q-T	C	25	0.2083	0.405	0.166

Table 14 (continued).

Pulmonary Valve. End systole.

	GROUP	N	MEDIAN	MAX	MIN
Length	C	16	2.105	3.28	1.13
Width	C	16	0.885	2.17	0.28
Area	C	16	1.632	5.11	0.57
Q-Onset	C	16	0.4660	1.200	0.426

Pulmonary Valve. Early systole.

	GROUP	N	MEDIAN	MAX	MIN
Length	C	2	-	2.82	1.72
Width	C	2	-	0.73	0.45
Area	C	2	-	1.55	0.51

Aortic Valve. End systole / Early diastole.

	GROUP	N	MEDIAN	MAX	MIN
Length	C	15	3.130	5.47	0.97
Width	C	15	0.850	2.28	0.34
Area	C	15	3.030	10.49	0.26

Aortic Valve. Pre systole.

	GROUP	N	MEDIAN	MAX	MIN
Length	C	8	2.890	4.16	1.19
Width	C	8	0.556	3.48	0.40
Area	C	8	1.240	2.38	0.45
Q-Onset	C	7	-0.0636	0.137	-0.354
Q-T	C	7	0.400	0.410	0.380

Table 15. Details of colour flow signals observed at the heart valves in a group of normal horses (group C) and in groups of horses with cardiac murmurs (groups 1-5). p = probability values from a Mann-Whitney test comparing groups 1-5 with the normal group C. p' = probability values from a Mann-Whitney test comparing group 3 with group 4. * indicates p or p' = <0.05. Significant values at this level are printed boldly. N = number of horses in which a signal was detected. Max = maximum value. Min = minimum value. Q-onset = time from the onset of the QRS signal to the onset of the colour flow signal. Dur/Q-T = duration of the colour flow signal divided by the Q-T interval. Time measurements are in seconds. Linear measurements are in centimetres. Area measurements are in square centimetres.

Tricuspid Valve. Pre systole / Systole.

	GROUP	N	MEDIAN	MAX	MIN	p	p'
Length	C	31	3.250	5.77	1.08	-	-
	2	**8**	**5.055**	**6.53**	**4.09**	**0.0004 ***	-
	3	8	4.075	5.06	2.54	0.0916	
	4	**8**	**5.885**	**7.00**	**4.09**	**0.0020 ***	**0.0136 ***
Width	C	31	1.346	3.89	0.53	-	-
	2	8	1.028	2.10	0.66	0.2809	-
	3	8	1.016	2.56	0.79	0.4038	-
	4	8	1.023	2.10	0.66	0.2967	0.9163
Area	C	31	3.636	10.44	0.52	-	-
	2	**8**	**6.110**	**12.88**	**4.39**	**0.0029 ***	-
	3	8	3.795	8.30	1.74	0.4545	-
	4	**8**	**9.220**	**12.88**	**4.39**	**0.0009 ***	**0.0180 ***
Q-Onset	C	31	0.045	0.113	-0.040	-	-
	2	8	0.082	0.184	0.014	0.3535	-
	3	8	0.048	0.122	-0.130	0.7177	-
	4	**8**	**0.107**	**0.184**	**0.015**	**0.0054 ***	0.1182
Dur/Q-T	C	31	0.2174	0.540	0.170	-	-
	2	**8**	**1.2068**	**1.346**	**0.959**	**0.0010 ***	**-**
	3	**8**	**1.7650**	**1.490**	**0.834**	**0.0000 ***	-
	4	**8**	**1.2122**	**1.251**	**0.959**	**0.0001 ***	0.7723

Table 15 (continued).

Mitral Valve. Pre systole / Systole.

	GROUP	N	MEDIAN	MAX	MIN	P
Length	C	27	3.180	9.84	2.02	
	1	8	5.250	9.52	3.65	0.0950
Width	C	27	1.373	2.22	0.57	
	1	8	1.026	2.03	0.71	0.4206
Area	C	27	4.260	23.82	1.57	
	1	8	7.93	26.71	2.04	0.4554
Q-Onset	C	23	0.0610	0.196	-0.072	
	1	7	0.0680	0.120	0.016	0.6239
Q-T	C	25	0.400	0.530	0.280	
	1	8	0.440	0.480	0.360	0.1889
Dur/Q-T	C	25	0.2083	0.405	0.166	
	1	**8**	**1.1566**	**1.482**	**0.630**	**0.0000** *

Aortic Valve. Early diastole/ Diastole.

	GROUP	N	MEDIAN	MAX	MIN	P
Length	C	15	3.130	5.47	0.97	
	5	**8**	**7.320**	**8.65**	**2.29**	**0.0074** *
Width	C	15	0.850	2.28	0.34	
	5	8	0.794	3.26	0.43	1.0000
Area	C	15	3.030	10.49	0.26	
	5	**8**	**15.77**	**27.50**	**1.29**	**0.0359** *

DISCUSSION

Flow patterns in the heart chambers

The flow signals recorded in this study were similar to those described in humans (Wittlich *et al.* 1988). The strong red signal in early diastole at the right and left atria, atrioventricular valves and ventricular inlets, represented blood flowing towards the transducer. The orientation of the transducer in relation to the cardiac structures indicated that the blood was flowing from the atria into the ventricles. The timing and direction of this inflow pattern is consistent with the rapid filling of the ventricle in early diastole. In some horses, aliased flow was observed. This was more common at the left ventricular inlet (20%) than at the right ventricular inlet (5%). As the Nyquist limit remained constant throughout the studies, the blood must enter the left ventricle at a higher velocity, or more accurate alignment with mitral inflow must be achieved in some horses. Reef *et al.* (1989) reported mean flow velocities of 0.7 m/s during rapid filling of the left ventricle and 0.49 m/s during rapid filling of the right ventricle. The angle between the two-dimensional image and flow was not measured in the present study, as it gives no indication of alignment in the azimuthal plane (Goldberg *et al.* 1988). Therefore it is not possible to compare the alignment with the left and right ventricle. In the study by Wittlich *et al.* (1988) 63% of the human subjects showed aliasing of the left ventricular inflow signal during early diastole, whereas none showed aliasing of the right ventricular inflow signal. The Nyquist limit in this study (0.65 m/s) was lower than in the present study, but more accurate alignment with inflow was probably achieved by using the four chambered plane. This image plane cannot be obtained in adult horses (Carlsten 1987).

In the majority of horses, no flow signals were recorded at the right and left atria, atrioventricular valves or ventricular inlets during diastasis, although some horses showed low velocity flow. It is possible that low velocity flow was present in all horses, but the use of low velocity filters, set at 0.31 m/s, filtered out these signals. Reef *et al.* (1989) recorded mean flow velocities of 0.19 m/s at the atrial side of the

tricuspid valve during diastasis, and a mean velocity of 0.27 m/s at the atrial side of the mitral valve during diastasis. These low flow velocities would have been filtered out in the present study.

In late diastole a red flow pattern was detected, indicating blood flow from the atria to the ventricles. The timing of this flow signal is suggestive of flow associated with atrial contraction. In some horses this was a small signal which did not extend into the ventricle. In other horses this signal was not detected.

Colour flow studies recorded from a short-axis view of the right ventricular inlet, showed a flow pattern during early and late diastole, that changed from blue to red. This signal was not of high velocity and was not aliased. It appeared to represent a genuine change in the direction of blood flow in the ventricles at this level. This may indicate that during ventricular filling, blood flows into the ventricle, flows towards the closed pulmonary valve and then curls back towards the tricuspid valve. A change in the direction of flow was also noted in the left ventricular outflow tract during early diastole.

During the second half of systole, a red flow pattern was observed in the right atrium indicative of atrial filling. This has also been reported by Wittlich *et al.* (1988). However, the blue signal recorded by these authors during late systole, suggestive of a reversal of atrial filling at the closed tricuspid valve, or two separate inflow streams, was not observed in the present study. The two-dimensional images used in this study do not allow full visualisation of the atria, especially the left atrium. Consequently the flow patterns recorded in this study can only represent those occurring in a small part of the equine atria.

During diastole, regurgitant flow was detected at the atrioventricular valves in horses with second degree atrioventricular block. The flow pattern occurred immediately after the red flow pattern associated with the non conducted P wave. Diastolic mitral and tricuspid valve regurgitation have also been reported in human subjects with first and second degree atrioventricular block (Rutishauser, Wirz,

Gander, and Luthy 1966; Panadis, Ross, and Mintz 1986; Covalesky *et al.* 1989) and in dogs with second degree atrioventricular block (Darke 1992). Tricuspid and mitral regurgitation also occur after atrial contractions that are not followed by a properly timed ventricular systole, unless the atrial systole occurs in the early part of diastole (Rutishauser *et al.* 1966). Diastolic mitral regurgitation has been reported following non-conducted atrial contractions in dogs with experimentally induced atrioventricular block (Williams, O'Donovan, Vandenberg, Sturm and Wood 1968a). It has been demonstrated that a pressure gradient develops between the left ventricle and left atrium after the atrial contraction, which causes premature closure of the mitral valve. This closure however is not as effective as closure caused by an optimally timed atrial and ventricular systole or ventricular systole, and results in valvular regurgitation (Williams, Vandenberg, Sturm and Wood 1968b). This mechanism may explain the diastolic atrioventricular valve regurgitation in the present study.

During systole an aliased blue pattern was present in the left ventricular outflow tract and through the open aortic valve. This pattern represented flow away from the transducer, from the left ventricular outflow tract to the aorta. In the right ventricular outflow tract during the first part of systole the flow pattern was predominantly blue, changing to an aliased signal in the second part of systole and back to a blue signal in late systole. This represents flow away from the transducer from the right ventricular outflow tract into the pulmonary artery. The velocity of flow must increase in the second part of systole and then decrease in late systole. Alignment with the right ventricular outflow appeared to be better than the left ventricular outflow, and therefore flow into the aorta must reach a higher velocity earlier in systole than flow into the pulmonary artery. Reef *et al.* (1989) although demonstrating no significant difference in the mean aortic and pulmonary artery flow velocities, indicated that flow velocities do peak earlier in the aorta than in the pulmonary artery. In human studies the velocity of flow has been shown to be higher

in the aorta than the pulmonary artery (Gardin *et al.* 1984). During early diastole, flow in the left ventricular outflow tract was coded primarily in red flowing away from the aortic valve towards the left ventricle. This direction of flow has also been recorded by Reef *et al.* (1989) using pulsed wave Doppler echocardiography.

Flow patterns at the heart valves

Control group

Flow patterns at the heart valves in the control horses are similar to those described in normal humans, associated with transvalvular regurgitation (Wittlich *et al.* 1988). In humans two distinct types of flow pattern are recognised at normal valves, a signal of brief duration associated with valve closure and a regurgitant signal of longer duration unassociated with valve closure (Sahn and Maciel 1988; Wittlich *et al.* 1988). Brief signals lasting up to 100 msecs at the aortic and mitral valve, and lasting up to 150 msecs at the tricuspid valve are more common than longer regurgitant signals (Wittlich *et al.* 1988). Brief signals were also common in the present study, although the duration of the signals was not accurately timed. A distinction was made between high velocity signals on the atrial side of the atrioventricular valves during valve closure and distinct jets directed into the atria. The timing of the signals was approximately determined by recording the number of frames in which the colour signal persisted. However, an event recorded in one frame could have occurred at any time during the previous frame and may have stopped at any time before the next frame change. Therefore, signals regarded as being present for only one frame may be of any duration up to approximately 200msecs. This causes difficulties in comparing the results of the present study to those of previous human studies. Difficulty has also been reported in comparing results from the various human studies (Berger *et al.* 1989; Choong *et al.* 1989) where different criteria were used for the diagnosis of regurgitant flow and different techniques used to detect it (Sahn and Maciel 1988). Berger *et al.* (1989) only considered valvular regurgitation to be present if it lasted for greater than 50% of systole. Pollak *et al.*

(1988) and Douglas *et al.* (1989) only included signals that persisted throughout systole or diastole. Kostucki *et al.* (1986) included all subjects where regurgitant flow was identified. Yoshida *et al.* (1988) and Choong *et al.* (1989) only included signals which persisted longer than 100-200 msecs. More accurate timing of the regurgitant flow signals in the present study may have been obtained using colour M-mode. However it can be difficult to maintain the M-mode cursor within a small regurgitant signal, due to movement of the heart during systole (Wittlich *et al.* 1988). This leads to inaccuracy in timing of the duration of the signal (Choong *et al.* 1989). This problem is more obvious when imaging the heart in a long-axis plane rather than a four-chambered plane, because the image tends to move from side to side on the screen as the heart contracts and relaxes. Pulsed wave or continuous wave Doppler echocardiography could also be used to time the duration of regurgitation more accurately, although the limitations noted above also apply.

When all signals are considered together, this study shows that valvular regurgitation in normal Thoroughbred and Thoroughbred cross horses is more common at the tricuspid valve than at the other heart valves. This is also the case if only the closure signals or signals less than one frame duration are analysed. Tricuspid regurgitation has also been shown to be the regurgitation most commonly associated with valve closure in humans (Wittlich *et al.* 1988).

When regurgitant signals of greater than one frame duration are classified as regurgitation unassociated with valve closure, aortic insufficiency is the most common finding in horses. This is contrary to the findings in humans where pulmonary insufficiency (Wittlich *et al.* 1988), tricuspid regurgitation (Berger *et al.* 1989) and mitral regurgitation (Choong *et al.* 1989) have been variously reported as the most common. Aortic insufficiency however, was only detected throughout diastole in two horses (5%). This is similar to the findings of Wittlich *et al.* (1988), Choong *et al.* (1989) and Berger *et al.* (1989) in humans, but in contrast to the

findings of Yoshida *et al.* (1988) who did not detect aortic insufficiency in any normal subjects.

If however discrete jets are considered indicative of valvular regurgitation unassociated with valve closure, mitral regurgitation is the most common finding in normal horses. Despite the high incidence of regurgitant signals at the tricuspid valve, these were primarily closure noises rather than discrete jets. In contrast, the signals detected at the mitral valve were discrete jets rather than closure noises. This may reflect a smaller orifice at the mitral valve at the onset of systole, due to more efficient atriogenic closure, or it may be associated with a more rapid rise in ventricular pressure allowing jet formation prior to effective valve closure. Signals at the tricuspid valve lasted for a greater proportion of systole than signals at the mitral valve. Fifty one percent of signals at the tricuspid valve lasted for greater than 20% of systole, whereas only 30% of the signals at the mitral valve lasted for greater than 20% of systole. Berger *et al.* (1989) studying the prevalence of valvular regurgitation in normal humans, only included tricuspid and mitral signals that lasted for greater than 50% of systole. Reassessment of the equine data using these criteria shows that there was no evidence of mitral regurgitation in the normal horses and only two horses showed evidence of tricuspid regurgitation. Using these criteria tricuspid regurgitation and aortic insufficiency are the most common regurgitant signals in normal horses, being present in 5% of horses studied. This level of valvular regurgitation in the normal group of horses is much lower than previously reported in humans. This is unlikely to be related to the athletic nature of horses, as the prevalence of valvular regurgitation has been shown to be higher in human athletes than in sedentary people (Pollak *et al.* 1988; Douglas *et al.* 1989). There is an increased incidence of valvular regurgitation with age in normal humans (Akasaka *et al.* 1987; Choong *et al.* 1989). It has been suggested that this is related to myxomatous degeneration of the valve leaflets and supporting structures (Akasaka *et al.* 1987) and increased intracardiac pressures with age (Choong *et al.* 1989). Choong

et al. (1989), found that the increase in tricuspid and mitral regurgitation occurred at a relatively young age (between birth to 19 years and 20 to 39 years), whereas the major increase in aortic insufficiency occurred later in life (after 40 years). Horses also show an increased incidence of aortic valve lesions with age, occurring most commonly in horses of 15 years and older (Else and Holmes 1972). Horses with aortic insufficiency in the present study were significantly older than control horses with a high velocity signal at the aortic valve. Horses with high grade murmurs of tricuspid regurgitation were significantly older than the control horses with a high velocity signal at the tricuspid valve. The normal horses in this study showed a similar incidence of aortic insufficiency not associated with valve closure, but a lower incidence of mitral and tricuspid regurgitation not associated with valve closure to that reported in humans (Wittlich *et al.* 1988). This may be explained by an earlier onset of age related mitral and tricuspid regurgitation in humans. It is also possible that a proportion of valvular regurgitation remained undetected in the normal horses. This may have resulted from the limited visualisation of the atria using the standardised image planes. Signals recorded as being of short duration may have moved out of the image plane but may still have been present. This may also explain the occurrence of tricuspid and mitral regurgitation after the T wave. Signals may have been within the image plane at the onset and end of systole but may have moved out of the image plane during midsystole. Therefore the incidence of mitral and tricuspid regurgitation may be higher in normal horses than reported in this study.

Pulmonary and aortic regurgitation was detected following atrial contraction. Late diastolic accentuation of aortic insufficiency murmurs has been described previously in horses (Smetzer, Bishop and Smith 1966). The origin of the presystolic accentuation has been shown to be the aortic valve and not the anterior mitral valve leaflet as reported in man (Smetzer, Bishop and Smith 1966). Two-dimensional short-axis studies of the aortic valve at high frame rates have shown apparent opening of the aortic valve between the right coronary and the non coronary cusp in normal horses

following atrial contraction (Long unpublished observations). Also, in some cases of aortic insufficiency an increase in intensity of the Doppler spectrum has been observed following the atrial contraction. It is possible that the increase in ventricular volume and pressure following atrial contraction results in distortion of the semilunar valves allowing valvular regurgitation. Smetzer, Bishop and Smith (1966) also propose that atrial systole may exert an external force on the aortic valve annulus leading to an increase in aortic insufficiency.

Takao, Miyatake, Izumi, Kinoshita, Sakakibara and Nimura (1985) suggested that physiological regurgitation at the pulmonary valve could be differentiated from pathological regurgitation by its limited jet length of less than 1cm. Other workers have also associated physiological regurgitation with signals that are located close to the cardiac valves (Kostucki *et al.* 1986. Choong *et al.* 1989 Berger *et al.* 1989). Wittlich *et al.* (1988) however, reported that most normal regurgitant signals are between 1 and 2cm in length and therefore are outwith the physiological classification of some authors. In horses regurgitant signals varied from 0.6 - 9.8cm in length, the longest signals being evident at the mitral valve. As jet length has been shown to relate to jet velocity and therefore the pressure gradient across the valve (Switzer *et al.* 1987), the larger jets at the mitral valve may reflect the rapid increase in the transvalvular pressure gradient at this valve during systole combined with a small regurgitant orifice (Bolger *et al.* 1988b).

Presystolic regurgitation was detected at the tricuspid and the mitral valve in two horses, occurring before the onset of the QRS complex. However there is a time lag between the onset of electrical activity and the rise in ventricular pressure which results in complete closure of the atrioventricular valves of approximately 28 msecs (Holmes 1987). The number of horses showing valvular regurgitation, prior to the onset of mechanical systole (defined as 28 msecs after the onset of the QRS complex) was determined. Eight horses (20%) showed presystolic regurgitation at the tricuspid valve and six horses of the thirty six in which the onset time was recorded (16.6%)

showed presystolic regurgitation at the mitral valve. Due to the inaccuracy of assessing onset time from colour frames of limited frame rate, the true prevalence of presystolic regurgitation may be higher. Presystolic mitral regurgitation has also been recorded in dogs when the P-R interval was lengthened (Williams *et al.* 1968b). The amount of regurgitation was not constant. It was suggested that the vigour of atrial contraction may alter the effectiveness of atriogenic valve closure. This had previously been demonstrated experimentally by Sarnoff, Gilmore and Mitchell (1962) in dogs. It is possible that the occurrence of valvular regurgitation in normal horses is related to the long P-R interval in this species. Blood inflow velocities have been shown to be higher at the mitral valve than at the tricuspid valve (Reef *et al.* 1989). Increased velocity of flow through the mitral valve may result in an increased efficiency of atriogenic closure. This may explain the higher incidence of tricuspid regurgitation associated with valve closure, compared to that at the mitral valve in normal horses.

Groups with cardiac murmurs

Comparison of the signals occurring at the tricuspid valve in normal horses (group C) with horses suspected of having tricuspid regurgitation (groups 2, 3 and 4) revealed that the signals in the latter horses occupied a significantly higher proportion of systole than signals recorded from the control horses. There was no overlap in this measurement between any of the horses in groups 2, 3 and 4 and the horses in group C. This demonstrates that horses in groups 2, 3 and 4 had more prolonged tricuspid regurgitation than the control group and suggests that auscultation of a systolic murmur over the right hemithorax at the level of the tricuspid valve is indicative of non-physiological tricuspid regurgitation. The length and area of the jet was significantly larger in groups 2 and 4 than in the control group, and these variables were also significantly larger in group 4 horses than in group 3. Although there was overlap in measurements between groups, the two horses with grade 5 murmurs, and two of the three horses with grade 4 murmurs, had jets of a length and area outwith

that reported for normal horses and greater than reported for horses in group 3. Although many limitations have been described in using jet dimensions to quantify regurgitant volume (Chen *et al.* 1990; Simpson and Sahn 1991), it is accepted that assessment of jet dimensions provides a means by which valvular regurgitation can be semiquantified (Switzer and Nanda 1985). The larger regurgitant jets in the horses with grade 5 tricuspid regurgitation and two of the three horses with grade 4 tricuspid regurgitation may indicate more severe valvular regurgitation in these horses. This study suggests that tricuspid regurgitation murmurs of grades 2 and 3 are associated with less severe tricuspid regurgitation. The grading of tricuspid regurgitation murmurs by auscultation appears to be a useful method of assessing the severity of regurgitation. However one horse with a grade 4 tricuspid regurgitation murmur could not be differentiated by colour flow Doppler from horses with less severe murmurs, implying that the severity of tricuspid regurgitation in this horse was wrongly assessed by one of the two techniques.

The regurgitant signal was significantly later in group 4 horses (horses with high grade tricuspid murmurs) than the control group. None of the other groups showed a significant difference in the onset time of the regurgitant signal. The onset time of the tricuspid regurgitation signal was longer in two horses with a grade 4 murmur and one horse with a grade 3 murmur. This later onset time may reflect errors associated with measurements at limited frame rates, causing overestimation of the onset time in these horses.

There was no significant difference in the length, width, area and time of onset of the signals in the group 1 horses when compared to the normal group. However the duration of signals relative to the duration of systole, were significantly longer in the group 1 horses. In all but one of the group 1 horses, the regurgitant signal lasted throughout systole, and in the remaining horse, the signal occupied a greater proportion of systole than any of the horses in the control group. These findings confirm that the horses in group 1, suspected on auscultation of having mitral

regurgitation, had more prolonged mitral regurgitation detected by colour flow Doppler echocardiography.

The area and length of the regurgitant signals at the aortic valve in group 5 horses were significantly larger than those found in the control group. However there was overlap between the two groups when the length of the jets were compared, although this was restricted to the horses with grade 2/6 aortic insufficiency. Horses with grades 3/6, 4/6 and 5/6 aortic insufficiency had jets which were longer than the maximum jet length recorded in the control group. The maximum jet areas recorded in the horses in the control group were less than the jet areas found in all horses in group 5, except for one of the horses with a grade 3/6 murmur of aortic insufficiency and both horses with a grade 2/6 murmur. Therefore the latter two horses had jet dimensions within the range of the normal group, and although the duration of the jets were longer in these horses than in most control horses, two of the control horses also had a regurgitant signal that lasted throughout systole. It is possible that these horses were wrongly suspected of having aortic insufficiency, or that the two horses in the control group had murmurs of aortic insufficiency which were not detected. Alternatively it is possible that overlap does occur in the colour flow findings between normal horses and horses with low grade aortic insufficiency, and that this technique is not sufficiently sensitive to differentiate between the two groups. These colour flow studies suggest that horses with audible diastolic murmurs of grade 3/6 and above have more severe aortic insufficiency than horses with grade 2/6 aortic insufficiency.

Summary

In agreement with human studies, regurgitant signals are a common finding at the heart valves in normal horses. However in horses, the regurgitant signals appear to be primarily associated with valve closure, with a low incidence of regurgitation unassociated with valve closure. Regurgitation at the mitral and tricuspid valve can

be differentiated from signals of physiological mitral and tricuspid regurgitation by the duration of the regurgitant signal.

It appears that horses with more severe tricuspid regurgitation and aortic regurgitation can be identified by the increase in length and area of the regurgitant signals but maximal signal dimensions cannot be used to determine the severity of mitral regurgitation. These studies support the clinical suspicion that horses with louder grade regurgitant murmurs have more severe valvular regurgitation, although this cannot be supported in cases of mitral regurgitation as the Doppler colour flow technique was unable to distinguish between grades. Colour flow Doppler echocardiography is a sensitive technique for identifying valvular regurgitation in horses and has been shown to be more sensitive than two-dimensional and M-mode echocardiography in differentiating horses with cardiac murmurs. Colour flow Doppler echocardiography is likely to be a useful technique for differentiating horses with systolic murmurs that are clinically ill defined.

CHAPTER 6. SPECTRAL DOPPLER RECORDINGS IN NORMAL HORSES AND HORSES WITH SPECIFIC CARDIAC MURMURS.

INTRODUCTION

In Chapter 5 colour flow Doppler studies were recorded from a group of normal horses and from a group of horses with suspected valvular disease. Intracardiac flow patterns were described, and the presence of valvular regurgitation was confirmed in the horses in groups 1-5. In humans blood flow velocities are often higher in patients with valvular disease. This chapter describes the use of pulsed wave Doppler echocardiography to record blood flow velocities in normal Thoroughbred and Thoroughbred cross horses and in horses with valvular disease.

METHODS

Blood inflow and outflow velocities were recorded from both sides of the heart using HPRF Doppler echocardiography (page 35). The velocity scale was set such that only one sample volume was available, to prevent range ambiguity. The baseline was placed near the top or bottom of the display so that the maximum velocity of the signal could be recorded without the signal aliasing. If the velocity of a signal exceeded the Nyquist limit, the velocity scale was increased by introducing another sample volume.

Recordings were made on completion of the corresponding colour flow study. The most accurate velocity recordings are obtained when the Doppler ultrasound beam is aligned parallel to the direction of blood flow (Hatle and Angelsen 1982). Initial alignment of the Doppler beam with blood flow was judged from the two-dimensional image. A colour flow study from that image was then used to guide placement of the sampling site into an area of maximal blood flow. Following the initial placement of the sample volume minor alterations in beam angulation were made to obtain good quality audio and visual signals.

Tricuspid Inflow

The tricuspid inflow was recorded from the right parasternal long-axis apical view (Figure 2e) and from the angled view dorsal location right ventricular outflow tract (Figure 4). For the long-axis apical view the image was adjusted so that the Doppler beam was held as parallel as possible to blood flow. The sample volume was placed on the ventricular side of the tricuspid valve at the valve tips (Figure 34), either in the area with the highest flow velocity on the colour flow map, or in the centre of a uniform colour flow signal. The sample volume remained between the valve leaflets in diastole. Care was taken to ensure the sample volume was not placed too close to the valve leaflets during systole, as translation of the heart during the cardiac cycle would cause it to enter the right atrium during diastole. The right ventricular inflow tract in the horse is relatively large compared to the sample volume; therefore, flow was recorded at a number of sites between the valve tips. Minor transducer adjustments were made to obtain the maximum flow velocity.

The peak velocity was measured during the rapid filling phase of the ventricle (E wave) and during the atrial contraction (A wave). The deceleration time of the E wave was measured from the peak of the rapid filling signal (E), to the point where the downstroke intercepted the baseline (Figure 35). When baseline crossing was obscured by low velocity signals or the use of low velocity filters, the point of baseline crossing was determined by extrapolation of the downstroke. The time taken from the onset of the QRS complex to the onset of the E and A waves was recorded. Simultaneous R-R intervals (RR) and the preceding R-R (RRp) intervals were also measured. Where peak E velocities were obtained from a different location than peak A velocities, the R-R intervals relate to the peak E recording. All measurements were made with electronic calipers as described in Chapter 2 (page 77).

In the right parasternal angled view, dorsal location right ventricular outflow tract, spectral Doppler traces showed E and A waveforms similar to those recorded from the long-axis view (Figure 36). This signal was measured as described above.

Pulmonary outflow

The pulmonary outflow velocity was recorded from the right parasternal short-axis view at the pulmonary artery level (Figure 3e). The transducer was adjusted until a strong blue outflow signal was visible on the colour flow image. The sample volume was then placed on the arterial side of the pulmonary valve. Minor adjustments were made as previously described to obtain the clearest signals with the maximum velocity (Figure 37). Care was taken to ensure that the sample volume remained in the centre of the vessel during systole. Electronic calipers were used to record the peak velocity (VMax), peak acceleration (dv/dt), velocity time integral (VTI), pre-ejection period (PEP) and ejection time (EjT) as shown in Figure 38. The acceleration time (dt), R-R interval (RR) and prior R-R interval (RRp) were also measured. The acceleration time was recorded from the onset of the Doppler waveform to the start of the maximum velocity plateau. The velocity time integral was measured by tracing the modal velocity of the Doppler signal. The modal velocity was represented by the brightest line in the spectral Doppler waveform (Goldberg *et al.* 1988). The pre-ejection period was measured from the onset of the QRS complex to the onset of the of the spectral waveform. The ejection time was measured from the onset to the end of the spectral waveform. For both measurements the caliper was placed in the centre of the ascending or descending limbs of the spectral waveform as it crossed the baseline. Where low velocity filters prevented display of the actual point where the baseline was crossed, the caliper was placed by visual extrapolation of the signal to the baseline. To minimise error during this procedure low velocity filters were set as low as possible.

Mitral Inflow

The mitral inflow velocity (Figure 39) was recorded from the left parasternal apical view of the left ventricular inlet (Figure 5b). The sample volume was located as described for the tricuspid inflow. Minor adjustments were made to obtain the

maximum velocities with the clearest audible signal. Measurements were made as described for the tricuspid inflow.

Aortic Outflow

The aortic outflow (Figure 40) was recorded from the left parasternal long-axis 5-chambered view (Figure 5c). The sample volume was located on the arterial side of the aortic valve as described for the pulmonary outflow. Measurements were made as described for the pulmonary outflow (Figure 38). All Doppler recordings were taken at resting heart rates. Measurements were made from five consecutive cardiac cycles. Where horses showed second degree atrioventricular block, the immediate post-block beat was not measured. In a number of horses five post-block beats were measured from the aortic and pulmonary outflow and from the mitral and tricuspid inflow, for comparison with non post-block beats. Doppler recordings were repeated at the aortic and pulmonary outflow and the tricuspid inflow, in a number of horses at elevated heart rates. High heart rates were recorded when they occurred naturally in the course of a study, or were produced by unplugging the headphones so the horses could hear the audible Doppler signal. Doppler recordings were made from six horses on three consecutive days to assess the repeatability of these measurements.

Statistical analysis of results.

Descriptive statistics were calculated for the maximum, minimum and median values of five consecutive Doppler spectra from 40 normal Thoroughbred and Thoroughbred cross horses (range, mean, standard deviation and coefficient of variation). Correlation was used to determine any relationship between measurements and bodyweight, and between measurements and age. A Student's t-test was used to compare measurements from; the pulmonary and aortic outflow, the tricuspid and mitral inflow and the tricuspid inflow waveforms recorded from the right parasternal long-axis and angled views. The peak velocity, peak acceleration and time to peak velocity of aortic and pulmonary waveforms in normal horses with

ejection murmurs were compared with measurements in normal horses without ejection murmurs (Student's t-test). The mitral E velocity and the deceleration time in horses with left sided early diastolic flow murmurs were compared to the same measurements in horses without such murmurs (Student's t-test). The tricuspid E velocity and deceleration time in horses with right sided early diastolic flow murmurs was compared to the same measurements in horses without such murmurs (Mann-Whitney test).

A Wilcoxon test was used to compare measurements obtained from the same group of horses on different days. This test was also used to compare waveforms recorded at elevated and resting heart rates and for comparison of measurements made following and not following second degree atrioventricular block. A Mann-Whitney test was used to compare measurements from a group of normal horses and from five groups of horses with cardiac murmurs.

RESULTS

Control group

The summary statistics of measurements from forty normal Thoroughbred and Thoroughbred cross horses are given in Table 16. Recording of tricuspid inflow velocities from the short-axis view was only attempted in twenty three horses. An A wave was recorded in seventeen of these horses. Many measurements had a large coefficient of variation, especially the peak velocities of the mitral and tricuspid A wave and the aortic and pulmonary pre-ejection periods (PEP). There was less variability in tricuspid inflow measurements from the short-axis view than from the long-axis view.

Comparisons between median measurements from the aortic and pulmonary artery are shown in Table 17. Details of comparisons between all aortic and pulmonary artery measurements are given in Appendix 8. The Doppler waveforms from the aortic outflow showed a significantly greater acceleration (dv/dt Max, Med, Min) and a shorter time to peak velocity (dt Max, Med, Min) than the pulmonary

artery waveforms. The aortic waveforms also had a significantly longer pre-ejection period (PEP Max, Med, Min) and a significantly shorter ejection time (EjT Max, Med, Min) than the pulmonary artery waveforms.

Comparisons between median measurements from the mitral and tricuspid inflow waveforms are shown in Table 18. Details of comparisons between all mitral and tricuspid measurements are given in Appendix 9. The peak velocity (A Max, Med, Min) of the tricuspid A signal was significantly higher than that of the mitral signal. There was no significant difference between the peak velocity of the mitral and tricuspid E signals but the deceleration time (dt Max, Med) of the mitral E signal was shorter. The time to onset of the tricuspid E and A waveforms (QE and QA Max, Med, Min) was shorter than the time to onset of the mitral E and A waveforms.

Table 19 compares median measurements of Doppler tricuspid inflow signals recorded from the right parasternal long-axis and angled views. Details of comparisons between all tricuspid measurements are given in Appendix 10. The velocity of the tricuspid E and A signals (E and A Max, Med, Min) was significantly greater when recorded from the angled view than the long-axis view. The time to onset of the tricuspid E and A signals (QE and QA Max, Med, Min) was significantly shorter when measured from the long-axis compared to the angled view.

There was no significant differences between measurements from geldings and mares (Appendix 11), with the following exceptions. The mitral E wave deceleration time (dt Med) was significantly shorter in mares than in geldings, and the aortic outflow peak acceleration (dv/dt Max) and the acceleration time (dt Min) was greater in mares than in geldings.

Correlation with age and bodyweight

Table 20 details the correlation coefficients and p values for the correlation of median measurements with age and bodyweight. Correlation data for all measurements is given in Appendix 12. A weak though significant negative correlation was detected between the peak E tricuspid inflow velocities (E Max, Med,

Min) and age when measured from the angled view. A weak negative correlation with age was found for the peak mitral and tricuspid E velocity (E Max) from the long-axis views. Very weak, but statistically significant, correlations were demonstrated between onset time of the tricuspid angled E signal (QE Max, Med) and age, and the R-R interval (RR Max, Med, Min) during left ventricular filling and age. No significant differences were demonstrated between any measured Doppler inflow variable and bodyweight.

The peak velocity of the pulmonary artery waveforms (VMax Max, Med, Min), showed a statistically significant correlation with bodyweight. Velocity time integral measurements, from pulmonary artery waveforms showed a very weak, but statistically significant, relationship with age (VTI Max, Med, Min) and bodyweight (VTI Med, Min). All R-R intervals (except RRp max) from the aortic outflow showed a very weak, but statistically significant, relationship with age. This relationship was mainly positive but was occasionally negative (RR Max). The minimum value for aortic acceleration (dv/dt Min) showed a weak, but statistically significant, relationship with bodyweight.

Comparisons in horses with functional murmurs

Table 21 shows the results of comparisons between median measurements from normal Thoroughbred and Thoroughbred cross horses with and without functional murmurs. Appendix 13 details the comparisons of all measurements from these horses. Horses with ejection murmurs showed a significantly lower peak aortic acceleration (dv/dt Med, Min) and a significantly longer time to peak velocity (dt Max, Med, Min) than horses with no ejection murmur. However, there were no significant differences in the aortic peak velocity between horses with and without ejection murmurs, nor in any of the measured variables from the pulmonary waveforms. Horses with left sided early diastolic filling murmurs had a significantly higher peak mitral E velocity than horses without murmurs. There was no significant difference in the mitral E signal deceleration time between these horses. Horses with

right sided early diastolic filling murmurs showed no significant differences in the peak tricuspid E velocity nor in the tricuspid E deceleration time when compared to horses without murmurs.

Repeatability study

Table 22 shows the results of the repeatability study for median measurements recorded from six horses on different days. The results for all measurements are given in Appendix 14. The mitral and tricuspid peak E (E Max, Med, Min), peak A (A Max, Med, Min) and deceleration times (dt Max, Med, Min) were not significantly different on different days. However the mitral R-R intervals (RR and RRp Max, Med, Min) were significantly different between days 1 and 3 and between days 2 and 3 (RRp Min, RR Max, Med, Min). The tricuspid R-R intervals also showed significant differences between days 2 and 3 (RRp Med, RR Max, Med) and between days 1 and 3 (RR Max, Med, Min, RRp Min). No significant differences were recorded between R-R intervals from days 1 and 2. The time to onset of the tricuspid A wave was significantly different between days 2 and 3 (QA Max, Med) and the time to onset of the mitral A wave was significantly different between days 1 and 3 (QA Max). There were no significant differences between measurements obtained on different days from the pulmonary artery and aortic Doppler signals, with the exception of the pulmonary artery RRp Med and the aortic dv/dt Max, which were significantly different on days 1 and 3.

Effects of increased heart rate on flow velocities

There was a significant difference in all tricuspid inflow measurements when the heart rate was increased, with the exception of the E/A Min (Table 23 Appendix 15). The peak E and A velocities increased and the deceleration time decreased at elevated heart rates. The R-R intervals and time to onset of the E and A signals were decreased with increased heart rate.

There was a significant increase in the aortic and pulmonary peak velocity (VMax Max, Med, Min) at elevated heart rates (Table 24 Appendix 16). All aortic

acceleration rates (dv/dt Max, Med, Min) were also increased; however only the pulmonary maximum peak acceleration was significantly increased. The median and minimum pulmonary acceleration measurements were not significantly increased. The aortic ejection time (EjT Max, Med, Min) and the pulmonary ejection time (EjT Med, Min) were significantly decreased and all R-R intervals were also significantly decreased. The aortic and pulmonary pre-ejection periods were not significantly different at increased heart rates.

Effects of atrioventricular block on flow velocities

There was no significant differences in mitral and tricuspid inflow velocities before and after atrioventricular block with the exception of the tricuspid peak E velocity (E Max). This was significantly higher in the immediate post-block beats (Table 25 Appendix 17). The preceding R-R intervals were significantly longer for tricuspid and mitral inflow signals following atrioventricular block. Significant differences were also recorded in the mitral RR Max and the tricuspid QA Med.

Table 26 compares the median measurements from the aortic and pulmonary artery signals recorded before and immediately after atrioventricular block. Details of all measurements are given in Appendix 18. The peak aortic velocity (VMax Max, Med, Min) and the velocity time integral (VTI Med, Min) were significantly higher in the post block beats. There were no significant differences in peak velocity measurements and VTI measurements from pulmonary artery signals before and after atrioventricular block. The acceleration time (dt Min) was significantly longer for the pulmonary artery post block signals. Significant differences were also recorded in the aortic and pulmonary R-R intervals before and after atrioventricular block.

Flow velocities in horses with specific cardiac murmurs

Appendix 19 shows the maximum, median and minimum values for all measurements from Doppler waveforms recorded in normal horses and horses with cardiac murmurs due to valvular regurgitation. Tricuspid inflow signals were not

recorded from the short-axis view in horses with cardiac murmurs. There was no significant difference in the tricuspid inflow signals between groups, except for the time to onset of the E wave (QE Max) which was significantly longer in groups 5 (horses with aortic insufficiency), 2 (horses with tricuspid regurgitation) and 4 (horses with more severe tricuspid regurgitation). However, only three horses had a QE interval longer than the maximum range of the control group. These horses had grade 4/6 mitral regurgitation, grade 3/6 aortic insufficiency and grade 5/6 tricuspid regurgitation. Figure 41 shows box and whisker plots of QE Med measurements for all groups. The RR Max was also significantly different between groups 2 and 3.

There were no significant differences in the mitral inflow recordings between groups, except for the peak E velocity (E Max, Med, Min) which was significantly lower in group 4 horses (horses with more severe tricuspid regurgitation) than the control group. Figure 42 shows box and whisker plots of E Med measurements for all groups. Horses in group 1 had a significantly higher RR Max than the control horses.

There was no significant differences in the pulmonary outflow measurements between groups except for the RR Max which was significantly longer in groups 1 and 3 than the control group, and the RR Med which was significantly longer in group 3 than the control group. Significant differences in the aortic outflow signals were noted between group 5 (horses with aortic insufficiency) and the control horses. The peak velocity (VMax Max, Med, Min), the peak acceleration (dv/dt Max, Med) and the VTI (Max, Med, Min) were significantly larger in group 5 horses than in the control group. Box and whisker plots of aortic VMax Med, dv/dt Med and VTI Med measurements for all groups are shown in Figures 43-45 respectively. Groups 2, 3 and 4 horses (all groups with tricuspid regurgitation) had a significantly longer pre-ejection period (PEP Max, Med) than the control group. Box and whisker plots of aortic PEP Med measurements for all groups are shown in Figure 46. The minimum pre-ejection period (PEP Min) was also longer in group 2 horses. The acceleration

time (dt Min) was significantly shorter in group 1 horses and the dt Max was significantly shorter in group 2 horses.

Table 16. Summary statistics of Doppler measurements from a group of normal horses. N= Number of horses. MIN = Minimum value. MAX = Maximum value. SD = Standard Deviation. CV = Coefficient of variation. SD/Mean. * = values where CV > 15%. E and A measurements are in metres/second all other measurements are in seconds.

Short-Axis Tricuspid inflow

Variable	N	MEAN	MAX	MIN	SD	CV
RRp Max	22	1.784	2.37	1.36	0.224	12.56
RRp Med	22	1.728	2.30	1.33	0.229	13.23
RRp Min	22	1.669	2.24	1.32	0.219	13.10
RR Max	22	2.069	4.43	1.36	0.224	12.56
RR Med	23	1.896	4.21	1.33	0.229	13.23
RR Min	23	1.781	3.88	1.32	0.219	13.10
E Max	23	0.959	1.14	0.81	0.099	10.36
E Med	23	0.899	1.05	0.77	0.097	10.74
E Min	23	0.812	1.01	0.67	0.102	12.53
A Max	17	0.770	1.07	0.62	0.130	**16.88** *
A Med	17	0.687	1.07	0.48	0.136	**19.87** *
A Min	17	0.598	1.01	0.39	0.151	**25.21** *
E/A Max	17	1.253	1.585	0.980	0.192	**15.32** *
E/A Med	17	1.400	1.974	0.870	0.292	**20.86** *
E/A Min	17	1.400	1.974	0.870	0.292	**20.86** *
QE Max	22	0.865	1.00	0.75	0.062	7.20
QE Med	22	0.831	0.96	0.73	0.058	6.98
QE Min	22	0.794	0.93	0.66	0.069	8.72
QA Max	16	1.667	2.03	1.20	0.217	13.04
QA Med	16	1.606	2.00	1.14	0.215	13.40
QA Min	16	1.515	1.88	1.10	0.206	13.63

Table 16 (continued). Summary statistics of Doppler measurements from a group of normal horses. N= Number of horses. MIN = Minimum value. MAX = Maximum value. SD = Standard Deviation. CV = Coefficient of variation SD/Mean. * = values where CV > 15%. E and A measurements are in metres/second all other measurements are in seconds.

Long-Axis Tricuspid inflow

Variable	N	MEAN	MAX	MIN	SD	CV	
RRp Max	40	1.695	2.63	1.27	0.224	12.56	
RRp Med	40	1.646	2.49	1.24	0.229	13.23	
RRp Min	40	1.601	2.35	1.20	0.219	13.10	
RR Max	40	1.695	2.63	1.27	0.256	**15.08**	*
RR Med	40	1.646	2.49	1.24	0.248	**15.03**	*
RR Min	40	1.601	2.24	1.32	0.219	13.65	
E Max	40	0.729	1.01	0.51	0.116	**15.50**	*
E Med	40	0.650	0.87	0.44	0.104	**16.02**	*
E Min	40	0.577	0.78	0.43	0.093	**16.13**	*
A Max	40	0.602	0.86	0.32	0.116	**19.36**	*
A Med	40	0.531	0.79	0.26	0.117	**22.01**	*
A Min	40	0.473	0.72	0.25	0.107	**22.71**	*
E/A Max	40	1.263	3.156	0.767	0.394	**31.19**	*
E/A Med	40	1.304	3.192	0.759	0.486	**37.26**	*
E/A Min	40	1.300	3.120	0.776	0.458	**35.23**	*
dt Max	40	0.291	0.37	0.23	0.039	13.56	
dt Med	40	0.238	0.34	0.16	0.041	**17.03**	*
dt Min	40	0.191	0.32	0.11	0.047	**24.79**	*
QE Max	40	0.597	0.77	0.51	0.058	9.66	
QE Med	40	0.566	0.67	0.46	0.046	8.06	
QE Min	40	0.542	0.62	0.43	0.043	7.86	
QA Max	40	1.418	2.32	1.05	0.258	**18.20**	*
QA Med	40	1.358	2.29	0.96	0.249	**18.32**	*
QA Min	40	1.310	2.00	0.94	0.216	**16.48**	*

Table 16 (continued). Summary statistics of Doppler measurements from a group of normal horses. N= Number of horses. MIN = Minimum value. MAX = Maximum value. SD = Standard Deviation. CV = Coefficient of variation SD/Mean. * = values where CV > 15%. E and A measurements are in metres/second all other measurements are in seconds.

Mitral inflow

Variable	N	MEAN	MAX	MIN	SD	CV	
RRp Max	22	1.691	2.37	1.36	0.224	12.56	
RRp Med	22	1.633	2.30	1.33	0.229	13.23	
RRp Min	22	1.590	2.24	1.32	0.219	13.10	
RR Max	39	1.751	4.20	1.10	0.300	**17.76**	*
RR Med	39	1.685	4.16	1.06	0.273	**16.70**	*
RR Min	39	1.625	3.98	1.00	0.271	**17.06**	*
E Max	40	0.753	1.12	0.41	0.144	**19.19**	*
E Med	40	0.697	1.12	0.41	0.139	**19.96**	*
E Min	40	0.617	0.88	0.33	0.126	**20.37**	*
A Max	40	0.481	0.71	0.27	0.111	**23.15**	*
A Med	40	0.417	0.63	0.24	0.099	**23.74**	*
A Min	40	0.348	0.59	0.20	0.078	**22.39**	*
E/A Max	40	1.662	3.407	0.804	0.566	**34.05**	*
E/A Med	40	1.781	3.560	0.953	0.631	**35.43**	*
E/A Min	40	1.879	3.391	0.892	0.622	**33.10**	*
dt Max	37	0.244	0.34	0.15	0.042	**17.08**	*
dt Med	37	0.216	0.27	0.14	0.032	14.97	
dt Min	37	0.189	0.26	0.10	0.038	**20.17**	*
QE Max	37	0.627	0.90	0.54	0.061	9.69	
QE Med	37	0.608	0.89	0.52	0.061	10.75	
QE Min	37	0.588	0.86	0.50	0.061	10.40	
QA Max	37	1.521	2.45	0.96	0.297	**19.53**	*
QA Med	37	1.442	2.23	0.92	0.271	**18.82**	*
QA Min	37	1.383	1.99	0.89	0.263	**19.00**	*

Table 16 (continued). Summary statistics of Doppler measurements from a group of normal horses. N= Number of horses. MIN = Minimum value. MAX = Maximum value. SD = Standard Deviation. CV = Coefficient of variation SD/Mean. * = values where CV > 15%. Velocity is measured in metres/second, dv/dt is measured in metres/second^2, VTI is measured in centimetres. All other measurements are in seconds.

Pulmonary outflow

Variable	N	MEAN	MAX	MIN	SD	CV	
RRp Max	40	1.738	2.94	1.32	0.302	**17.36**	*
RRp Med	40	1.688	2.53	1.31	0.256	**15.18**	*
RRp Min	40	1.649	2.32	1.31	0.239	14.50	
RR Max	40	1.850	5.07	1.32	0.224	12.56	
RR Med	40	1.744	4.09	1.31	0.229	13.23	
RR Min	40	1.691	3.94	1.31	0.219	13.10	
VMax Max	40	0.952	1.11	0.81	0.091	10.09	
VMax Med	40	0.906	1.04	0.78	0.082	9.09	
VMax Min	40	0.866	1.01	0.73	0.081	9.38	
dv/dt Max	40	5.032	8.50	3.40	0.968	**19.24**	*
dv/dt Med	40	4.453	6.71	2.99	0.742	**16.66**	*
dv/dt Min	40	4.004	6.31	2.89	0.679	**16.96**	*
dt Max	40	0.231	0.29	0.16	0.030	12.96	
dt Med	40	0.208	0.27	0.16	0.027	13.19	
dt Min	40	0.184	0.25	0.13	0.027	14.83	
VTI Max	40	27.277	37.580	22.260	3.063	11.23	
VTI Med	40	25.740	36.710	20.370	3.072	11.93	
VTI Min	40	24.549	-33.930	19.540	2.814	11.46	
PEP Max	40	0.073	0.12	0.04	0.019	**26.40**	*
PEP Med	40	0.061	0.10	0.02	0.017	**28.23**	*
PEP Min	40	0.049	0.09	0.01	0.016	**32.65**	*
EjT Max	40	0.521	0.60	0.46	0.031	5.87	
EjT Med	40	0.501	0.58	0.45	0.030	6.03	
EjT Min	40	0.483	0.55	0.43	0.028	5.77	

Table 16 (continued). Summary statistics of Doppler measurements from a group of normal horses. N= Number of horses. MIN = Minimum value. MAX = Maximum value. SD = Standard Deviation. CV = Coefficient of variation SD/Mean. * = values where CV > 15%. Velocity is measured in metres/second, dv/dt is measured in metres/second^2, VTI is measured in centimetres. All other measurements are in seconds.

Aortic outflow

Variable	N	MEAN	MAX	MIN	SD	CV	
RRp Max	40	1.710	3.16	1.31	0.330	**19.32**	*
RRp Med	40	1.615	2.08	1.30	0.203	12.59	
RRp Min	40	1.562	2.03	1.27	0.192	12.32	
RR Max	40	1.761	4.26	1.31	0.224	12.56	
RR Med	40	1.672	4.17	1.29	0.229	13.23	
RR Min	40	1.562	2.12	1.27	0.219	13.10	
VMax Max	40	0.979	1.16	0.79	0.089	9.14	
VMax Med	40	0.937	1.15	0.78	0.094	10.07	
VMax Min	40	0.890	1.10	0.72	0.097	10.87	
dv/dt Max	40	9.123	13.16	5.32	1.796	**19.69**	*
dv/dt Med	40	8.015	10.83	5.19	1.448	**18.07**	*
dv/dt Min	40	7.047	9.81	4.66	1.418	**20.12**	*
dt Max	40	0.138	0.21	0.10	0.025	**18.42**	*
dt Med	40	0.122	0.17	0.09	0.021	**16.93**	*
dt Min	40	0.107	0.16	0.08	0.019	**17.86**	*
VTI Max	40	27.427	35.610	22.760	2.845	10.37	
VTI Med	40	25.369	32.900	20.610	3.209	12.65	
VTI Min	40	23.921	32.550	19.610	3.130	13.08	
PEP Max	37	0.087	0.12	0.05	0.019	**21.71**	*
PEP Med	37	0.075	0.11	0.04	0.018	**24.80**	*
PEP Min	37	0.064	0.10	0.01	0.018	**28.56**	*
EjT Max	40	0.488	0.60	0.42	0.038	7.70	
EjT Med	40	0.467	0.55	0.41	0.031	6.72	
EjT Min	40	0.449	0.53	0.39	0.031	6.89	

Table 17. Comparison of median measurements from pulmonary artery Doppler waveforms and aortic Doppler waveforms by a Student's t-test. Differences were considered significant (*) when $p<0.05$. N = number of horses studied.

Variable	N	Pulmonary Artery (Mean)	N	Aorta (Mean)	p value	
RRp Med	**40**	**1.688**	**40**	**1.615**	**0.0034**	*
RR Med	**40**	**1.744**	**40**	**1.672**	**0.0016**	*
VMax Med	40	0.906	40	0.937	0.061	
dv/dt Med	**40**	**4.453**	**40**	**8.015**	**0.0000**	*
dt Med	**40**	**0.208**	**40**	**0.122**	**0.0000**	*
VTI Med	40	25.740	40	25.369	0.58	
PEP Med	**40**	**0.061**	**37**	**0.075**	**0.0001**	*
EjT Med	**40**	**0.501**	**40**	**0.467**	**0.0000**	*

Table 18. Comparison of median measurements from the tricuspid (long-axis) and mitral inflow using a Student's t-test. Differences were considered significant (*) when $p<0.05$. N = number of horses studied.

Variable	N	Tricuspid Inflow (Mean)	N	Mitral Inflow (Mean)	p value	
RR Med	40	1.646	39	1.633	0.600	
RR Med	40	1.695	40	1.685	0.730	
E Med	40	0.650	40	0.697	0.1000	
A Med	**40**	**0.531**	**40**	**0.417**	**0.0000**	*
E/A Med	**40**	**1.305**	**40**	**1.781**	**0.0003**	*
dt Med	**40**	**0.238**	**37**	**0.216**	**0.0130**	*
QE Med	**40**	**0.566**	**37**	**0.608**	**0.0002**	*
QA Med	**40**	**1.358**	**37**	**1.442**	**0.0044**	*

Table 19. Comparison of median measurements using a Student's t-test from tricuspid inflow velocity waveforms obtained from the right parasternal long-axis view and the right parasternal angled view. Differences were considered significant (*) when $p<0.05$. N = number of horses studied.

Tricuspid Inflow Variable	N	Long-axis (Mean)	N	Angled View (Mean)	p value
RR Med	40	1.646	22	1.728	0.290
RR Med	40	1.695	23	1.896	0.089
E Med	**40**	**0.650**	**23**	**0.899**	**0.0000** *
A Med	**40**	**0.531**	**17**	**0.687**	**0.0005** *
E/A Med	40	1.305	17	1.328	0.8100
QE Med	**40**	**0.566**	**22**	**0.831**	**0.0000** *
QA Med	**40**	**1.358**	**17**	**1.606**	**0.0000** *

Table 20. Probability values (p) for correlation (r) of median Doppler measurements against bodyweight (kg) and age (years). Values are considered significant (*) when $p < 0.05$.

Tricuspid inflow (angled view).

Variable	Correlation vs bodyweight r	p	Correlation vs age r	p
RRp Med	0.194	0.387	0.358	0.102
RR Med	-0.018	0.936	0.271	0.211
E Med	**-0.463**	**0.026** *	**-0.552**	**0.006** *
A Med	-0.092	0.726	-0.344	0.177
E/A Med	-0.051	0.846	-0.109	0.678
QE Med	0.371	0.090	**0.437**	**0.042** *
QA Med	0.101	0.711	0.210	0.435

Table 20 (continued). Probability values (p) for correlation (r) of median Doppler measurements against bodyweight (kg) and age (years). Values are considered significant (*) when $p < 0.05$.

Tricuspid inflow (long-axis view).

Variable	Correlation vs bodyweight		Correlation vs age	
	r	p	r	p
RRp Med	-0.212	0.190	0.143	0.379
RR Med	-0.091	0.575	0.296	0.063
E Med	-0.205	0.205	-0.231	0.152
A Med	-0.219	0.174	0.006	0.973
E/A Med	**-0.313**	**0.049** *	-0.122	0.454
dt Med	0.033	0.838	0.249	0.122
QE Med	0.135	0.405	0.203	0.210
QA Med	-0.226	0.161	0.150	0.355

Mitral inflow.

Variable	Correlation vs bodyweight		Correlation vs age	
	r	p	r	p
RRp Med	-0.148	0.370	0.285	0.078
RR Med	-0.001	0.996	**0.378**	**0.016** *
E Med	-0.172	0.290	**-0.318**	**0.045** *
A Med	0.141	0.385	-0.077	0.637
E/A Med	-0.172	0.288	-0.116	0.475
dt Med	0.081	0.632	-0.010	0.955
QE Med	0.050	0.769	0.041	0.810
QA Med	0.211	0.210	0.182	0.281

Table 20 (continued). Probability values (p) for correlation (r) of median Doppler measurements against bodyweight (kg) and age (years). Values are considered significant (*) when $p < 0.05$.

Pulmonary outflow.

Variable	Correlation vs bodyweight		Correlation vs age	
	r	p	r	p
RRp Med	-0.145	0.544	0.174	0.087
RR Med	-0.057	0.727	**0.324**	**0.041** *
VMax Med	0.509	0.984	0.153	0.258
dv/dt Med	**0.192**	**0.042** *	0.262	0.979
dt Med	**0.087**	**0.012** *	-0.174	0.944
VTI Med	0.361	0.258	0.383	0.317
PEP Med	0.090	0.290	-0.268	0.592
EjT Med	-0.153	0.177	0.243	0.767

Aortic outflow.

Variable	Correlation vs bodyweight		Correlation vs age	
	r	p	r	p
RRp Med	-0.023	0.888	**0.330**	**0.037** *
RR Med	0.017	0.918	**0.386**	**0.014** *
VMax Med	0.139	0.394	-0.182	0.262
dv/dt Med	0.278	0.082	0.101	0.536
dt Med	0.278	0.083	-0.191	0.237
VTI Med	0.012	0.943	-0.219	0.174
PEP Med	0.098	0.564	-0.122	0.472
EjT Med	-0.230	0.153	0.171	0.292

Table 21. Comparison of the mean values of median measurements from the pulmonary artery and aorta in horses with and without systolic ejection murmurs, using a Student's t-test. Differences were considered significant (*) when $p<0.05$. N = number of horses studied.

Variable	N	No Murmur	N	Ejection Murmur	P value	
Aortic Outflow						
VMax Med	26	0.934	14	0.939	0.860	
dv/dt Med	**26**	**8.430**	**14**	**7.240**	**0.019**	*
dt Med	**26**	**0.115**	**14**	**0.135**	**0.0044**	*
VTI Med	26	24.630	**14**	26.750	0.064	
Pulmonary Artery						
VMax Med	26	0.895	14	0.927	0.260	
dv/dt Med	26	4.486	14	4.390	0.750	
dt Med	26	0.202	14	0.218	0.150	
VTI Med	26	25.830	14	25.570	0.790	

Table 21 (continued) Comparison of mean values of median measurements from the mitral and tricuspid inflow in horses with and without early diastolic filling murmurs using a Student's t-test. Mitral flow velocities are compared in horses with and without left sided murmurs, tricuspid flow velocities are compared in horses with and without right sided murmurs. Differences are considered significant (*) when $p<0.05$. N = number of horses studied.

Variable	N	No Murmur	N	Filling Murmur	P value	
Tricuspid Inflow (Long-axis)						
E Med	33	0.655	7	0.627	0.500	
dt Med	33	0.240	7	0.231	0.590	
Mitral Inflow						
E Med	**26**	**0.649**	**14**	**0.786**	**0.0060**	*
dt Med	26	0.213	14	0.223	0.4000	

Table 22. Results of Wilcoxon tests performed on median measurements from six horses on three consecutive days. * indicates a significant difference ($p<0.05$) between the measurements obtained on the different days.

Tricuspid Inflow (Long-axis)

Variable	Day 1 vs Day 2	Day 2 vs Day 3	Day 1 vs Day 3
RRp Med	0.142	**0.036** *	0.295
RR Med	0.208	**0.036** *	**0.036** *
E Med	0.675	0.201	0.142
A Med	0.142	0.345	0.675
E/A Med	1.000	0.142	0.418
dt Med	0.917	0.345	0.529
QE Med	0.787	0.093	0.281
QA Med	0.138	**0.036** *	0.059

Mitral Inflow

Variable	Day 1 vs Day 2	Day 2 vs Day 3	Day 1 vs Day 3
RRp Med	0.529	0.059	**0.036** *
RR Med	1.000	**0.036** *	**0.036** *
E Med	0.600	0.787	0.529
A Med	0.208	0.834	0.463
E/A Med	0.295	1.000	0.402
dt Med	0.208	0.753	0.178
QE Med	0.839	0.584	0.584
QA Med	0.584	0.281	0.059

Pulmonary outflow

Variable	Day 1 vs Day 2	Day 2 vs Day 3	Day 1 vs Day 3
RRp Med	1.000	0.418	**0.036** *
RR Med	1.000	0.529	0.173
VMax Med	0.201	0.463	0.059
dv/dt Med	0.529	0.463	0.402
dt Med	0.753	0.463	0.834
VTI Med	0.834	0.834	0.402
PEP Med	0.100	0.423	0.273
EjT Med	0.273	1.000	0.500

Table 22 (Continued). Results of Wilcoxon tests performed on median measurements from six horses on three consecutive days. * indicates a significant difference ($p<0.05$) between the measurements obtained on the different days.

Aortic outflow

Variable	Day 1 vs Day 2	Day 2 vs Day 3	Day 1 vs Day 3
RRp Med	0.834	0.295	0.590
RR Med	0.600	0.208	0.093
VMax Med	0.787	0.059	0.208
dv/dt Med	0.402	0.208	0.142
dt Med	1.000	0.715	0.402
VTI Med	0.675	0.281	0.281
PEP Med	0.917	0.834	1.000
EjT Med	1.000	0.787	0.715

Table 23. Comparison of median tricuspid inflow measurements recorded at resting and elevated heart rates using a Wilcoxon test. Differences were considered significant (*) when $p<0.05$. N = number of horses studied.

Tricuspid Inflow Measurements	N	Resting Heart Rate	Elevated Heart Rate	p	
RRp Med	**8**	**1.625**	**1.070**	**0.003**	*
RR Med	**8**	**1.625**	**1.050**	**0.000**	*
E Med	**8**	**0.670**	**0.890**	**0.017**	*
A Med	**8**	**0.510**	**0.740**	**0.007**	*
E/A Med	**8**	**1.491**	**1.145**	**0.022**	*
dt Med	**8**	**0.235**	**0.200**	**0.021**	*
QE Med	**8**	**0.570**	**0.535**	**0.044**	*
QA Med	**8**	**1.290**	**0.900**	**0.002**	*

Table 24. Comparison of median aortic outflow measurements recorded at resting and elevated heart rates using a Wilcoxon test. Differences were considered significant (*) when $p<0.05$. N = number of horses studied.

Aortic Measurements	N	Resting Heart Rate	Elevated Heart Rate	p	
RRp Med	**8**	**1.605**	**1.145**	**0.014**	*
RR Med	**8**	**1.600**	**1.135**	**0.014**	*
VMax Med	**8**	**0.860**	**1.150**	**0.014**	*
dv/dt Med	**8**	**7.645**	**11.070**	**0.014**	*
dt Med	8	0.120	0.100	0.116	
VTI Med	8	24.855	27.940	0.107	
PEP Med	8	0.800	0.065	0.141	
EjT Med	**8**	**0.465**	**0.415**	**0.014**	*

Table 24 (continued). Comparison of median pulmonary outflow measurements recorded at resting and elevated heart rates using a Wilcoxon test. Differences were considered significant (*) when $p<0.05$. N = number of horses studied.

Pulmonary artery Measurements	N	Resting Heart Rate	Elevated Heart Rate	p	
RRp Med	**6**	**1.420**	**1.150**	**0.036**	*
RR Med	**6**	**1.490**	**1.190**	**0.036**	*
VMax Med	**6**	**0.855**	**1.035**	**0.036**	*
dv/dt Med	6	4.165	5.565	0.059	
dt Med	6	0.200	0.185	0.138	
VTI Med	6	25.440	26.42	0.402	
PEP Med	6	0.060	0.070	0.402	
EjT Med	**6**	**0.480**	**0.455**	**0.036**	*

Table 25. Comparison of median mitral inflow measurements before and immediately following second degree atrioventricular block using a Wilcoxon test. Differences were considered significant (*) when $p<0.05$. N = number of horses studied.

Mitral Inflow Measurements	N	Post block beats	Preceding beats	p value	
RRp Med	**8**	**3.585**	**1.725**	**0.036**	*
RR Med	8	1.640	1.730	0.093	
E Med	8	0.570	0.685	0.402	
A Med	8	0.365	0.360	0.142	
E/A Med	8	1.740	2.027	0.183	
dt Med	8	0.175	0.225	0.173	
QE Med	8	0.620	0.620	0.834	
QA Med	8	1.420	1.460	0.142	

Table 25 (continued). Comparison of median tricuspid inflow long-axis measurements before and immediately following second degree atrioventricular block using a Wilcoxon test. Differences were considered significant (*) when $p<0.05$.
N = number of horses studied.

Tricuspid Inflow Measurements	N	Post block beats	Preceding beats	p value	
RRp Med	**6**	**3.000**	**1.375**	**0.036**	*
RR Med	6	1.395	1.740	0.834	
E Med	6	0.770	0.670	0.059	
A Med	6	0.625	0.540	0.402	
E/A Med	6	1.068	1.109	0.295	
dt Med	6	0.220	0.200	1.000	
QE Med	6	0.565	0.575	0.059	
QA Med	**6**	**1.115**	**1.135**	**0.036**	*

Table 26. Comparison of median aortic outflow measurements before and immediately following second degree atrioventricular block using a Wilcoxon test. Differences were considered significant (*) when $p<0.05$. N = number of horses studied.

Aortic Measurements	N	Post block beats	Preceding beats	P	
RRp Med	**7**	**3.840**	**1.710**	**0.022**	*
RR Med	7	1.690	2.100	0.076	
VMax Med	**7**	**0.930**	**0.860**	**0.035**	*
dv/dt Med	7	8.250	7.520	0.205	
dt Med	7	0.110	0.110	0.855	
VTI Med	**7**	**25.170**	**22.520**	**0.022**	*
PEP Med	7	0.060	0.090	0.075	
EjT Med	7	0.490	0.450	0.789	

Table 26 (continued). Comparison of median pulmonary outflow measurements before and immediately following second degree atrioventricular block using a Wilcoxon test. Differences were considered significant (*) when $p<0.05$.
N = number of horses studied.

Pulmonary artery Measurements	N	Post block beats	Preceding beats	P	
RRp Med	**8**	**3.610**	**1.545**	**0.014**	*
RR Med	**8**	**1.520**	**1.700**	**0.030**	*
VMax Med	8	0.960	0.855	0.363	
dv/dt Med	8	3.230	4.145	0.529	
dt Med	8	0.260	0.210	1.00	
VTI Med	8	21.68	24.960	0.234	
PEP Med	8	0.040	0.054	0.272	
EjT Med	8	0.500	0.504	0.866	

DISCUSSION

Flow velocities in normal Thoroughbred and Thoroughbred cross horses.

The Doppler waveforms and peak flow velocities recorded from normal Thoroughbred and Thoroughbred cross horses are similar to those described in normal humans (Gardin *et al.* 1984; Hatle and Angelsen 1985; Pye, Pringle and Cobbe 1991) and dogs (Brown, Knight and King 1991).

The analysis of the Doppler measurements in the present study differs from that of previous reports. In previous studies, the average of three (Harrison *et al.* 1991), five (Zoghbi, Habib and Quinones 1990) or ten (Bennet, Barclay, Davis, Mannering and Mehta 1984) Doppler measurements from consecutive cardiac cycles, or the single largest Doppler measurement (Gardin *et al.* 1984b) have been used in analysis. Other workers have calculated the average value from three of the largest Doppler waveforms (Reef *et al.* 1989). In the present study the median value of five consecutive cardiac cycles was used for analysis. If the data was normally distributed, the mean and median values should be similar. If the data is not normally distributed, the median value will be a more appropriate representation of the data. The maximum value of five consecutive cardiac cycles was also analysed, so that the present data could be compared more closely with studies in which the average value was calculated from the largest Doppler waveforms.

Analysis of the maximum and minimum measurements in the present study shows the true range of Doppler measurements in normal Thoroughbred and Thoroughbred cross horses, and gives an indication of the normal variability in measurement between beats.

The peak flow velocities from the aortic outflow and mitral inflow in the present study are similar to those recorded in conscious horses using invasive techniques (Nerem *et al.* 1974). Table 27 (page 187) shows the results of these two studies, with the findings of a previous investigation in normal Standardbred horses using Doppler echocardiography (Reef *et al.* 1989).

Tricuspid inflow velocities

Reef *et al.* (1989) used the average of three maximum flow signals for statistical analysis. The maximum tricuspid inflow velocity occurred during inspiration (Reef *et al.* 1989). In normal resting horses at least one respiratory cycle occurs during five consecutive cardiac cycles. Therefore for the tricuspid inflow signals, summary statistics of the maximum flow velocities in the present study, may be the most suitable values for comparison with the study by Reef *et al.* (1989).

The mean tricuspid inflow velocities were higher in the present study than in the study by Reef *et al.* (1989). This may reflect differences in sampling site between the two studies, or more accurate alignment with flow in the present study. Reef *et al.* (1989) recorded the tricuspid inflow velocity from the atrial side of the tricuspid valve at the junction of the valve leaflets, whereas in the present study, tricuspid inflow velocities were recorded from the ventricular side of the valve at the valve tips. Studies in humans have shown that inflow velocities are higher when recorded at the valve tips, than when recorded at the valve annulus or within the atrium (Gardin *et al.* 1986; Jaffe *et al.* 1991). This is probably due to the smaller cross-sectional area at the valve tips compared to the annulus (Gardin *et al.* 1986). For flow to remain constant through the valve, the velocity at the valve tips must increase. Reef *et al.* (1989) reported a mean angle of 40.86° (range 40° - 86°) between the ultrasound beam and the tricuspid inflow. The apical view in the present study may have allowed more accurate alignment with flow, however the angle between the ultrasound beam and the blood flow was not measured. Although this angle can be assessed from the two-dimensional image, this only indicates alignment in two planes, and therefore the angle between the ultrasound beam and blood flow was not measured. In addition the two-dimensional image is fixed during the final adjustment of the transducer, when the most accurate alignment is achieved.

Mitral inflow velocities

The mean velocity of rapid filling of the left ventricle reported by Reef *et al.* (1989), was similar to the mean velocity of median measurements in the present study, but smaller than that of the maximum measurements. The mean velocity of the atrial contribution to left ventricular filling was lower in the study by Reef *et al.* (1989) than the minimum velocities reported in the present study. This may again reflect differences in sample volume location between the two studies or improved alignment with flow in the present study as discussed previously. Reef *et al.* (1989) recorded mitral inflow velocities from the atrial side of the mitral valve whereas in the present study velocities were recorded at the valve tips. In some horses in the present study, the peak A velocities of the mitral and tricuspid inflows, were not recorded from the same site as the peak E velocities. In these horses the sites of the maximum E and A flow velocities were indicated by the colour flow image. The peak A flow was often recorded nearer to the interventricular septum whereas the peak E flow was recorded from the centre of the ventricular inlet. The peak E and A velocities were recorded separately in these horses. Underestimation of the peak A velocity may have occurred in the study by Reef *et al.* (1989) if the transducer was adjusted to ensure optimum E waveforms.

Ventricular outflow velocities

The peak velocity of the pulmonary outflow reported by Reef *et al.* (1989) was slightly higher than in the present study. The values for the aortic outflow were also slightly higher. However the values reported by Reef *et al.* (1989) were calculated, to allow for the angle between the ultrasound beam and blood flow, whereas the values reported in the present study assumed accurate alignment with flow. If accurate alignment with flow is not achieved, the true flow velocity is underestimated. This underestimation can be corrected if the angle between the ultrasound beam and the blood flow is known. However, estimation of the angle between the ultrasound beam and blood flow, from a two-dimensional image, can

lead to overestimation of the true flow velocity (Hatle and Angelsen 1985). Re-analysis of the results reported by Reef *et al.* (1989), without correction for the angle between the ultrasound beam and blood flow (Table 27), revealed that the pulmonary and aortic blood flow velocities were considerably lower than those reported in the present study. If it is assumed that the actual flow velocity in the horses in the two studies was similar, this suggests more accurate alignment with blood flow in the present study. Reef *et al.* (1989) recorded aortic outflow velocities from a right parasternal long-axis view similar to the right parasternal long-axis view of the aorta described in Chapter 3 (Figure 2c). In the present study aortic outflow velocities were recorded from the left hemithorax from a 5-chambered view (Figure 5c). Reef *et al.* (1989) recorded pulmonary outflow velocities from the left hemithorax from a view similar to the left parasternal angled view of the right ventricular inlet and outlet (Figure 7a). In the current study pulmonary artery velocities were recorded from the right hemithorax from a short-axis view at the pulmonary artery level (Figure 3e). Considerable angulation of the transducer was used in the present investigation to obtain good alignment with flow. This was facilitated by the small rounded head of the Vingmed transducer. It would be difficult to maintain skin contact at such acute angulation with a flat transducer as used in the study by Reef *et al.* (1989). The small transducer used in the present study also facilitated recording of pulmonary outflow velocities, for which it was necessary to push the transducer between the triceps mass and the thoracic wall.

Ventricular inflow velocities (E/A ratios)

In most horses the peak velocity of ventricular filling (E) was higher than the peak A velocity for both the mitral and tricuspid inflow. However, in some normal Thoroughbred and Thoroughbred cross horses this was reversed. Reversal of the E to A ratio was detected most commonly during right ventricular filling (E/A Med <1 in 8 horses), although it also occurred in one horse during filling of the left ventricle. In human medicine the E velocity is normally higher than the A velocity (Nishimura *et*

al. 1989b; Pye, Pringle and Cobbe 1991), while reversal of the E to A ratio is commonly associated with abnormal ventricular diastolic function (Gottdiener 1991). In the present study, reversal of the E/A ratio may reflect more accurate alignment with the A waveform (atrial contraction) than the E wave of rapid filling. An apical four chambered plane would allow more accurate alignment with both the E and A waveforms, however this cannot be achieved in adult horses.

Variability

Many of the Doppler measurements had a large coefficient of variation, especially the A waveforms of the mitral and tricuspid inflows. This indicates a large variation in these values between individual horses, possibly due to poor alignment with flow in certain animals. The coefficient of variation of the tricuspid E wave was lower for measurements obtained from the angled view than for those obtained from the long-axis view. This suggests more consistent alignment with flow between individual horses from this view. The pulmonary artery and aortic pre-ejection period also showed marked variability between horses. This may be associated with inaccuracies, caused by extrapolation of outflow signals to the baseline.

Comparison of tricuspid inflow velocities from long-axis and angled views

The maximum velocity of the rapid filling phase (E) and of the atrial contribution to filling (A) were significantly higher when measured from the angled view. This implies that more accurate alignment with the right ventricular inflow was achieved from this view, unless the flow velocity actually increases within the ventricle at this site. In the angled view, the blood is flowing towards the transducer at the sampling site. However the expected direction of flow, from the two-dimensional image, would be from right to left, perpendicular to the transducer (Figure 4). Flow in this direction would have resulted in a low Doppler frequency shift and poor signal quality. In contrast, the signal was strong and of high velocity. Comparison of the onset times of the E and A waveforms obtained from the angled

view shows that they occur significantly later than the E and A waveforms recorded from the long-axis view (Table 19). This finding suggests that in the angled view the right ventricular inflow signal is not recorded as soon as it passes through the tricuspid valve, as in the long-axis view, but is recorded later in diastole. The colour flow studies from this view suggest that blood flows into the right ventricle towards the apex and up towards the right ventricular outflow tract. It then appears to curl back and flows towards but perpendicular to the tricuspid valve at which point the angled recordings were made. Due to the higher velocities and lower coefficient of variation for E wave measurements from this site, this may be a more appropriate image from which to measure the equine E wave.

Comparison of mitral and tricuspid inflow velocities

Comparison of the waveforms obtained from the long-axis views of the left and right ventricular inlets, showed that the peak A velocity of the mitral inflow was significantly higher than the tricuspid inflow. This has also been reported in normal humans (Nishimura *et al.* 1989b; Pye, Pringle and Cobbe 1991), however, although the mitral A wave was reported to be higher in the previous equine study (Reef *et al.* 1989) this was not statistically significant. Studies in humans (Nishimura *et al.* 1989; Pye, Pringle and Cobbe 1991) and horses (Reef *et al.* 1989) have also shown the mitral E wave to be significantly higher than the tricuspid E wave. The mean E velocities were higher at the mitral valve in this study, but this was not statistically significant. Human studies (Zoghbi, Habib and Quinones 1990; Pye, Pringle and Cobbe 1991) suggest that the lower inflow velocities at the tricuspid valve reflect the greater area of the tricuspid valve annulus compared to the mitral valve annulus. Zoghbi, Habib and Quinones (1990) suggest that the early filling rate of the right ventricle is lower than that of the left ventricle. Tricuspid valve and mitral valve areas were not evaluated in the present study, nor was the rate of ventricular filling.

The onset of rapid filling and atrial contraction was earlier for the tricuspid inflow compared to the mitral inflow, despite similar R-R intervals. Earlier onset of

right atrial contraction would be expected, as the sino-atrial node located in the right atrial wall will cause earlier stimulation of the right atrium (Holmes 1987). The difference in onset time between the two inflows was approximately equal to half the maximum reported P wave duration for normal horses (Fregin 1982). Therefore the earlier stimulation of the right atrium may explain the difference in onset time between the mitral and tricuspid A waves. The E signal begins when the ventricular pressure falls below the atrial pressure. This study suggests right ventricular filling precedes left ventricular filling in normal horses. The deceleration time of the E signal was significantly shorter for the mitral inflow compared to the tricuspid inflow. This has also been reported in humans (Pye, Pringle and Cobbe 1991). The deceleration time of the left ventricle reflects the decrease in the left atrial, left ventricular pressure gradient and is related to the peak E velocity (Nishimura *et al.* 1989). A decrease in the peak E velocity results in a prolonged deceleration time whereas an increase in the peak E velocity decreases the deceleration time. If similar factors affect the right ventricular deceleration time, the lower peak E velocity of right ventricular inflow would cause a prolonged deceleration time compared to the left ventricular inflow. Although the difference in peak E velocities between the two ventricles was not statistically significant, the peak E velocity of the mitral inflow tended to be larger, which may have resulted in the significantly reduced deceleration time.

Comparison of aortic and pulmonary outflow velocities

Comparison of the pulmonary and aortic flow signals showed that the peak acceleration was significantly higher and the time to peak velocity significantly lower, for the aortic outflow compared to the pulmonary outflow. Although the R-R intervals (RRp Med, Min, RR Med, Min) were significantly longer for the pulmonary artery studies, the measured increase in R-R interval was slight and is unlikely to have significantly influenced the results. The RR Max was not significantly different between studies from the two outflows. Reef *et al.* (1989) described the aortic

waveform as peaking earlier in systole than the pulmonary waveform, but the time to peak velocity was not measured. Gardin *et al.* (1984) demonstrated significant differences between flow variables recorded from the pulmonary artery and aorta in humans. The time to peak velocity and average acceleration were higher in the human aorta compared to the pulmonary artery. Whereas the ejection time was significantly longer and the peak velocity lower in the pulmonary artery compared to the aorta. A significantly longer ejection time was recorded in the present study, but there was no difference in the peak velocity of flow in the two vessels. Reef *et al.* (1989) also failed to show a difference in peak flow velocity between the equine pulmonary artery and aorta. This is probably due to more accurate alignment with pulmonary artery flow in horses compared to aortic flow. However it is possible that this represents a species variation in actual flow velocities. The pre-ejection period of the pulmonary artery signals was significantly shorter than for the aortic signals. Right ventricular ejection begins very early in systole because the diastolic pulmonary artery pressure is low.

Correlation between flow velocities and age

A significant modest negative correlation was found between peak tricuspid E velocity (E Max, Med, Min) recorded from the angled view, and age. A weaker negative correlation was demonstrated between the peak tricuspid E Max velocity measurements from the long-axis view, and age. However, the E Med and E Min measurements were not significantly correlated. There was no correlation between the peak E mitral velocity measurements, and age. In human studies the peak E velocity of the tricuspid and mitral inflows has been shown to decrease with age (Zoghbi, Habib and Quinones 1990; Kitzman *et al.* 1991) These studies also demonstrated a significant increase in the peak A velocity with age. This was not demonstrated in the present study for the mitral or tricuspid inflow. The decrease in the peak E velocity with age shown in humans, is similar to that which is recorded in patients with prolonged ventricular relaxation (Zoghbi, Habib and Quinones 1990).

Although horses of a wide age range were investigated in the present study (2 to 17 years), it is possible that older horses would need to be examined to determine the effects of ageing on intracardiac flow velocities in this species.

A significant weak correlation with age was shown by R-R intervals measured during the mitral inflow and the aortic outflow studies. However the previous mitral R-R intervals were not significantly related to age, neither were R-R intervals that had been recorded during the pulmonary outflow studies or the tricuspid long-axis and angled studies. It is unlikely that these findings represent a true relationship between R-R interval and age. A similar weak correlation was detected between the pulmonary artery velocity time integral and age.

Repeatability study

There were no significant differences between measurements made on three separate days, with the exception of the aortic peak acceleration (VMax Max), the time to the onset of the mitral A wave (QA Max) and the time to onset of the tricuspid A wave (QA Max, Med). Differences were noted however, between R-R intervals on the days in which the E and A onset times differed. Changes in R-R intervals probably caused the observed changes in the onset time of the mitral and tricuspid A waves. This study indicates that statistically significant differences detected in Doppler flow variables at resting heart rates on different days (with the exception of R-R intervals and QA intervals), probably represent significant changes in cardiac function outwith normal day to day variation.

Effects of increased heart rate on ventricular inflow velocities

The peak E and peak A tricuspid inflow velocities were significantly increased at increased heart rates. The median values of E Med and E Min measurements at elevated heart rates exceeded the normal range of these measurements at resting heart rates. The increase in the peak tricuspid E velocity with increased heart rate shown in the present study is in contrast to the findings of Zoghbi, Habib and Quinones (1990) who, in humans, demonstrated a weak negative correlation ($r = -0.20$) of tricuspid E

velocity with heart rate. Harrison *et al.* (1991) recorded no change in mitral inflow velocities in humans during electrical pacing at elevated heart rates. This is in contrast to the studies of Zoghbi, Habib and Quinones (1990) and Benjamin *et al.* (1992), who demonstrated a weak negative correlation of mitral E velocity with heart rate. Johannessen *et al.* (1991) demonstrated a decrease in peak mitral E velocity following the administration of atropine to healthy human volunteers, but an increase in peak E velocity following the administration of adrenaline. The mitral peak E velocity in the study by Johannessen *et al.* (1991) was significantly related to measures of systolic function. However sympathetic stimulation also increases the rate of relaxation of myocytes (Levick 1991). The active relaxation of the ventricle has been shown to influence the peak E velocity (Devereux 1989). The elevation in heart rate in the present study would probably result from parasympathetic withdrawal and sympathetic stimulation. An increase in the rate of relaxation of the right ventricle caused by sympathetic stimulation may explain the increase in peak tricuspid E velocity with increased heart rate demonstrated in the present study. The deceleration time of the E signal was significantly decreased at increased heart rates. This is probably caused by the increase in the peak E velocity (Nishimura *et al.* 1989).

The increase in the peak tricuspid A velocity in horses at elevated heart rates is similar to that reported in humans (Zoghbi, Habib and Quinones 1990). An increase in right atrial preload due to the reduction in diastolic filling time, may cause an increase in right atrial contractility. This mechanism has been proposed for the increase in peak mitral A velocities at increased heart rates (Harrison *et. al.* 1991; Johannessen *et al.* 1991). The increased A velocity may also reflect an increase in ventricular compliance at the onset of atrial contraction, due to reduced ventricular filling in early diastole (Harrison *et. al.* 1991). Sympathetic stimulation may also have a positive inotropic effect on the atrial myocardium.

Effects of increased heart rate on ventricular outflow velocities

Increases in heart rate also resulted in significant changes in the aortic and pulmonary flow velocities. The aortic peak flow velocity and peak acceleration were increased at elevated heart rates, whereas the aortic ejection time was significantly decreased. The median values for VMax (Max, Med) and the dv/dt (Max, Med) measurements at elevated heart rates exceeded or equalled the maximum range of resting values in normal horses. The peak velocity of the pulmonary outflow was also significantly increased at elevated heart rates. The pulmonary artery dv/dt increased with increased heart rate, but this was only statistically significant for the dv/dt Max.

Studies in humans have shown a decrease in aortic peak velocity and peak acceleration at elevated heart rates, induced by electrical pacing (Harrison *et al.* 1989). Electrical pacing also caused a decrease in ventricular preload. Invasive indices of myocardial contractility (dp/dt) increase with increasing heart rate only when preload is maintained (Schaefer *et al.* 1988). The peak velocity and peak acceleration of flow have been shown to be sensitive to changes in inotropic state in humans (Bennet, Barclay, Davis Mannering and Mehta 1984) and dogs (Noble, Trenchard and Guz 1966) but are also influenced by changes in preload (Bedotto, Eichorn and Grayburn 1989). It is therefore possible that the reduction in peak velocity and peak acceleration recorded in the study by Harrison *et al.* (1989) was due to reductions in preload. During exercise, in humans, peak acceleration has been shown to increase linearly with increases in heart rate (Mehta *et al.* 1988). This increase in acceleration is thought to be caused by a major inotropic effect which is absent during electrical pacing. Peak flow velocity was shown initially to increase during exercise, before reaching a plateau. In the present study the increase in heart rate was likely to be caused by parasympathetic withdrawal and sympathetic stimulation. Sympathetic stimulation would increase myocardial contractility and help to maintain ventricular preload. This may explain the increased acceleration and peak velocity recorded at elevated heart rates in these horses.

The pulmonary ejection time (EjT Med, Min) was significantly decreased at elevated heart rates. Increased sympathetic tone and decreased vagal tone at elevated heart rates causes shortening of the work cell action potential thus shortening systole (Levick 1991).

Effects of atrioventricular block on recorded velocities

The mitral and tricuspid inflow velocities of cardiac cycles which followed second degree atrioventricular block were not significantly different from those which followed normally conducted beats, with the exception of the tricuspid E Max and the tricuspid QA Med. However, significant differences have previously been described in QA measurements on a day to day basis in the same horse, and therefore this may not reflect changes associated with atrioventricular block. The median tricuspid E velocities were higher in the post block beats than in the preceding beats. This was only statistically significant for the E Max, although the E Med velocities approached statistical significance. Examination of a greater number of horses may determine whether this increase in tricuspid E velocity following atrioventricular block is a significant finding. During atrioventricular block, the right atrial pressure gradually increases during the prolonged diastole (Brown and Holmes 1979), presumably due to an increase in right atrial preload from venous return and diastolic tricuspid regurgitation. This will cause an increase in the transvalvular pressure gradient at the onset of rapid filling, which will influence the peak E velocity (Devereux 1989).

The peak aortic flow velocities were significantly increased in the post block beats (VMax Max, Med, Min), and the aortic velocity time integral (VTI Med, Min) was also significantly increased. Second degree atrioventricular block is a normal finding in adult horses (Smetzer, Smith and Senta 1969). The large resting stroke volume in normal horses causes a progressive increase in arterial blood pressure at rest. This leads to increased baroreceptor activity causing stimulation of the vagus nerve which blocks electrical conduction at the atrioventricular node. During the blocked beat the arterial blood pressure falls, baroreceptor activity wanes and

electrical conduction at the atrioventricular node is facilitated. The decrease in afterload during atrioventricular block and the increased ventricular preload (Brown and Holmes 1979) caused by the prolonged diastole may explain the observed elevations in peak velocity and velocity time integral. No significant differences were noted in the pulmonary artery waveforms following second degree atrioventricular block, with the exception of the dt Min. The reason for this difference between the two ventricular outflows is not clear. It is possible that changes in preload during respiration also influence the pulmonary artery flow velocities. The timing of the blocked beats in relation to respiration was not determined. Respiratory variation may have masked variation in pulmonary flow velocities associated with atrioventricular block.

Flow velocities in horses with functional murmurs

Comparison of Doppler waveforms in horses with functional flow murmurs showed that horses with left sided early diastolic murmurs had a significantly higher peak mitral E velocity (E Max, Med, Min) than horses without left sided early diastolic murmurs. An increase in the velocity of blood flow into the ventricle during rapid filling may cause the Reynolds number to be exceeded such that turbulence develops. However the audible Doppler signal in these horses was clear and the spectral Doppler tracing did not show spectral broadening. In many horses with early diastolic murmurs, the murmur only occurs at the end of rapid filling, at the time of the third heart sound. The E waveform is dependent on the active relaxation of the ventricle (Devereux 1989), therefore early diastolic murmurs may be related to the abrupt end of rapid active ventricular relaxation in some horses, rather than turbulence of the inflow itself.

Comparison of Doppler outflow measurements in horses with systolic ejection murmurs, to those with no ejection murmurs revealed no differences in the maximum recorded velocity in the aorta or pulmonary artery. This is in contrast to human studies where aortic velocity was significantly related to the production of flow

murmurs following the administration of the inotrope dobutamine (Klewer, Donnerstein and Goldberg 1991).

The peak acceleration (dv/dt Med, Min) of the aortic waveforms was significantly lower, and the time to peak aortic acceleration (dt Max, Med, Min) was significantly longer, in horses with ejection murmurs. Acceleration has been shown to have a stabilising effect on fluid flowing above the critical Reynolds number for turbulent flow (Yoganathan, Cape, Sung, Williams and Jimoh 1988). It is possible that the peak blood flow velocity recorded in both groups of horses was sufficiently high to produce turbulent aortic flow. However the higher flow acceleration in the group without murmurs may have prevented the formation of turbulent flow. No differences were detected between the pulmonary waveforms in the two groups of horses.

Flow velocities in horses with cardiac murmurs

There was no evidence of valvular stenosis in any of the control horses or in any of the horses with cardiac murmurs, using the criterion of a post valvular flow velocity of greater than 2.2 metres per second (Hoffmann and Burckhardt 1983).

Tricuspid inflow velocities

The tricuspid inflow velocities did not differ significantly between any of the groups of horses with cardiac murmurs and the control group, with the exception of the QE Max, Med. Horses with tricuspid regurgitation may be expected to have a higher tricuspid inflow velocity due to-increased preload of the right atrium at end systole; however this was not detected. It is likely that the degree of tricuspid regurgitation, in horses in the present study, was not sufficiently severe to cause volume overload of the right side of the heart. Measurements of tricuspid inflow velocities from the right parasternal angled view show less variation than measurements from the long-axis view in normal horses. Therefore it may have been

more appropriate to use the angled view to measure tricuspid inflow velocities in horses with cardiac murmurs.

Mitral inflow velocities

The peak mitral E wave (E Max, Med, Min) was significantly lower in group 4 (horses with more severe tricuspid regurgitation) than in the control group (Figure 42). However, only one horse with grade 5/6 tricuspid regurgitation had a peak E velocity below the normal range (E Med). The reduced mitral E wave in this group may be due to a reduction in the left atrial preload caused by low right ventricular outflow secondary to tricuspid regurgitation. Reduction in the peak mitral A velocity has been reported in humans with severe tricuspid regurgitation (Louie *et al.* 1990). This was not detected in the horses in this study. No other significant differences were detected in the mitral flow velocities between groups, although the horse with grade 5/6 mitral regurgitation had peak mitral E velocities which exceeded the range of the control group. Increased mitral E peak velocities have been reported in human patients with severe mitral regurgitation (Appleton *et al.* 1990). Elevated peak E velocities would result from the increase in left atrial preload caused by more severe mitral regurgitation.

Pulmonary outflow velocities

There were no significant differences in the pulmonary artery waveforms between groups. However six horses had a peak velocity below that of the control group, six horses had a low velocity time integral and four horses had low pulmonary artery acceleration. Although the horses with grade 5/6 tricuspid regurgitation were included in this group of horses, animals with mitral regurgitation and aortic insufficiency were also found to have low pulmonary flow velocities, acceleration and velocity time integrals. It is possible that these results merely reflect poor alignment with flow in these horses.

Aortic outflow velocities

The aortic peak velocity (VMax Max, Med, Min), velocity time integral (VTI Max, Med, Min) and peak acceleration (dv/dt Max, Med) were significantly increased in group 5 horses (horses with aortic insufficiency) compared to the control group. Three horses with grades 3/6, 4/6 and 5/6 aortic insufficiency had peak velocities which exceeded the range of the control group, however two other horses (one from group 1 and one from group 2) also had high peak velocities. Two horses with aortic insufficiency (grades 3/6 and 4/6) and three other horses, (two with mitral regurgitation and one with tricuspid regurgitation) had a peak acceleration outwith the range of the normal group. However only horses with grades 4/6 and 5/6 aortic insufficiency had a velocity time integral which exceeded the range of the normal group. Increased flow velocities have been reported in human patients with severe aortic insufficiency (Goldberg *et al.* 1988). The increase in velocity, acceleration and velocity time integral is due to the increased preload and decreased afterload in cases with aortic insufficiency. No differences were noted in the ejection time in the horses with aortic insufficiency.

Horses in groups 2, 3 and 4 showed a significantly longer aortic pre-ejection period than horses in the control group. Only five horses had a pre-ejection period which exceeded the maximum value of the control group. All of these horses had tricuspid regurgitation. Two horses were grade 2/6, two grade 3/6 and one grade 5/6. The cause of this finding is not clear.

Summary

Spectral Doppler waveforms could be recorded in all horses. However few differences were found in the recorded flow velocities between the normal horses and horses with cardiac murmurs. Horses with severe aortic insufficiency, based on the grade of the murmur (grade 4/6 and 5/6) and suggested by colour flow studies, showed significant changes in aortic waveforms which could be identified as exceeding the normal range. Similarly the horse with the most severe mitral

regurgitation based on the grade of the murmur (5/6) also had spectral Doppler waveforms which exceeded the normal range, the mitral inflow velocity being increased. The severity of regurgitation in this horse could not be identified by colour flow Doppler echocardiography. The horses with more severe tricuspid regurgitation in this study did not show specific changes in the tricuspid inflow velocities. It appears that horses with less severe valvular regurgitation do not show large changes in Doppler waveforms. The large variation in flow velocities between horses may have masked changes in Doppler waveforms in individual horses with valvular regurgitation. It is possible that Doppler echocardiography may be more useful in monitoring the progression of valvular disease in individual cases. However, spectral Doppler recordings appear to be useful in identifying cases with more severe valvular regurgitation. Examination of a larger group of horses with more severe valvular regurgitation may have shown a greater variation in Doppler waveforms between groups.

Table 27. Comparison of the results of the present study with those of previous workers. Values reported for the present study are the medians of maximum and median measurements. For the study by Nerem *et al.* (1974), only measurements recorded at resting heart rates (<45) have been shown. Reef *et al.* (1989) angle correction, shows the values reported by these workers. The cosine of the angle between the ultrasound beam and blood flow was included in the calculation of these velocities. Reef *et al.* (1989) no angle correction, details blood flow velocities which have been recalculated excluding the cosine of the angle between the ultrasound beam and blood flow from the Doppler equation. The angles between the ultrasound beam and blood flow, reported by these workers, were used for recalculation of the Doppler flow velocities. The number of horses studied is recorded in brackets ().

	Present Study		**Reef *et al.* (1989)**		**Nerem *et al.* (1974)**
Flow Velocity	**Median measurements (Median)**	**Maximum measurements (Median)**	**angle correction**	**No angle correction**	**Invasive measurement**
Tricuspid E	0.65 (40)	0.73 (40)	0.49 (29)	0.39 (29)	-
Tricuspid A	0.53 (40)	0.60 (40)	0.39 (29)	0.31 (29)	-
Mitral E	0.70 (40)	0.75 (40)	0.70 (27)	0.46 (27)	0.70 (1)
Mitral A	0.42 (40)	0.48 (40)	0.31 (27)	0.20 (27)	-
Pulmonary VMax	0.91 (40)	0.95 (40)	1.09 (18)	0.56 (18)	-
Aortic Vmax	0.94 (40)	0.98 (40)	1.01 (27)	0.67 (27)	0.90 (2)

PART 2

CHAPTER 1. REVIEW OF THE LITERATURE

INTRODUCTION

In Part 1 of this thesis, normal horses and horses with suspected valvular disease were studied using echocardiography. Colour flow Doppler echocardiography was shown to be a sensitive technique for the diagnosis of valvular regurgitation. Spectral Doppler studies identified horses with more severe valvular regurgitation. Horses with severe aortic insufficiency had an increased heart size, aortic blood flow velocity and acceleration. It was suggested that the increase in aortic blood flow velocity and acceleration were due to an increase in stroke volume, however the ability to measure stroke volume with Doppler echocardiography was not evaluated. The peak velocity of blood flow and the integral of the velocity profile have been shown to reflect accurately changes in stroke volume (Colocousis, Huntsman and Curreri 1977). The peak acceleration of blood flow in the ascending aorta has been shown to be a sensitive index of global left ventricular function in dogs (Stein and Sabbah 1976).

Part 2 of this thesis evaluates the use of Doppler echocardiography to assess cardiac function in horses.

ASSESSMENT OF VENTRICULAR FUNCTION

The function of the ventricle is to pump blood into the systemic or pulmonary circulation in response to the body's requirements. The ability of the ventricle to perform this task can be assessed by measuring the stroke volume or cardiac output. However, the stroke volume of the ventricle is influenced by changes in preload, afterload and myocardial contractility (Levick 1991). Cardiac output is further influenced by changes in heart rate. If the contractile state of the heart remains constant, stroke volume will decrease if preload is reduced, and will increase if afterload is reduced (Braunwald 1971). Therefore, cardiac output alone, although reflecting overall cardiac performance, gives limited information on the contractile state of the cardiac muscle (Braunwald 1971). A comprehensive assessment of

cardiac function should involve measurement of cardiac output, and also assessment of the loading conditions and contractility of the myocardium (Braunwald 1988).

MEASUREMENT OF CARDIAC OUTPUT

In 1733 Stephen Hales published an estimation of the cardiac output of a 14 hands 3 inches (1.5 metres) high white mare (page 191) (Hamilton and Richards 1964). Following exsanguination of the horse, he filled the left ventricle with melted beeswax, and measured the volume from the solidified cast. He assumed the ventricle emptied completely during systole, despite William Harvey's previous observations to the contrary (Hamilton and Richards 1964), and therefore calculated the cardiac output by multiplying the measured volume by the "resting heart rate". The volume of the cast was 160cc, the resting heart rate 36 beats per minute and therefore cardiac output was calculated as six litres per minute. Hamilton and Richards (1964) suggest this low value was related to the cause of death of the horse (exsanguination), but the ventricular volume would also be low as it is likely that the heart was arrested in systole (O'Callaghan 1985). It was many years before cardiac output was measured in the living animal.

Fick Principle

In 1870, Adolf Fick proposed that the rate of oxygen uptake from alveolar gas must equal the pulmonary blood flow multiplied by the arteriovenous difference in oxygen concentration (Levick 1991). To calculate cardiac output using this principle, it is necessary to measure venous oxygen concentration from mixed venous samples. These can only be obtained from the right ventricle or pulmonary artery and therefore cardiac catheterisation is necessary. Cardiac catheterisation had been performed in 1844 by Claude Bernard (Cournand 1975) to measure the temperature of ventricular blood. Bernard had passed a long mercury thermometer into the right and left ventricles of a horse, via the jugular vein and carotid artery. However it was not until 1886 that the Fick principle was first used to measure cardiac output (Cournand

1975). The cardiac output of resting horses was measured, using the Fick principle, by Zuntz and Hagemann (Stewart 1897). To ensure valid results Zuntz ensured that the expired air was collected simultaneously with mixed venous and arterial blood samples, over a period of one and a half to two minutes, with the horse standing quietly (Cournand 1975). The average cardiac output of conscious horses was reported as 75mls/kg/min (Zuntz and Hagermann 1898). Using these values, the cardiac output of Hales' white mare would be approximately 36 litres per minute, rather than the six litres per minute previously reported (assuming the weight of a 14 hand 3 inch mare to be approximately 480kg). The Fick principle has since been used to measure cardiac output in anaesthetised horses (Stowe and Good 1961).

The Fick principle has several limitations in measuring cardiac output. Cardiac catheterisation for mixed venous oxygen sampling may result in arrhythmias at elevated heart rates (Levick 1991). It has been suggested that the need for simultaneous mixed blood and arterial sampling is a limitation to the use of the Fick principle in horses (Muir, Skarda and Milne 1976), although this appears to be a minor problem. The measurement of oxygen consumption is slow: therefore steady state conditions are required for valid determinations (Stowe and Good 1961). This prevents assessment of beat to beat changes in stroke volume (Levick 1991). A facemask is necessary for cardiac output determinations in conscious horses, which may result in inaccurate measurement of oxygen consumption (Muir, Skarda and Milne 1976). There must be no leakage of gas between the subject and the flow transducer (Davies, Jebson, Glasgow and Hess 1986). Fisher and Dalton (1961) consider facemasks to be a limitation of the technique in horses, as few horses will tolerate facemasks for a sufficient time for accurate ventilation measurements to be made. These authors suggest that this difficulty can be overcome by tracheotomy (Fisher and Dalton 1961). Even in human patients, without endotracheal intubation, considerable patient cooperation is required to obtain a complete gas sample (Mackenzie, Haites and Rawles 1985). Incomplete gas collection has been reported

as a source of error in humans. Stowe and Good (1961) reported a measurement error of up to 10% in the methods used to measure oxygen consumption. These workers also reported a variation of 15% and greater when measurements were made in quick succession, despite attempts to measure output during steady state conditions. This may be due to the phasic nature in which oxygen enters the circulation (Mackenzie, Haites and Rawles 1986). Phasic variations in flow rate and arteriovenous oxygen concentrations can cause large errors in the calculation of cardiac output (Visscher and Johnson 1952).

Indicator dilution techniques.

Dye dilution method

Measurement of cardiac output by the dye dilution method involves the injection of a known mass of indicator (dye) into the venous system. The indicator is diluted in the venous blood and passes through the lungs into the systemic circulation. The concentration of dye in the arterial blood is measured from consecutive samples. A graph is plotted of concentration against time, and cardiac output is calculated from the area under the concentration curve.

Stewart (1897) first proposed an injection method to measure cardiac output. Using saline as the indicator, concentration was determined from the change in electrical conductance of blood in the femoral artery. A modification of this technique was later used to measure cardiac output in humans (Hamilton, Moore, Kinsman and Spurling 1928). An indicator dye was used, and cardiac output was determined by colorimetry of consecutive arterial blood samples. In vitro experiments showed the method predicted flow with an average error of 3.2% (Kinsman, Moore and Hamilton 1929). However when used in vivo, following the initial decay of the concentration curve there was a secondary increase in dye concentration due to recirculation of the indicator. As cardiac output is calculated from the concentration curve of dye from the first circulation, it was important to identify accurately the part of the curve associated with recirculation. In a study in

anaesthetised dogs the increase in concentration caused by recirculation was readily identified by plotting the concentration of dye on semi-logarithmic paper (Moore, Kinsman, Hamilton and Spurling 1929). Results showed the average difference between dye dilution and Fick measurements of cardiac output to be -4.5%. However the range of differences was between +2.5% and -29%. Comparison of the Fick method and the dye dilution method in humans revealed that in most cases, estimations by the dye technique were within 25% of the Fick measurement (Hamilton, Riley, Attyah, Cournand, Himmelstein, Fowell, Noble, Remington, Richards, Wheeler and Witham 1948).

Fisher and Dalton (1959a) used the dye dilution technique to record cardiac output in horses. The dye (Evans blue) was injected into the jugular vein (Fisher and Dalton 1959b), and serial arterial blood samples were collected from the brachial artery at 2 second intervals. The mean cardiac output recorded in this study (74 mls/kg/min) was similar to that measured in horses by Zuntz and Hagermann (1898) using the Fick principle (75 mls/kg/min). Dye dilution techniques have also been used to study changes in cardiac output in horses under general anaesthesia (Eberley, Gillespie, Tyler and Fowler 1968; Hall, Gillespie and Tyler 1968; Gillespie, Tyler and Hall 1969; Hillidge and Lees 1975). However, no direct comparisons with the Fick method have been made, to determine the accuracy of the technique in horses.

Dye dilution methods have the advantage over the Fick technique, that face masks and ventricular catheterisation are not required. Furthermore, dye dilution methods have an improved time resolution compared to Fick measurements (Levick 1991). However recirculation of dye is a problem which causes error from extrapolation of dye concentration decay curves, especially in diseased hearts (Levick 1991). Additionally, some indicators have strict dosage limitations, and others may be lost during passage through the lungs (Goodyer, Huvos, Eckhardt and Ostberg 1959). The blood loss during estimations is not considered to be a significant problem (Hillidge and Lees 1975).

Thermodilution method

Fegler (1954) used a thermal indicator to measure cardiac output in dogs. This method, which he termed thermodilution, is based on the same principle as the dye dilution technique. Rather than measuring the time concentration curve of an injected dye, a similar curve is obtained for changes in blood temperature, following the injection of a known volume of cold fluid.

The accuracy of the technique evaluated in vitro was from 0.2% to 8.9% of the actual flow rate. The mean error was 4.2%, with the thermodilution measurements nearly always higher than direct measurements (Fegler 1954). However in a different in vitro model which allowed greater heat exchange of the injectate in the mixing chamber with the surrounding environment, there was a greater overestimation of flow (range 15% to 42%). Fegler (1954) compared the thermodilution method and the Fick method of measuring cardiac output in six dogs. He concluded that cardiac output measurements by the two methods agreed, as well as two measurements by the Fick method agreed. The difference between measurements was less than 7% in all but one case. The thermodilution studies of Fegler, were not widely accepted (Goodyer *et al.* 1959). The main criticisms of the technique were associated with potential losses of cold indicator to the tissues.

Heat loss to the tissues has been shown to occur, but this is usually reversible, and does not appreciably affect estimations of cardiac output (Goodyer *et al.* 1959). Reversible heat loss to the tissues causes the prolonged downstroke of the thermal indicator curve (Goodyer *et al.* 1959; Hosie 1962). This results in an insignificant overestimation of cardiac output at normal flow rates, however, as cardiac output falls, thermodilution progressively overestimates cardiac output (Runciman, Isley and Roberts 1981). This is due to increased heat loss to the tissues between the site of injection and the thermistor, recording changes in blood temperature, as blood flow slows (Runciman, Isley and Roberts 1981). Injection of the thermal indicator directly

into the right atrium, rather than a central vein, prevents overestimation of cardiac output at low flow rates (Runciman, Isley and Roberts 1981).

Recirculation of indicator, a problem in the dye dilution technique is not a major problem in the thermodilution technique because of heat loss in the periphery, especially in capillary beds and through thin walled veins where flow rates are slower (Hosie 1962).

Incomplete mixing of the cold indicator with blood causes error in estimations of cardiac output by thermodilution (Dow 1956; Hosie 1962). Incomplete mixing is a problem in all indicator dilution methods, however mixing appears to be more efficient in the thermodilution method than in the dye dilution method (Branthwaite and Bradley 1967; Pavek, Pavek and Boska 1970). Inadequate mixing will be evident from the bizarre shape of the dilution curves (Goodyer *et al.* 1959). The shape of dilution curves also changes if the thermocouple is lying against the vessel wall. The rise and fall of the curves is sluggish and cardiac output will be overestimated (Fegler 1954).

A further problem associated with the use of a thermal indicator is the heat gained by the indicator, from the catheter before injection: the lower the temperature of the injectate, the more pronounced the problem (Evonuk, Imig, Greenfield and Eckstein 1960; Hosie 1962). Correction can be made for the loss of indicator during injection (Goodyer *et al.* 1959) or a second thermocouple can be used to record the temperature of the injectate as it leaves the catheter (Ganz, Donoso, Marcus, Forrester and Swan 1971). It is essential that the exact temperature of the injectate is known, to ensure accurate cardiac output estimations (Levett and Repogle 1979). Solutions placed in an ice bath for cooling prior to injection require 45 to 60 minutes to reach a steady-state temperature. Temperature probes inserted directly into the ice will underestimate the temperature of the injectate even after this time (Levett and Repogle 1979). A more accurate determination of injectate temperature is possible when an injectate at room temperature is used (Evonuk *et al.* 1960).

Continued cooling of the blood by the cold injectate left in the catheter also represents a potential source of error in cardiac output estimations by thermodilution. However, studies by Goodyer *et al.* (1959) failed to demonstrate a difference between thermodilution curves, when cool injectate remained in the catheter and when the injectate was immediately withdrawn. This is in contrast to the findings of Ganz *et al.* (1971) who demonstrated distortion of the exponential downslope when iced saline was allowed to remain in the catheter. The difference in the results of these workers probably reflects the different temperature of the injectates used. Goodyer *et al.* (1959) used injectate at room temperature whereas Ganz *et al.* (1971) used an ice cold indicator. The room temperature indicator would produce a lower temperature gradient between the catheter and the blood therefore heat transfer would be reduced.

The pulmonary artery temperature has been shown to fluctuate in phase with respiration (Afonso, Herrick, Youmans, Rowe and Crumpton 1961). This represents an unstable base line against which changes in temperature are recorded. To minimise errors associated with this baseline fluctuation, cardiac output estimations can be performed at the same time in the respiratory cycle. However, as the variation in temperature is caused by changes in the venous return, this would bias the cardiac output estimation (Afonso *et al.* 1961). Errors can be minimised by using an increased amount of indicator to increase the signal to noise ratio (Ganz *et al.* 1971). This is achieved either by increasing the volume of fluid injected, or by increasing the temperature difference between the injectate and the blood. The disadvantage of increasing the volume of the injectate is the associated risk of altering the cardiac output. Furthermore a large volume takes longer to inject whereas the indicator should be injected as rapidly as possible (Fegler 1954). A disadvantage of the use of iced indicators is a possible decrease in body temperature if a large number of determinations are made (Fegler 1954). Also potential errors due to increased loss of indicator prior to injection and inaccurate measurement of injectate temperature are greater when an iced indicator is used (Evonuk *et al.* 1960). When the accuracy and

reproducibility of various cardiac output studies were analysed no difference was noted between measurements obtained with ice cold or room temperature indicators (Stetz, Millar, Kelly and Raffin 1982). Further work has shown that some cardiac output computers show a reduced variability when ice cold indicators are used, although this can be associated with reduced accuracy (Mackenzie, Haites and Rawles 1986). A greater variability was found when room temperature indicators were used to measure cardiac output in horses (Muir, Skarda and Milne 1976). However, the temperature of the room was high (27°C).

Cardiac output computers from different manufacturers differ in the methods used to estimate the area under the curve, especially the method used to define the tail of the curve. The shorter the time base of this calculation, the greater the sampling error, especially with pulsatile flow (Mackenzie, Haites and Rawles 1986). This may present a problem in the use of the thermodilution technique to measure cardiac output in horses with second degree atrioventricular block. If cardiac output is measured during the conducted beats, flow will be overestimated, whereas if cardiac output is measured during the prolonged diastole, flow will be underestimated.

The thermodilution technique has the advantage of avoiding the build up of foreign substances in the body, which occur with the dye dilution techniques (Fegler 1954; Hosie 1962). Repeated estimations can be made quickly (Fegler 1954; Ganz *et al.* 1971) and if the thermistor has a rapid response time, beat to beat changes in stroke volume can be measured. In contrast to other indicator dilution techniques, blood samples are not required for calibration or measurements, and calibration of thermodilution curves is simple (Ganz *et al.* 1971). The calibration variability was significantly better for the thermodilution method than the dye dilution method (Fischer, Benis, Jurado, Seely, Tierstein and Litwak 1978). Estimations of cardiac output by thermodilution have correlated closely with estimations by the Fick technique in anaesthetised dogs (Goodyer *et al.* 1959) and humans (Branthwaite and Bradley 1967). Estimations of cardiac output by thermodilution have correlated

closely with dye dilution techniques in humans (Ganz *et al.* 1971) and horses (Muir, Skarda and Milne 1976; Dunlop, Hodgson, Chapman and Grandy 1992). However correlation only gives a measure of the linear relationship between the two techniques (Altman 1980). It would be expected that two techniques designed to record the same variable would show a close correlation (Bland and Altman 1986). A close correlation would be expected between thermodilution and dye dilution, as both are based on the same principle, and therefore both are subject to similar errors (Schuster and Nanda 1984; Mackenzie, Haites and Rawles 1986). Assessing the accuracy of a new technique is difficult when the comparative technique is also subject to error. Previous comparative studies of thermodilution with either dye dilution or the Fick principle, have been reanalysed to determine more fully the accuracy and reproducibility of the thermodilution technique (Stetz *et al.* 1982). Measurements of cardiac output using custom made thermodilution equipment were more reproducible than those obtained from commercial machines. However many commercial machines do not show the thermodilution curves from which the cardiac output estimation is derived. Owing to the problems associated with inadequate mixing, loss of indicator and poor positioning of thermistors, it is important to assess the quality of the thermodilution curve before computing the cardiac output (Levett and Repogle 1979). Inadequate thermodilution curves caused by technical error, are the likely source of the "nonsense" readings reported by some authors (Stetz *et al.* 1982). Despite the technical limitations, many authors believe that with due precautions and attention to detail, thermodilution provides an accurate means of measuring cardiac output (Ganz *et al.* 1971; Levett and Repogle 1979) and may be considered a reference technique (Stetz *et al.* 1982). Other authors have concluded that little reliance can be placed on any absolute measurement of cardiac output by thermodilution, due to large errors in conditions of pulsatile flow (Mackenzie, Haites and Rawles 1986).

Doppler ultrasound method

Light (1969b) first reported the potential value of Doppler echocardiography for measuring left ventricular stroke volume noninvasively in humans. Flow through a vessel can be calculated from the product of the mean flow velocity and the cross sectional area of the vessel, measured at the same site. The early development and validation of the technique was discussed in Part 1 (page 28). The limitations of Doppler echocardiography in measuring absolute flow velocity and therefore absolute flow in vivo have been discussed. However the technique is considered useful for recording relative changes in blood flow in a given patient (Angelsen and Brubakk 1976; Sequeira *et al.* 1976).

Calculation of flow by Doppler echocardiography is based on a number of assumptions. The cross sectional area of the vessel is assumed to be constant during ventricular ejection (Light 1969a). However, the area of the aorta and pulmonary artery have been shown to vary in phase with the cardiac cycle (Loeber, Goldberg, Marx, Carrier and Emery 1987). It has been assumed that most of this increase occurs early in systole, simultaneously with the upstroke of the pressure tracing (Stewart, Jiang, Mich, Pandian, Guerrero and Weyman 1985). Although, studies in dogs have shown that the maximum vessel area occurs in mid to late systole (Loeber *et al.* 1987). It is not necessary to measure vessel area during this maximal distension, as this only occurs transiently during systole (Goldberg *et al.* 1988).

The velocity profile at the sampling site is assumed to be flat and the measured velocity is assumed to be accurate. The latter assumption requires the ultrasound beam to be parallel with flow. The validity of a centre-line aortic velocity measurement to approximate the average velocity across the vessel, has been demonstrated in dogs (Seed and Wood 1971). It has been suggested that in horses, the aortic velocity profile of the ascending aorta may not be flat (Skjaerpe 1988). However, the equine aortic velocity profile has only been measured distal to the aortic arch where it does have a flat velocity profile (Nerem *et al.* 1974).

The velocity time integral (VTI) is determined by measuring the area under the velocity curve. The modal velocity (the whitest or darkest line in the spectral display) is usually integrated (Goldberg *et al.* 1988). Some workers estimate the area under the maximum velocity curve, assuming that the average maximum velocity across the vessel will closely approximate the average mean velocity across the vessel, if the velocity profile is flat (Ihlen, Amlie, Dale, Forfang, Nitter-Hauge, Otterstad, Simonsen and Myhre 1984).

Velocity waveforms are only integrated during systole (aortic or pulmonary waveform), or during diastole (mitral and tricuspid waveform). This assumes no forward or backward flow during the opposite phase of the cardiac cycle (Goldberg *et al.* 1988).

In theory the velocity profile should be recorded at the same level as the area measurement. However for aortic flow, the velocity has been shown to be constant when measured at different centre-line positions in the vessel (Fisher, Sahn, Friedman, Larson, Valdes-Cruz, Horowitz, Goldberg and Allen 1983a; Ihlen *et al.* 1984), due to conservation of the blood jet velocity upwards from the valve (Eriksen and Walloe 1990). This suggests that the exact sampling site of aortic velocities is relatively unimportant. Recent work has demonstrated that the velocity time integral is within 90% of its maximum value, up to 30 millimetres above the aortic valve in humans (Eriksen and Walloe 1990). Other authors stress the importance of measuring the vessel area from the exact Doppler sampling site, as flow velocity is a direct function of flow area (Goldberg *et al.* 1988).

The area of a vessel is usually calculated from a measure of its diameter, by assuming the vessel to be circular. The diameter can be measured in a number of ways. The measurement may include the thickness of one vessel wall (leading edge to leading edge method), both vessel walls (leading edge to trailing edge method) or neither wall (trailing edge to leading edge method). Aortic measurements from two-dimensional images, have been shown to be superior to those from M-mode images

(Gardin, Tobis, Dabestani, Smith, Elkayam, Castleman, White, Allfie and Henry 1985). Any error in measurement of the diameter of a vessel will be magnified in the calculation of the vessel area.

Estimation of the area of the mitral orifice is difficult due to its non circular geometry and its change in size during the cardiac cycle (Goldberg *et al.* 1988). Various methods have been used to estimate the mean area of the mitral orifice during diastole (Ascah, Stewart, Gillam, Triulzi, Newall and Weyman 1989). Changes in area during diastole have been estimated from M-mode studies (Fisher, Sahn, Friedman, Larson, Valdes-Cruz, Horowitz, Goldberg and Allen 1983b; Zhang, Nitter-Hauge, Ihlen and Myhre 1985). Accurate measurement of flow area is considered by many to be the major source of error in the calculation of cardiac output by Doppler echocardiography (Gardin *et al.* 1985; Ihlen, Myhre, Amlie, Forfang and Larson, 1985; Sahn 1985).

Owing to the difficulties in recording the flow area accurately, and the strong relationship demonstrated between Doppler aortic velocity time integrals and stroke volume (Sequeira *et al.* 1976; Colocousis, Huntsman and Curreri 1977; Steingart, Meller, Barovick, Patterson, Herman and Teichholz 1980; Gisvold and Brubakk 1982; Mehta and Bennett 1986), some workers have proposed that the velocity time integral (stroke distance), can be used to accurately reflect changes in stroke volume without the need to measure flow area (Haites, Mowat, McLennan and Rawles 1985). The product of the velocity time integral and heart rate (minute distance) has also been suggested to be an accurate indicator of cardiac output. In vitro, this method detects changes in flow rate of 33%, with 95% certainty. This is similar to the results obtained in vitro for the thermodilution technique under conditions of pulsatile flow (McLennan, Haites, MacKenzie, Daniel and Rawles 1986). Measurements of aortic velocity time integrals show limited day to day variability (Gardin, Dabestani, Matin, Allfie, Russell and Henry 1984b), and therefore measurement of this variable has been considered a valid way of monitoring cardiac output (Haites, McLennan, Mowat

and Rawles 1985; Northridge, Findlay, Wilson, Henderson and Dargie 1990; Moulinier, Venet, Schiller, Kurtz, Morris and Sebastian 1991). It is also useful in assessing the response to medical treatment (Elkayam, Gardin, Berkley, Hughes and Henry 1983). Although it is generally accepted that changes in the velocity time integral alone indicate changes in cardiac output, some workers consider the omission of the vessel area from the calculation of cardiac output introduces further error when monitoring cardiac output (Gibson 1985; Stewart *et al.* 1985; Mehta and Bennett 1986). The area of the pulmonary artery, aorta and mitral valve have been shown to increase with increases in stroke volume causing significant error in cardiac output estimations if a standard area is used (Stewart *et al.* 1985). However Loeber *et al.* (1987) failed to demonstrate a significant change in baseline cyclical variation of aortic area during changes in preload, afterload and during dobutamine infusion. A significant decrease in the cyclical change in aortic size did occur during clamping of the inferior vena cava.

In vitro studies, have shown good correlations between direct recordings of cardiac output and measurements by Doppler echocardiography (Fisher *et al.* 1983; Stewart *et al.* 1985). Estimations from the aorta showed a closer relationship to actual flow than those from the mitral valve and pulmonary artery (Stewart *et al.* 1985). The poorer relationship between actual flow and flow estimated from the pulmonary artery, probably reflects the increased difficulty in measuring the pulmonary artery diameter. The relationship between Doppler aortic measurements was closer to unity when the vessel diameter was measured from the valve annulus, rather than from the sinotubular junction (Stewart *et al.* 1985). Measurements from the sinotubular junction caused underestimation of flow. This is in contrast to in vivo studies in humans, where Doppler estimations of cardiac output grossly overestimated flow measured by thermodilution when vessel area was measured at this location (Ihlen *et al.* 1984). It was suggested that the effective area of blood flow in the aortic root is smaller than the anatomical root area. The closest relationship with cardiac output,

measured by thermodilution, occurred when the vessel area was calculated from the diameter of the aortic annulus using the leading edge to leading edge method (Ihlen *et al.* 1984; Christie, Sheldahl, Tristani, Sagar, Ptacin and Wann 1987). Correlation of Doppler measurements of cardiac output with the thermodilution and Fick methods shows considerable intersubject variation (Christie *et al.* 1987). This variability is not altered by removing the vessel area from the calculation. The intersubject variability is thought to be due to differences in alignment of the ultrasound beam in different individuals. This variability between subjects has been reported previously (Angelsen and Brubakk 1976; Sequeira *et al.* 1976).

Numerous studies have compared various Doppler echocardiographic measurements of stroke volume with an invasive reference standard in humans (Huntsman, Stewart, Barnes, Franklin, Colocousis and Hessel 1983; Chandraratna, Nanna, McKay, Nimalasuriya, Swinney, Elkayam and Rahimtoola 1984; Ihlen *et al.* 1984; Zhang *et al.* 1985; Ihlen *et al.* 1985). Although good correlations have been reported between the techniques, few studies have assessed the accuracy of the Doppler methods. This is probably due to inaccuracies of the invasive standard against which the Doppler techniques are compared (Schuster and Nanda 1984). A statistical technique has been proposed to determine how well two methods agree, when neither gives an absolute measurement (Bland and Altman 1986). A recent study, using this method of analysis, has assessed the relative accuracy of the more common Doppler estimations with thermodilution (Dubin, Wallerson, Cody and Devereux 1990). There was no significant difference between stroke volume and cardiac output measured by Doppler echocardiography and thermodilution, when the velocity time integral was derived from the maximum aortic velocity waveform, and the area was measured at the aortic valve annulus (trailing edge to leading edge method). Integration of the modal velocity systematically underestimated the thermodilution stroke volume. This may reflect the finding that maximum velocity

signals are not as sensitive to poor angulation as modal velocity signals (Gisvold and Brubakk 1982).

The Doppler method of measuring cardiac output has many advantages over conventional invasive techniques. It avoids the need for catheterisation, is safe, and allows repeated measurements to be made (Light 1969a; Colocousis, Huntsman and Curreri 1977; Ihlen *et al.* 1984; Haites *et al.* 1985). The Doppler technique is as accurate as conventional invasive methods at measuring cardiac output (Schuster and Nanda 1984; Northridge *et al.* 1990) and allows beat to beat changes in stroke volume to be determined (Eriksen and Walloe 1990). The use of a Doppler ultrasound transducer located on a flexible endoscope introduced transoesophageally allows the continuous monitoring of cardiac output during anaesthesia in humans (Perrino, Fleming and LaMantia 1990).

Doppler echocardiography has been used to measure cardiac output in dogs (Bonagura, Darke, Long and Haigh 1990) and anaesthetised foals (Fenger, Bertone and Bonagura 1991). Good correlations have been recorded with thermodilution but the absolute accuracy of the technique was not reported.

Haemodynamic monitoring in veterinary medicine is often based on the mistaken assumption that an adequate blood pressure represents an adequate cardiac output. The tendency to resort to this simplistic approach is probably a reflection of the complex and invasive nature of cardiac output determinations. A noninvasive method of measuring cardiac output in horses would be valuable in the assessment of cardiac performance in horses with cardiovascular disease, and would enable continuous monitoring of critically ill horses and those under general anaesthesia.

DOPPLER ECHOCARDIOGRAPHIC ASSESSMENT OF VENTRICULAR SYSTOLIC FUNCTION

In addition to measurement of cardiac output or stroke volume, comprehensive assessment of cardiac function requires an evaluation of the loading conditions of the heart and the contractility of the myocardium (Braunwald 1988). Assessment of contractility is valuable, as cardiac output is often maintained at rest, even when patients have severe cardiac disease (Rushmer 1964). Many indices purported to reflect changes in contractility, are also sensitive to changes in ventricular preload and afterload (Van den Bos, Elzinga, Westerhof and Noble 1973; Borow 1989). Most invasive indices of ventricular contractility are based on the rate of rise of ventricular pressure (dp/dt) or derived from changes in flow. However, the large number of invasive indices available indicates that none is entirely satisfactory (Milnor 1990).

Rushmer (1964) used the term "initial ventricular impulse" to describe the dynamic properties of ventricular ejection. Impulse is the product of force and time, with force defined as the product of mass and acceleration. Changes in ventricular impulse were demonstrated in dogs during exercise, general anaesthesia and following acute coronary occlusion. The ventricular impulse was considered potentially valuable in assessing ventricular performance, although it was shown to be preload dependent. The value of peak acceleration in assessing ventricular function was demonstrated in further canine studies. Following coronary artery occlusion, the peak acceleration was reduced despite maintenance of cardiac output (Rushmer, Watson, Harding and Baker 1963). At constant stroke volume, myocardial stimulation causes an increase in the maximum acceleration of blood from the canine left ventricle (Noble, Trenchard and Guz 1966). The maximum acceleration of blood flow in dogs is considered to be a sensitive index of myocardial contractile function (Noble, Trenchard and Guz 1966). Maximum acceleration and peak velocity of flow have been shown to be useful for assessing ventricular performance in humans with coronary artery disease (Bennett, Else, Miller, Sutton, Miller and Noble 1974).

However, these indices have failed to differentiate between patients, grouped according to left ventricular function by conventional invasive indices (Kolettis, Jenkins and Webb-Peploe 1976).

Doppler echocardiography allows peak flow velocity and acceleration to be recorded noninvasively. Various studies have compared the Doppler flow indices with conventional invasive indices of ventricular function under conditions of changing preload and afterload.

Peak flow velocity is inversely related to afterload (Bedotto, Eichhorn and Grayburn 1989; Harrison, Clifton, Berk and DeMaria 1989) and systemic vascular resistance (Elkayam *et al.* 1983). However, it is sensitive to changes in inotropic state over a range of increasing arterial blood pressures (Wallmeyer, Wann, Sagar, Csakanski, Kalbfleisch and Klopfenstein 1988). Although changes in peak flow velocity are inversely related to changes in systemic vascular resistance, absolute measurements are not related (Elkayam *et al.* 1983). Peak flow velocity is influenced by a number of factors. It is dependent on the force generated by the contracting myocardium (Elkayam *et al.* 1983; DeMaria, Smith and Harrison 1989), but is also influenced by the reduction in cross sectional area from left ventricle to aorta at the time of peak blood velocity (Isaaz, Ethevenot, Admant, Brembilla and Pernot (1990). Therefore patients with depressed contractility may still maintain peak velocity within a normal range if the ventricle is dilated (Isaaz *et al.* 1990).

Peak velocity is directly related to changes in preload (Bedotto, Eichhorn and Grayburn 1989) and stroke volume. The relationship with stroke volume is linear until high cardiac outputs are obtained, when the peak velocity reaches a plateau and stroke volume is augmented by increases in the ejection time (Colocousis, Huntsman and Curreri 1977). An increase in the ejection time maintains the velocity time integral when the peak velocity is reduced by an increase in afterload (Harrison *et al.* 1989).

Peak and mean acceleration are inversely related to afterload (Bedotto, Eichhorn and Grayburn 1989; Harrison *et al.* 1989) and are directly related to preload (Bedotto, Eichhorn and Grayburn 1989). However, moderate increases in preload, which increase the stroke volume, cause only a limited increase in maximum acceleration compared to the large increases which occur following the administration of inotropes (Bennett, Barclay, Davis, Mannering and Mehta 1984). In anaesthetised dogs, the peak acceleration of blood flow is less responsive to changes in afterload than to changes in inotropic state (Stein and Sabbah 1976). The mean acceleration of flow also remains sensitive to changes in inotropic state over a range of increasing arterial blood pressures (Wallmeyer *et al.* 1988).

The mean acceleration (peak velocity divided by the time to peak velocity) may be less sensitive to changes in inotropic state than the maximum acceleration, measured directly from the Doppler signal (Wallmeyer, Wann, Sagar, Kalbfleisch and Klopfenstein 1986). Small errors in measurement of the acceleration time result in relatively large errors in calculation of the mean acceleration (Gardin *et al.* 1983). Measurement of the true maximum aortic acceleration requires special instrumentation that differentiates the initial portion of the Doppler signal (Bedotto, Eichhorn and Grayburn 1989). Most modern, commercially available, Doppler equipment does not allow this. The mean acceleration can be determined from Doppler waveforms by measuring from zero velocity to the peak velocity (Bedotto, Eichhorn and Grayburn 1989). Alternatively the peak or maximal acceleration can be measured from the maximum upstroke of the Doppler signal (Harrison, Smith, Friedman and DeMaria 1987). This does not represent the maximum acceleration at the onset of ventricular ejection (Bedotto, Eichhorn and Grayburn 1989).

The velocity time integral, an index strongly related to stroke volume (Mehta and Bennett 1986), is sensitive to changes in preload (Bedotto, Eichhorn and Grayburn 1989).

The time to peak velocity, although preload and heart rate dependent correlates with the maximum rate of rise of ventricular pressure during changes in afterload (Berman and Alverson 1985). The peak velocity and maximal acceleration were shown to correlate with the invasive index dp/dt max, during changes in preload, heart rate and myocardial contractility, but not during increases in afterload (Berman and Alverson 1985). It is suggested therefore, that the combined use of these noninvasive indices will provide as much information on ventricular performance as the most commonly used invasive index of ventricular performance, dp/dt max (Berman and Alverson 1985).

The relative sensitivity of the peak velocity of flow and the maximum acceleration to changes in ventricular performance is not clear. Some authors consider the maximum acceleration to be the most sensitive indicator of ventricular performance (Noble, Trenchard and Guz 1966; Sabbah, Khaja, Brymer, McFarland, Albert, Snyder, Goldstein and Stein 1986; Stein and Sabbah 1976), whereas others consider the peak flow velocity to be more indicative of changes in ventricular performance (Gardin *et al.* 1983; Wallmeyer *et al.* 1988). Some authors consider both indices to be equally valuable (Bennett *et al.* 1973), although the mean acceleration is subject to greater variability (Wallmeyer *et al.* 1986).

The systolic time intervals (pre-ejection period and ejection time), have also been used to assess changes in ventricular performance (Borow 1989). The ejection time is heart rate dependent (Elkayam *et al.* 1983) and is influenced by changes in preload, afterload, stroke volume and contractile state (Borow 1989). The ventricular ejection time shortens in patients with cardiomyopathy (Riggs, Hirschfeld, Borkat, Knoke and Liebman 1977), but it is also shortened in patients with reduced forward stroke volume, due to mitral regurgitation (Borow 1989). High cardiac output states such as aortic insufficiency cause an increase in the ejection time (Borow 1989). The ratio of right ventricular pre-ejection period to ejection time has been shown to be independent of heart rate (Riggs *et al.* 1977), although it remains dependent on

preload, afterload and contractility (Borow 1989). This ratio is used to evaluate patients with pulmonary hypertension (Senecal, Weyman, Pyhel, Dillon, Feigenbaum and Stewart 1977), indicating its sensitivity to changes in afterload. The pre-ejection period is inversely related to heart rate (Riggs *et al.* 1977) and contractility (Talley, Meyer and McNay 1971). It is also related to preload and the duration of intraventricular conduction (Borow 1989).

Despite the load dependence of Doppler ejection phase indices, they have proved useful in distinguishing patients with normal left ventricular function from those with global dysfunction (Gardin, Iseri, Elkayam, Tobis, Childs, Burn and Henry 1983; Sabbah *et al.* 1986). They have proved useful for monitoring clinical response to therapy (Elkayam *et al.* 1983; Bennett *et al.* 1984; Sabbah, Gheorghiade, Smith, Frank and Stein, 1988), and are of value in assessing prognosis in patients with acute myocardial infarction (Mehta and Bennett 1986). Doppler indices do not measure contractility directly (Bedotto, Eichhorn and Grayburn 1989) and therefore cannot be used to compare contractility between individuals. They are sensitive to changes in ventricular systolic function (Stein and Sabbah 1976), and are no more influenced by changes in preload, afterload and heart rate than are conventional invasive indices (Wallmeyer *et al.* 1986; Wallmeyer *et al.* 1988). Doppler indices of ventricular function appear to be equally sensitive or in some instances more sensitive than invasive indices in detecting changes in left ventricular function resulting from myocardial ischaemia (Mathias, Wann, Sagar and Klopfenstein 1988). They also provide an insight into the mechanism and haemodynamic effects of therapeutic agents (Harrison, Smith, Nissen, Grayburn and DeMaria 1988; Harrison, Clifton and DeMaria 1991).

The advantage of Doppler indices of ventricular function over M-mode indices, is that the former reflect global ventricular function rather than regional function which may be influenced by local wall motion abnormalities (Gardin *et al.* 1983).

AIMS OF PART 2 OF THIS WORK

1) To determine the accuracy of Doppler echocardiography to measure cardiac output in horses.

2) To assess whether Doppler flow indices are sensitive to changes in inotropic state in horses.

CHAPTER 2. MATERIALS AND METHODS

HORSES

The horses studied in Part 2 of this thesis fulfilled the entry criteria of the control group described in Part 1. The group studied by thermodilution and Doppler echocardiography consisted of nine adult horses, two mares, seven geldings weighing between 381kg and 630kg (mean 516kg).

MEASUREMENT OF CARDIAC OUTPUT

Thermodilution Technique

Cardiac output was measured by the thermodilution technique using a Cardiomax II cardiac output computer (Columbus Instruments)[a] (Figure 47). In this technique a known volume of cold dextrose saline is injected into the right atrium. A temperature sensor positioned in the pulmonary artery monitors the change in blood temperature caused by dilution of the injectate. The computer records the temperature changes over time and integrates the area under the dilution curve. Cardiac output (CO) is calculated from the following equation

$$CO = \frac{(\text{Blood Temp} - \text{Injectate Temp}) \times \text{Injectate volume}}{\text{Integral of the dilution curve}}$$

A calorific difference of at least 100 Calories is required to obtain adequate thermodilution curves. The calorific difference is calculated as follows.

Calorific difference = (Blood Temp - Injectate Temp) x Injectate volume

The thermistor used to record changes in blood temperature, is located near the tip of a 7 french Swan-Ganz catheter (Columbus instruments)[a]. The catheter also has a proximal port for the injection of cold indicator. However, in vitro studies demonstrated that it took twelve seconds to inject 45 millilitres of indicator through the narrow lumen of the Swan-Ganz catheter using a pressure injector (pressure 30 pounds per square inch). This injection time was considered too long to ensure

a Columbus Instruments. North Hague Avenue, Columbus, Ohio, U.S.A.

optimal thermodilution estimations. Injection of the indicator through a wider bore (1.6mm) polyethylene catheter using a pressure injector at the same pressure, allowed the injection of 45 millilitres of indicator in three seconds. Injection by hand doubled the injection time. Consequently in all subsequent studies, the thermal indicator was injected, by a pressure injector, through a polyethylene catheter placed in the right atrium.

A pilot study in one horse, using iced indicator, caused shivering and piloerection after four thermodilution estimations. The volume of injectate administered, did not cause a fall in the core temperature of the horse, but may have caused a reduction in temperature of carotid arterial blood, leading to central stimulation of thermoreceptors. In all subsequent studies the thermal indicator was kept at room temperature which still ensured an adequate calorific difference. The room temperature was measured directly by the Cardiomax II through a platinum probe (Figure 47). During the experimental procedures the recorded room temperature varied between 12^{o}C and 15^{o}C.

To facilitate the introduction of catheters, the horses were lightly sedated using 0.3mg/kg xylazine (Rompun)[b] immediately prior to catheterisation. Both catheters were inserted into the jugular vein under local anaesthesia, using a percutaneous Seldinger technique. The catheters were introduced through an 8.5 French percutaneous sheath introducer set[c]. The polyethylene catheter was advanced into the right atrium for injection of the cold indicator. The catheter had multiple terminal side ports to promote adequate mixing of the indicator and to reduce jet formation during injection. The 7 French Swan-Ganz catheter was advanced into the pulmonary artery. Correct placement of the catheters was verified by analysis of the pressure waveforms and by two-dimensional echocardiography. Pressure waveforms were obtained by connecting the catheters via a fluid line to a Bell and Howell strain

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b Rompun. Bayer U.K. Limited, Eastern Way, Bury St. Edmunds, Suffolk.
c (CI-09800) Arrow International Inc.

gauge. The pressure waveforms were recorded on a strip chart recorder (Lectromed Multitrace 2)[d] (Figure 47).

The self test feature of the Cardiomax II was performed prior to each study to confirm that the equipment was functioning within the manufacturers specifications.

The indicator volume used throughout the study was 45 millilitres. This volume was preset on the Cardiomax II using a thumb wheel selector (Figure 47). The sterile injectate (dextrose saline) was drawn into sterile syringes from fluid bags which were kept at room temperature. The volume of fluid injected by the pressure injector had been measured repeatedly prior to the experiments, using a calibrated measuring cylinder.

Cardiac output estimations were displayed digitally in litres per minute on the front panel of the Cardiomax II, for each output estimation (Figure 47). The dilution curve (Figures 48 and 49) for each cardiac output was recorded on the strip chart recorder. Thermodilution curves have a characteristic shape, a rapid downstroke, followed by a smooth decay. Cardiac output estimations from abnormally shaped dilution curves were not accepted and the estimation was repeated.

Thermodilution injections were not timed with respiration or with the cardiac cycle, except where horses showed second degree atrioventricular block. In these horses the injection of indicator was timed to coincide with the first conducted P wave following the blocked beat.

On completion of the study, the catheters and introducers were removed and heamostasis was achieved by manual pressure.

Doppler echocardiographic technique

The mean velocity time integral (VTI) was computed by manual planimetry with electronic calipers. Five consecutive signals were averaged to allow for respiratory variation. Heart rate was calculated from ten consecutive R-R intervals.

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d Lectromed U.K. Limited. Welwyn Garden City, Letchworth, Herts. U.K.

Poor quality traces and signals from premature ventricular contractions were not measured.

Doppler spectra from the pulmonary artery were enhanced by the injection of the cold indicator (Figure 50). Therefore five Doppler waveforms immediately prior to the thermodilution injection, and five enhanced waveforms, were measured. Heart rate was calculated from the average R-R interval of the ten beats. This heart rate was used to estimate cardiac output from both the enhanced and normal signals.

The diameter of the aorta and pulmonary artery were measured from a two-dimensional image recorded at the start of each study period. The aortic diameter was measured from a right parasternal long-axis view (Figure 2c) and from an M-mode derived from a right parasternal short-axis view (Figures 3d and 9). The aortic diameter was measured from the long-axis image at the following locations (Figure 51);

1) The sinus of Valsalva
2) The valve tips
3) The ascending aorta.

Measurements from locations 2 and 3 were made by the leading edge to leading edge method, and by the trailing edge to leading edge method. Measurements from the sinus of Valsalva were made by the leading edge to leading edge method. A further measurement was taken from an M-mode image derived from a cross-section of the vessel which included the valve leaflets.

The pulmonary artery was measured from a right parasternal angled view (Figure 4). The diameter of the pulmonary artery was measured at the level of the valve tips in systole. The diameter was measured as follows (Figure 52);

1) Including both walls
2) Including the right wall
3) Including neither wall.

Three measurements were taken during systole from three cardiac cycles. The average of these nine measurements was then used to calculate the area of the vessel using the following equation

$$\text{Vessel area} = 3.14 \times \text{radius}^2$$

Cardiac output by Doppler echocardiography was calculated as follows

$$\text{Cardiac output} = \text{VTI} \times \text{vessel area} \times \text{heart rate.}$$

CHAPTER 3. ASSESSMENT OF VENTRICULAR FUNCTION BY DOPPLER ECHOCARDIOGRAPHY

INTRODUCTION

In Part 1 of this thesis normal Doppler measurements were established for a group of Thoroughbred or Thoroughbred cross horses with no evidence of cardiovascular disease. These measurements were compared with data from five groups of horses with valvular disease. No attempt was made to assess ventricular function in these horses by Doppler echocardiography.

In Part 2 of this thesis cardiac output measured by Doppler echocardiography will be compared to that measured by an invasive thermodilution technique. The effect of inotropes on Doppler indices of ventricular function will be studied.

METHODS

Nine horses were studied. Doppler waveforms were recorded from the aorta and pulmonary artery, simultaneous to the injection of cold indicator for measurement of cardiac output as described in the previous chapter. Cardiac output was manipulated by the administration of drugs, to compare the relative accuracy of the measurement techniques over a wide range of outputs. Cardiac output was increased by the infusion of two inotropes (dopamine[e] and dobutamine[f]), and was decreased by the administration of the sedative combination detomidine[g] and butorphanol[h]. Cardiac output was measured; a) with the horses standing quietly, b) during the intravenous infusion of 4μg/kg/min dopamine, c) during the intravenous infusion of 4μg/kg/min dobutamine and d) following the intramuscular administration of a combination of 10μg/kg detomidine and 20μg/kg butorphanol. Inotropes were administered for at least ten minutes prior to the simultaneous Doppler and

e Intropin. Du pont (UK) Limited, Wedgwood way, Stevenage, Hertfordshire.
f Dobutrex. Eli Lilly and Company Limited, Kingsclere Road, Basingstoke, Hampshire.
g Domosedan. Smithkline Beecham Animal Health, Walton Oaks, Dorking Road, Tadworth, Surrey.
h Torbugesic. Willows Francis Veterinary, 3 Charlwood Court, County Oak Way, Crawley, West Sussex.

thermodilution recordings, and ten minutes elapsed following the injection of detomidine and butorphanol. The administration of inotropes was discontinued for at least ten minutes before the onset of the next treatment. Six thermodilution estimations were performed in each horse during each study period. Three estimations coincided with Doppler recordings taken from the pulmonary artery and three coincided with Doppler recordings from the aorta.

M-mode studies of the left ventricle were recorded from the right parasternal short-axis view, chordal level. The left ventricular internal dimension was measured at end diastole, as described in Chapter 4. The average value from five consecutive cardiac cycles was used for statistical analysis.

Doppler indices of ventricular function

To investigate the effects of inotropes and the sedative combination detomidine and butorphanol on Doppler indices of ventricular function, the following Doppler variables were measured during each study period;

1) Peak velocity
2) Peak acceleration
3) Time to peak velocity (acceleration time)
4) Pre-ejection period
5) Ejection time.

Measurements were made from five consecutive Doppler waveforms from the aorta and pulmonary artery. Doppler waveforms from the pulmonary artery were recorded immediately prior to the injection of dextrose saline used for thermodilution measurements. The average value of the five measurements was used in statistical analysis.

Statistical Analysis of Results

Scatter plots were produced of simultaneous Doppler and thermodilution cardiac output estimations to study the relation between the two techniques. The

presence of a linear relationship was determined by calculation of the product-moment correlation coefficient. Agreement between the two techniques was assessed by the methods of Bland and Altman (1986). The differences between the two methods were expressed as a percentage of the thermodilution estimations. Summary statistics (maximum, minimum and median) of the percentage differences were described.

Any significant differences in vessel diameter and left ventricular internal dimensions between study periods were assessed by a Kruskal-Wallace test. Differences between Doppler variables measured during different study periods were also determined by a Kruskal-Wallace test. Where significant differences were detected, a paired Wilcoxon Rank test was used to locate the differences between groups. A paired Wilcoxon Rank test was used to compare cardiac output measurements during the different study periods. The cardiac output measurements used for analysis were; thermodilution measurements made during the recording of Doppler pulmonary artery and aortic waveforms, and Doppler estimations of cardiac output using the leading edge aorta and the pulmonary artery both walls (before injection) methods.

RESULTS

The cardiac output measured by thermodilution, during Doppler studies of aortic flow, varied between 15.8 and 68.0 l/min (mean 37.6 l/min). This represented a mean cardiac index (cardiac output divided by bodyweight) of 72mls/kg/hr. During Doppler pulmonary artery recordings, the cardiac output measured by thermodilution, varied between 15.2 and 77.0 l/min (mean 37.96 l/min). Figures 53a to 64a show scatter plots of cardiac output estimations by the various Doppler methods, against thermodilution. The correlation coefficients of these relationships are detailed in Table 28. Doppler measurements of cardiac output from the aorta show a closer correlation to thermodilution, than those from the pulmonary artery. The correlations with thermodilution estimations were slightly improved when pulmonary artery

waveforms were measured before the injection of saline. The highest correlation using Doppler pulmonary artery waveforms was achieved when the measured vessel diameter included the thickness of both vessel walls. The highest correlations for aortic waveforms occurred when the vessel diameter was measured by the leading edge method from the ascending aorta or from the sinus of Valsalva.

Data were omitted from six horses as follows: Doppler pulmonary artery waveforms were not measured in five horses during various study periods, due to the poor quality of spectral Doppler signals. Twelve data points were omitted from studies prior to the thermodilution injection, and eight data points were omitted from studies during the thermodilution injection. Six data points were omitted from two horses, in which poor aortic Doppler waveforms were recorded.

Figures 53b to 64b show the differences in cardiac output estimations plotted against the mean of the two techniques, according to the methods of Bland and Altman (1986). Summary statistics of these differences are given in Table 29. The limits of agreement of the two techniques, that is the mean difference +/- 1.96 x the standard deviation of the differences, are recorded in Table 30. The limits of agreement between the two techniques were wider when cardiac output by Doppler echocardiography was estimated from the pulmonary artery rather than from the aorta. The pulmonary artery estimations showed similar limits of agreement both before and during the injection of saline. The closest agreement between techniques occurred when cardiac output was estimated from Doppler aortic waveforms, with the vessel diameter measured by the leading edge method from the ascending aorta.

The 95% confidence intervals, for the mean of the differences between the two techniques (bias), shows that when aortic diameter was measured from the sinus of Valsalva or from an M-mode image, Doppler echocardiography overestimated the cardiac output compared to thermodilution (Table 30). There was no significant bias between the two techniques when the aortic diameter was measured from the ascending aorta using the leading edge method. All other Doppler methods from the

aorta resulted in underestimation of cardiac output compared to thermodilution (Table 30). Doppler cardiac output estimations from the pulmonary artery showed a significant underestimation of cardiac output compared to thermodilution, when pulmonary artery diameter was measured by the inner edge method. Cardiac output by Doppler echocardiography was also underestimated compared to thermodilution when the pulmonary artery measurement only included the right vessel wall. Inclusion of both vessel walls in the measurement of the diameter did not cause a significant bias in the estimation of cardiac output compared to thermodilution.

The differences between measurements by Doppler and by thermodilution increased with increasing cardiac output for all Doppler techniques (Figures 53b to 64b). Summary statistics of the differences, expressed as a percentage of the thermodilution measurements, are detailed in Table 31. Doppler measurements of cardiac output, using the leading edge diameter at the ascending aorta, overestimated the thermodilution measurement by a maximum of 36% and underestimated the thermodilution by a maximum of 31%. The median value showed no difference between the two techniques. Comparison of the differences between thermodilution measurements and Doppler measurements from the pulmonary artery (both walls), showed that Doppler echocardiography overestimated the thermodilution measurement by a maximum of 114% and underestimated thermodilution by a maximum of 37%. The median difference between the two techniques expressed as a percentage of the thermodilution measurement was 3%. The percentage differences of the other Doppler measurement techniques compared to thermodilution, were between those reported for the leading edge aorta method and the pulmonary artery (both walls) method (Table 31).

The vessel diameters were not significantly different during the different study periods (Table 32). The left ventricular internal dimension was only recorded in six horses, due to the limitation of imaging depth. There was no significant differences in this measurement during the different study periods (Table 32).

Table 28. Correlation coefficients and probability values for the relationship between Doppler estimations of cardiac output and thermodilution. N = number of cardiac output estimations by both techniques. A significant correlation ($p<0.05$) is denoted * and printed boldly.

Aorta

2-D measurement	N	Correlation	p	Graph No.
Leading edge	**102**	**0.89**	**0.0000 ***	**53a**
Inner edge	**102**	**0.86**	**0.0000 ***	**54a**
Leading valve	**102**	**0.87**	**0.0000 ***	**55a**
Trailing valve	**102**	**0.87**	**0.0000 ***	**56a**
Sinus	**102**	**0.89**	**0.0000 ***	**57a**
M-mode	**102**	**0.87**	**0.0000 ***	**58a**

Pulmonary Artery

2-D measurement	N	Correlation	p	Graph No.
Before Injection				
Both walls	**97**	**0.77**	**0.0000 ***	**59a**
Right wall	**97**	**0.75**	**0.0000 ***	**60a**
Inner edge	**97**	**0.74**	**0.0000 ***	**61a**
During Injection				
Both walls	**98**	**0.76**	**0.0000 ***	**62a**
Right wall	**98**	**0.71**	**0.0000 ***	**63a**
Inner edge	**98**	**0.75**	**0.0000 ***	**64a**

Table 29. Summary statistics of the differences between cardiac output measurements by thermodilution and Doppler echocardiography (litres per minute). N = number of cardiac output estimations by both techniques. MIN = minimum difference between measurements. MAX = maximum difference between measurements. S.D. = Standard deviation of the differences between measurements.

Aorta

Two-dimensional Measurement	N	Mean	S.D.	MIN	MAX	1.96 x S.D.
Leading edge	102	-0.43	6.26	-20.00	14.56	12.26
Inner edge	102	4.11	6.72	-14.40	19.98	13.17
Leading valve	102	4.01	6.61	-13.00	17.95	12.95
Trailing valve	102	8.65	6.84	-4.34	24.69	13.41
Sinus	102	-12.27	8.07	-39.19	8.15	15.82
M-mode	102	-9.17	7.71	-30.00	6.48	15.11

Pulmonary Artery

Two-dimensional Measurement	N	Mean	S.D.	MIN	MAX	1.96 x S.D.
Before Injection						
Both walls	97	-2.07	9.56	-26.64	23.90	18.74
Right wall	97	4.78	9.66	-18.64	34.01	18.93
Inner edge	97	11.64	9.55	-7.93	41.02	18.72
During Injection						
Both walls	98	-1.08	8.88	-26.62	19.00	17.40
Right wall	98	4.73	9.94	-31.95	30.09	19.48
Inner edge	98	11.54	9.38	-8.31	37.78	18.38

Table 30. Confidence intervals for the bias between Doppler and thermodilution techniques for measurement of cardiac output (litres per minute). Limits of agreement represent the mean difference +/- 1.96 x the standard deviation.

Aorta

Two-dimensional Measurement	N	Mean	95% Confidence Intervals of mean	Limits of agreement
Leading edge	102	-0.43	-1.67 to 0.81	-12.69 to 11.83
Inner edge	102	4.11	2.77 to 5.45	-9.30 to 17.28
Inner valve	102	8.65	7.29 to 10.01	-4.76 to 21.82
Outer valve	102	4.01	2.71 to 5.31	-8.94 to 16.96
Sinus	102	-12.27	-13.87 to -10.67	-28.09 to 3.55
M-mode	102	-9.17	-10.71 to -7.63	-24.28 to 5.94

Pulmonary Artery

Two-dimensional Measurement	N	Mean	95% Confidence Intervals of mean	Limits of agreement
Before Injection				
Both walls	97	-2.07	-4.01 to -0.13	-20.81 to 16.67
Right wall	97	4.78	2.82 to 6.74	-14.15 to 23.71
Inner edge	97	11.64	9.70 to 13.58	-7.08 to 30.36
During Injection				
Both walls	98	-1.08	-3.54 to 0.06	-19.20 to 15.60
Right wall	98	4.73	2.73 to 6.73	-14.75 to 24.21
Inner edge	98	11.54	9.64 to 13.44	-6.84 to 29.92

Table 31. Differences between Doppler measurements from the aorta and thermodilution, expressed as a percentage of the thermodilution measurements. N = number of cardiac output estimations by the two techniques. Median = Median measurements. Max % Overest. = Maximum percentage overestimation. Max % Underest. = Maximum percentage underestimation.

Aorta

2-D measurement	N	Median	Max % Overest.	Max % Underest.
Leading edge	102	0	36	31
Inner edge	102	13	43	39
Leading valve	102	12	50	37
Trailing valve	102	24	13	48
Sinus	102	-34	95	33
M-mode	102	-26	79	12

Table 31 (continued). Differences between Doppler measurements from the pulmonary artery and thermodilution, expressed as a percentage of the thermodilution measurements. N = number of cardiac output estimations by the two techniques. Median = Median measurements. Max % Overest. = Maximum percentage overestimation. Max % Underest. = Maximum percentage underestimation.

Pulmonary artery

2-D measurement	N	Median	Max % Overest.	Max % Underest.
Before Injection				
Both walls	97	-3	114	37
Right wall	97	12	79	46
Inner edge	97	29	37	58
During Injection				
Both walls	98	-2	112	40
Right wall	98	16	79	51
Inner edge	98	31	39	61

Table 32. Median values for vessel diameters and left ventricular internal dimensions during the four study periods. 1 = control, 2 = dopamine, 3 = dobutamine, 4 = detomidine and butorphanol. Kruskal-Wallace tests revealed no significant differences in vessel diameters or ventricular dimensions during the different study periods, p values are given in column five.

Aorta

Vessel Diameter	Median 1	Median 2	Median 3	Median 4	Kruskal-Wallace
Leading edge	7.815	7.900	8.170	7.980	0.891
Inner edge	7.235	7.400	7.550	7.250	0.839
Leading valve	7.410	7.240	7.310	7.310	0.856
Trailing valve	7.130	6.890	6.900	6.950	0.795
Sinus of Valsalva	9.285	9.400	9.150	9.160	0.990
M-mode	8.920	8.900	9.020	8.935	0.959

Pulmonary artery

Vessel Diameter	Median 1	Median 2	Median 3	Median 4	Kruskal-Wallace
Both walls	7.840	8.190	7.695	7.690	0.224
Right wall	7.220	7.410	6.940	7.020	0.473
Inner edge	6.39	6.720	6.170	6.390	0.427

Left ventricle

Dimension	Median 1	Median 2	Median 3	Median 4	Kruskal-Wallace
LVIDd	12.01	12.20	10.97	11.20	0.853

Doppler indices of ventricular function

Figures 65 to 70 show the effects of the different inotropes, and the sedative combination detomidine and butorphanol on specific Doppler variables. The mean values and results of statistical analyses are listed in Appendix 20. The peak velocity (VMax) and the peak acceleration (dv/dt) of flow in the aorta (Figures 65 and 66) and pulmonary artery were highest during the infusion of dobutamine. Figure 71 shows Doppler spectra, recorded during the dobutamine infusion. The peak aortic acceleration and peak velocity were also significantly higher during the infusion of dopamine, than during the control period (Figures 65 and 66). There were no significant differences in the peak velocity and peak acceleration of flow in the pulmonary artery, between the infusion of dopamine and during the control period. Following the administration of detomidine and butorphanol the peak velocity and peak acceleration of aortic (Figures 65 and 66) and pulmonary flow, decreased significantly compared to the control period. Figure 72 shows Doppler spectra recorded from the aorta after the administration of detomidine and butorphanol.

The aortic velocity time integral was not significantly changed by the infusion of the inotropes. However following the administration of detomidine and butorphanol, the velocity time integral was significantly decreased (Figure 67). The aortic velocity time integral showed a weak but statistically significant correlation with cardiac output measured by thermodilution ($r = 0.47$). No significant differences were detected in the pulmonary velocity time integral during any study period.

The aortic (Figure 68) and pulmonary acceleration times were significantly longer following the administration of detomidine and butorphanol, and were significantly shorter during the infusion of dobutamine than during the control period or during the infusion of dopamine.

Dobutamine infusion caused a significant decrease in the pulmonary and aortic pre-ejection period and ejection time compared to the control values (Figures 69 and 70). There was no significant difference in the aortic and pulmonary pre-

ejection period, during the control period and during dopamine infusion. However, the aortic ejection time was significantly reduced. The administration of detomidine and butorphanol resulted in a significantly longer aortic and pulmonary pre-ejection period and ejection time.

Heart rate changes during the different study periods

During the recording of Doppler aortic waveforms, the heart rate was significantly decreased following the administration of detomidine and butorphanol, but was not significantly different during the infusion of dobutamine. The heart rate was significantly increased during the infusion of dopamine. During the recording of Doppler pulmonary artery waveforms, the heart rate was significantly decreased during the infusion of dobutamine, compared to control values. This decrease in heart rate was not as great as that which followed the administration of detomidine and butorphanol. The infusion of dopamine caused a significant increase in heart rate.

Cardiac output measurements during the different study periods

Cardiac output measured by Doppler echocardiography and thermodilution during the different study periods is shown in Table 33. Cardiac output measured by thermodilution and Doppler echocardiography during the recording of aortic flow velocities, was significantly increased during the infusion of dopamine and dobutamine compared to control values. Following the administration of detomidine and butorphanol, cardiac output was significantly decreased. There was no difference between cardiac output measurements during the infusion of dobutamine and dopamine.

Cardiac output measured by thermodilution and Doppler echocardiography during the recording of pulmonary flow velocities, was significantly increased during the infusion of dopamine compared to control values. There was no difference in cardiac output during the infusion of dobutamine compared to the control period. Administration of detomidine and butorphanol significantly decreased cardiac output.

Table 33. Cardiac output data by thermodilution and Doppler echocardiography (leading edge aorta and pulmonary artery before infusion both walls) during Doppler recordings of the aortic and pulmonary outflow, for study periods 1 to 4. 1 = control period, 2 = dopamine infusion, 3 = dobutamine infusion, 4 = detomidine and butorphanol administration. N = number of horses. Med = median measurement. Min = minimum measurement. Max = maximum measurement. Significant differences between groups were determined by a Wilcoxon test. p values in collumn 1 VS represent comparisons between group 1 and the horizontal groups. p values in collumn 2 VS represent comparisons between group 2 and the horizontal groups and collumn 3 VS represents comparisons between group 3 and group 4. * denotes a significant difference between groups.

Cardiac output during Doppler recordings from the aortic outflow.

Group	N	Med	Min	Max	Wilcoxon Test		
					1 VS	2 VS	3 VS
Thermodilution							
1	27	37.20	22.3	54.8	-	-	-
2	27	43.40	26.9	64.0	**0.000***	-	-
3	27	48.90	22.2	68.0	**0.006***	0.819	-
4	27	21.90	22.2	48.4	**0.000***	**0.000***	**0.000***
Doppler echocardiography (leading edge aorta)							
1	26	39.69	22.11	53.83	-	-	-
2	25	48.38	26.02	66.92	**0.011***	-	-
3	24	44.71	15.56	74.53	**0.027***	0.294	-
4	27	24.33	14.09	34.21	**0.000***	**0.000***	**0.000***

Table 33 (continued).

Cardiac output during Doppler recordings from the pulmonary outflow.

Group	N	Med	Min	Max	Wilcoxon Test 1VS	2VS	3VS
Thermodilution							
1	27	35.70	21.4	60.8	-	-	-
2	27	46.80	27.6	77.0	**0.004***	-	-
3	27	42.10	21.2	74.9	0.216	**0.034***	-
4	27	22.10	15.2	38.6	**0.000***	**0.000***	**0.000***
Doppler echocardiography (Before injection both walls)							
1	24	41.36	33.16	59.24	-	-	-
2	24	52.52	27.04	79.86	**0.017***	-	-
3	23	38.47	19.38	32.95	0.097	**0.001***	-
4	21	25.09	13.38	17.59	**0.000***	**0.000***	**0.003***

DISCUSSION

Doppler estimations of cardiac output showed a close linear relationship with thermodilution estimations. The correlations between thermodilution measurements and Doppler echocardiographic measurements of cardiac output are similar to those reported in previous human studies (Schuster and Nanda 1984; Dubin *et al.* 1990).

The best correlation with thermodilution was achieved when Doppler recordings were made from the aorta, with vessel diameter measured by the leading edge method at the sinus of Valsalva or ascending aorta. However, measurement of the vessel diameter at the sinus of Valsalva resulted in the poorest agreement with thermodilution of any of the Doppler aortic estimations. Calculation of the vessel area from this diameter caused a significant bias, with cardiac output consistently overestimated compared to thermodilution. Measurement of vessel diameter at the sinus of Valsalva overestimates the flow area, as flow is funnelled by the valve leaflets. Human studies have suggested that the actual flow area in the aortic root is much less than the anatomical area, due to the formation of a central core of flow (Ihlen *et al.* 1984). The M-mode measurement also showed a large bias. This would be expected, as the M-mode image was derived from a cross-section of the aorta at the level of the valve and the diameter was measured by the leading edge method from the vessel walls. Overestimation of the flow area will cause overestimation of the cardiac output by Doppler echocardiography. This will result in a consistent bias between Doppler and thermodilution estimations, when vessel diameter is measured at the sinus. However this does not explain the poor agreement which is obtained if vessel area is calculated from this site. Differences in the agreement between the different Doppler methods of cardiac output estimation and thermodilution, must be related to the measurement of the vessel diameter, as the velocity time integral and heart rate were the same for each group of measurements. Doppler estimations, with vessel diameter measured from the M-mode image, also showed wide limits of agreement when compared to thermodilution. The wide limits of agreement which

occur when aortic diameter is measured at the level of the sinus may reflect poor repeatability in obtaining this image. They may also reflect differences in the relationship between the anatomic and actual flow areas in different individuals.

Measurement of the vessel diameter at the ascending aorta by the leading edge method, resulted in the most accurate estimations of cardiac output. The limits of agreement were lower than with other Doppler methods, and there was no bias compared to measurement by thermodilution. The cardiac output estimation by Doppler using this diameter would be expected, in 95% of cases, to be within 12.62 litres of the thermodilution estimation.

The limits of agreement were wider for Doppler cardiac output estimations from the pulmonary artery than from the aorta. This may be due to increased difficulty in aligning the Doppler ultrasound beam with pulmonary blood flow compared to aortic flow. Poor alignment with flow by Doppler echocardiography would result in a consistent underestimation of flow velocity and therefore cardiac output. This would result in a significant bias between the two techniques, but it would not necessarily cause widening of the limits of agreement. The wide limits of agreement may be caused by differences in the alignment with flow between individual horses. Alignment with pulmonary flow may be less consistent between individuals than alignment with aortic flow. It is possible that the Doppler technique which agrees most closely with thermodilution differs in different individuals. This was not assessed in this study. The wider limits of agreement found with Doppler estimations from the pulmonary artery may also reflect the difficulty in measuring pulmonary artery diameter. This has also been reported in human echocardiography (Loeber, Goldberg and Allen 1984). Two-dimensional ultrasound equipment has poor lateral resolution compared to axial resolution. It is more difficult therefore to measure structures accurately that are aligned parallel to the ultrasound beam. The pulmonary artery diameter might have been measured more accurately from the

image of the right ventricular inlet/outlet, recorded from the left hemithorax (Figure 7a). In this image the vessel walls are perpendicular to the ultrasound beam.

All Doppler estimations of cardiac output from the pulmonary artery underestimated flow, except when the vessel area was calculated from a diameter which included the width of both vessel walls. It is likely that this measurement overestimated the vessel diameter, which offset the underestimation of flow caused by poor alignment of the ultrasound beam. Thermodilution has been shown to overestimate the actual flow, due to loss of the thermal indicator (Elkayam, Berkley, Azen, Weber, Geva and Henry 1983). It is therefore possible that Doppler methods which underestimate flow compared to thermodilution give a more accurate measure of actual blood flow.

The correlations with thermodilution estimations were slightly improved when pulmonary artery waveforms were measured before the injection of saline. However the limits of agreement were similar for waveforms measured before and during the saline injection. The injection of saline caused enhancement of the Doppler spectra, but this does not appear to cause overestimation of the velocity time integral.

Scatter plots of the differences between the two techniques against the mean of the two techniques (Figures 53b to 64b) show that the differences increase as cardiac output increases. This may be caused by increased error of the thermodilution technique at increased flow rates (Powner and Snyder 1978; Jebson and Karkow 1986). The cardiac index by thermodilution reported in this study (72mls/kg/min), is similar to that found by previous workers in conscious horses using the Fick principle, 75mls/kg/min (Zuntz and Hagemann 1892, quoted by Stewart 1897) and the dye dilution technique, 74mls/kg/min (Fisher and Dalton 1961).

Potential sources of error in this study

The volume of injectate was measured from a calibrated syringe. Although this is less accurate than measurement by weight, the volume of injectate was large

and any error in estimation of the volume would be relatively small (Pearl, Rosenthal, Nielson, Ashton and Brown 1986).

The errors associated with the use of the thermodilution technique are reduced if the average of three measurements is used (Stetz *et al.* 1982). This method was not used in this study, as the heart rate of the horses was very labile under normal resting conditions. The heart rate increased rapidly in response to external auditory stimuli even when the horses appeared calm. This prevented the assumption of steady state cardiac output in these horses and therefore it was not considered appropriate to average three consecutive thermodilution estimations. If this method had been used, this may have reduced the errors associated with thermodilution and resulted in closer agreement between the two techniques.

In cases with atrioventricular block, the thermodilution indicator was injected to coincide with the conducted beats. This may have caused overestimation of cardiac output, as flow during the conducted beats would be higher than during the blocked beats (Mackenzie, Haites and Rawles 1986). Thermodilution injections were made to coincide with the conducted beats, to minimise loss of thermal indicator during the non-conducted beats. Poor quality thermodilution curves were obtained when the thermodilution injection coincided with the onset of atrioventricular block.

Occasional premature beats occurred during thermodilution estimations. The Doppler spectra from these beats were excluded from analysis. Velocity time integrals from five Doppler spectra were used to indicate the mean flow velocity per minute. Inclusion of the velocity time integral from an isolated premature contraction would bias this estimation. However the premature contraction would contribute to the flow measured by the thermodilution technique. Isolated premature contractions have been reported in humans associated with the passage of thermodilution catheters (Forrester, Ganz, Diamond, McHugh, Chonette and Swan 1972). One horse developed a large number of premature contractions, often in series, following the insertion of the Swan-Ganz catheter. Two-dimensional echocardiography revealed

the tip of the catheter at the level of the pulmonary valve. The catheter was advanced further into the pulmonary artery, and the premature contractions ceased.

One horse developed occasional transient atrioventricular block following the injection of cold saline. The beat immediately following the injection was conducted, and the subsequent beat was blocked. The thermodilution curves appeared to be of suitable quality and therefore these estimations were not excluded. A transient bradycardia has been reported in a human patient immediately following thermodilution estimations with cold dextrose solution (Nishikawa and Dohi 1982). It was suggested that the transient bradycardia in this case was caused by local cooling of the sinus node by the cold injectate and stimulation of vagal receptors in the heart (Nishikawa and Dohi 1982). In humans, stimulation of atrial type B mechanoreceptors causes bradycardia via sympathetic inhibition and vagal stimulation (Milnor 1990). It is possible that similar receptors in horses may have caused the bradycardia observed in this study. Although the thermodilution curves appeared to be of good quality, the transient atrioventricular block may have resulted in the loss of thermal indicator and greater innaccuracy in the thermodilution recording. Transient atrioventricular block will alter the heart rate used for Doppler estimations. Small changes in heart rate cause large changes in the estimate of cardiac output by Doppler echocardiography.

Significant errors occur in the measurement of cardiac output by thermodilution during the rapid infusion of intravenous fluids. This is due to fluctuations in the baseline blood temperature (Wetzel and Latson 1985). In the present study dopamine and dobutamine were administered in normal saline, as intravenous infusions. This is unlikely to have caused significant errors in thermodilution estimations, as the volume of the infusion was low (0.016mls/kg/min dobutamine, 0.008mls/kg/min dopamine). Furthermore the infusion rate had remained constant for ten minutes prior to the first thermodilution measurement, allowing time for the baseline temperature to stabilise (Wetzel and Latson 1985).

Errors associated with indicator loss prior to injection were minimised in this study by the use of injectate at room temperature (Evonuk *et al.* 1960). A large volume of indicator was used to improve the signal to noise ratio (Nadeau and Noble 1986). A previous study in horses comparing the thermodilution technique to the dye dilution technique, demonstrated significant differences between the two techniques when volumes of less than 20ml of iced saline were used (Muir, Skarda and Milne 1976). The use of volumes of 30 to 50 ml iced saline resulted in no significant differences between the two techniques. When room temperature injectates were used, significant differences were found between dye dilution techniques at all injectate volumes (10 to 50 millilitres). However, the temperature of the room was high (27°C), and the baseline blood temperature was not measured directly. Therefore, error may have been caused by baseline fluctuations in pulmonary artery temperature and by the use of a low thermal signal. Studies in dogs have demonstrated decreased loss of thermal indicator when an injectate of room temperature is used (Meisner, Glanert, Steckmeier, Gams, Hagl, Heimisch, Sebening and Messmer 1973). Studies in humans have shown no significant differences in the use of indicators at room temperature compared to iced indicators (Stetz *et al.* 1982; Shellock and Riedinger 1983). The use of 45ml of injectate at room temperature (12°C to 15°C) produced a calorific difference well above the minimum requirement of the cardiomax II cardiac output computer and the thermodilution curves appeared to be of good quality. In the present study the injectate was kept at room temperature, and the temperature of the room was measured. More accurate measurement of injectate temperature may have been achieved if the temperature probe had been placed in a bag of saline alongside the bag of injectate. However as the injectate was kept in the experimental room for at least two hours prior to each study, error in the measurement of injectate temperature should have been minimal.

Increased variability has been reported in cardiac output estimations by thermodilution in horses when 10, 20 or 50 ml of iced saline were used rather than 30

or 40 ml (Muir, Skarda and Milne 1976). These authors used a separate polyethylene catheter in the right atrium for injection of indicator, but injections were made manually. Hand injection of 45 ml of indicator through a similar catheter took six seconds and injection of 50 ml of indicator would take slightly longer. The longer the injection time, the greater would be the loss of thermal indicator to the surrounding tissues (Meisner *et al.* 1973). In the present study the use of a pressure injector ensured that the injection rate remained constant at three seconds.

When recording aortic outflow velocities from the left hemithorax, the aortic root moves towards the ultrasound transducer during systole. This will lead to underestimation of the actual flow velocity (Eriksen and Walloe 1990). The movement of the heart increased during the infusion of the inotropes which would cause an increased error at the higher flow rates.

Comparison with previous studies

There have been no previous studies to compare the accuracy of Doppler echocardiography for measurement of cardiac output in standing horses. In previous work in anaesthetised foals, a higher correlation between Doppler echocardiography and thermodilution was reported for measurement of cardiac output. A higher correlation was demonstrated for Doppler measurements from the aorta (r=0.92) than for measurements from the pulmonary artery (r=0.86). This is similar to the findings of the present study. The higher correlations found between measurement techniques in anaesthetised foals, may have resulted from more accurate measurement of cardiac output by Doppler echocardiography. Improved alignment of the Doppler ultrasound beam with blood flow may be possible in small foals compared to that achieved in larger horses. Restraint of the foals in lateral recumbency may also improve the ability to align the ultrasound beam with aortic and pulmonary blood flow. However, the accuracy of the Doppler technique was not discussed. The thermodilution estimations in the anaesthetised foals were calculated by averaging three recordings. This method has been shown to improve the accuracy and reproducibility of the

thermodilution technique (Stetz *et al.* 1982). This method would have been possible in anaesthetised animals due to the more stable heart rate. This may have resulted in an improved correlation between the two techniques. The foals were examined under general anaesthesia, following the administration of fluids and inotropes. The use of fluids and general anaesthesia may have resulted in a larger range of cardiac output over which the two techniques could have been evaluated. This will result in an improved correlation between the two techniques (Northridge *et al.* 1990). The higher correlation coefficients in the study on foals were achieved with continuous wave Doppler echocardiography. Pulsed wave Doppler echocardiography yielded a lower correlation. The use of continuous wave Doppler echocardiography would only allow the maximum velocity time integral to be determined. Improved accuracy has been shown between Doppler echocardiography and thermodilution recordings in humans, when the maximum rather than the modal velocity time integral is measured (Dubin *et al.* 1990).

Studies in humans comparing Doppler measurement of cardiac output with thermodilution have reported limits of agreement (mean difference + 1.96 x S.D) of -31% to +33% of the mean cardiac output (Northridge *et al.* 1990). This is similar to the results of the present study, the limits of agreement for Doppler (leading edge aorta) measurements expressed as a percentage of the mean cardiac output by thermodilution was +/-32.6%). The differences between Doppler (leading edge aorta) and thermodilution measurements expressed as a percentage of the mean of the two techniques gave a maximum range of -46% to +46% (mean difference + 1.96 x S.D. = +/- 34%). The Doppler method (leading edge aorta) showed a maximum range of -36% to +31% when the differences between the two techniques were expressed as a percentage of thermodilution. Recent studies in humans showed that the mean cardiac output measurement by thermodilution was 31% greater than that by dye dilution (Russell, Smith, West, Aylward, McRitchie, Hassam, Minson, Wing and Chalmers 1990). As thermodilution and dye dilution are based on the same principle

and are subject to similar errors (Mackenzie, Haites and Rawles 1986), such a difference between these two techniques is surprising.

Effect of the drug treatments on Doppler flow variables.

The increase in the peak velocity and peak acceleration of blood flow, during the infusion of dobutamine, may be due to changes in ventricular contractility. An increase in contractility may also explain the decrease in the acceleration time. Dobutamine a synthetic catecholamine (Tuttle and Mills 1975), increases ventricular contractility in conscious (Hinchcliff, McKeever and Muir 1991) and anaesthetised horses (Swanson, Muir, Bednarski, Skarda and Hubbell 1985). Peak velocity, peak acceleration and acceleration time are reported to be sensitive indicators of inotropic state in humans and dogs (Bennett *et al.* 1974; Stein and Sabbah 1976; Wallmeyer *et al.* 1988). However peak velocity, peak acceleration and acceleration time are also sensitive to changes in heart rate, preload and afterload (Bedotto *et al.* 1989; Wallmeyer *et al.* 1988). The heart rate, during the infusion of dobutamine, was not significantly different from that of the control group for aortic measurements. For pulmonary artery measurements, the heart rate during the infusion of dobutamine was significantly lower than during the control period. Part 1 of this thesis showed that the peak velocity and peak acceleration of blood flow increased with increased heart rate. Therefore differences in heart rate do not explain the increase in peak velocity and peak acceleration which occurred during the infusion of dobutamine.

The left ventricular end diastolic diameter, measured from an M-mode recorded from the right hemithorax was not significantly different between groups. This suggests that preload was not significantly altered by the drug treatments. However this is an insensitive measure of changes in ventricular volume (Elkayam *et al.* 1983b).

Unfortunately, afterload was not measured during this study. In a previous study, the infusion of dobutamine to conscious horses, increased mean and systolic arterial blood pressures but did not affect systemic vascular resistance (Hinchcliff,

McKeever and Muir 1991). Such changes, if present in the current study, would tend to decrease the peak velocity and peak acceleration, and increase the acceleration time.

Dopamine is also a positive inotropic agent. At a dose rate of 5μg/kg/min in anaesthetised horses, it increases left ventricular dp/dt (Trim, Moore and White 1985; Swanson *et al.* 1985). In the present study, the peak velocity and peak acceleration increased during the infusion of dopamine, although there was no change in the acceleration time. The infusion of dopamine did not significantly increase the pulmonary artery peak velocity or the peak acceleration compared to the control level. The reason for the different response of the two ventricles to the infusion of dopamine is not known.

The heart rates in the control horses ranged from 32 to 51 beats per minute during recordings from the pulmonary outflow. Although the median heart rate of 37 beats per minute represents a normal resting rate, a heart rate of 51 beats per minute would not be considered a normal resting rate for an adult horse. Measurement of the Doppler waveforms at these elevated heart rates would cause an increase in the peak velocity and peak acceleration, as demonstrated in Part 1 of this thesis. If these horses had been excluded, a larger difference might have been recorded between the dopamine group and the control group.

The heart rate was significantly higher during the infusion of dopamine than during the other study periods. In normal horses the peak velocity and peak acceleration increase at elevated heart rates. This is probably due to increased inotropy associated with sympathetic stimulation. An increased stimulation frequency of the myocardium also causes increased myocardial contractility, due to the accumulation of intracellular calcium. However, this effect is thought to be minimal compared to that caused by increased sympathetic nerve stimulation (Levick 1991). It is not clear whether the increases in peak acceleration and peak velocity during the infusion of dopamine are due to the drugs chronotropic or inotropic effects.

In anaesthetised horses, dopamine infusion at 5μg/kg/min causes a significant decrease in peripheral vascular resistance (Trim, Moore and White 1985; Swanson *et al.* 1985), whereas arterial blood pressure remains unchanged. A similar finding in conscious horses may contribute to the observed increase in peak velocity and peak acceleration in the present study.

The peak velocity and peak acceleration in the dobutamine group were significantly higher than in the dopamine group. Dobutamine infusion did not significantly increase the heart rate during aortic measurements. Previous studies in conscious horses indicate that dobutamine infusion does not decrease afterload (Hinchcliff, McKeever and Muir 1991). This suggests that there is a greater increase in contractility during the infusion of dobutamine than is produced by the infusion of dopamine. This effect has also been demonstrated in anaesthetised horses by conventional invasive indices (Swanson *et al.* 1985).

It is unclear whether the reduction in peak velocity and peak acceleration, and the increase in acceleration time, following the administration of detomidine and butorphanol, is a result of decreased ventricular contractility or increased ventricular afterload. Butorphanol administration in conscious horses causes no significant change in cardiac output, heart rate, or mean and diastolic arterial blood pressure (Robertson, Muir and Sams 1981). The injection of a dosage ten times that used in the present study in conscious horses has resulted in an increase in systolic blood pressure (Robertson, Muir and Sams 1981). Detomidine administration decreases left and right ventricular dp/dt in conscious horses and increases systemic and pulmonary vascular resistance (Wagner, Muir and Hinchcliff 1991). Following the administration of detomidine at 10 μg/kg, arterial blood pressure initially rises but decreases to control levels after 10 minutes (Clarke and Taylor 1986). Other studies have shown that following the administration of detomidine at 10 μg/kg, arterial blood pressure remains unchanged for the first 15 minutes, and then it is significantly reduced (Wagner, Muir and Hinchcliff 1991). Afterload is influenced by the arterial

blood pressure (Braunwald 1988), but it is not synonymous with it (Borow 1989). The systemic vascular resistance is also an incomplete way of expressing ventricular afterload (Milnor 1990). It is therefore difficult to be sure whether afterload was increased or decreased following the administration of detomidine and butorphanol. Systemic vascular resistance may influence afterload through its effects on arterial blood pressure. Following detomidine administration, the systemic vascular resistance remains high, but the arterial blood pressure falls; therefore afterload may be reduced. The decrease in peak velocity and peak acceleration and the increase in acceleration time reflect a decrease in ventricular function which may be due to a decrease in contractility.

The administration of detomidine and butorphanol also caused a significant decrease in heart rate. This has been reported previously and it is caused by sinoatrial block and second degree atrioventricular block (Clarke and Taylor 1986). However, as demonstrated in Part 1 of this thesis, this is unlikely to significantly decrease peak velocity or peak acceleration.

The pre-ejection period and acceleration time appeared to be less sensitive to changes in inotropic state than the peak velocity and peak acceleration.

The velocity time integrals were not significantly different during the infusion of the inotropic agents. In the case of dobutamine, an increase in the velocity time integral, which would be caused by the large increase in peak velocity, was offset by the decrease in the ejection time. The velocity time integral was significantly reduced following the administration of detomidine and butorphanol. The ejection time was prolonged, but the peak velocity was reduced.

Cardiac output by Doppler echocardiography is calculated from the mean flow velocity, the heart rate and the area of flow. As no significant change in the diameter of the aorta and pulmonary artery occurred in this study, the cardiac output becomes proportional to the velocity time integral and heart rate. During the infusion of dopamine there was a significant increase in heart rate. Therefore, although the

velocity time integral was unchanged, the cardiac output, calculated by Doppler echocardiography increased. The infusion of dobutamine, did not cause an elevation in heart rate. In recordings from the pulmonary artery, heart rate was significantly decreased during the infusion of dobutamine. Despite this decrease in heart rate, the cardiac output was not significantly different during the infusion of dobutamine. The Doppler measurements suggest, that in conscious horses, dobutamine causes an increase in contractility, and therefore increases cardiac work with little effect on the cardiac output. These findings are similar to a previous study, which demonstrated that the infusion of dobutamine at 5µg/kg/min to conscious horses caused an increase in right ventricular dp/dt max but did not cause an increase in heart rate or cardiac output (Hinchcliff, McKeever and Muir 1991). The decrease in heart rate, compared to the control group, for recordings from the pulmonary artery during the infusion of dobutamine probably reflects the increased heart rate in the control group as discussed previously. In addition, the minimum rate (24) during the dobutamine infusion was lower than that of the control group. This was caused by the second degree atrioventricular block demonstrated by one horse during the dobutamine infusion.

During the recording of aortic waveforms, the heart rate was not significantly altered by the infusion of dobutamine when compared to the control group. However, the infusion of dobutamine caused a significant increase in the cardiac output which was not significantly different to that produced by the infusion of dopamine. The infusion of dopamine caused a significant increase in heart rate compared to both the control group and the group receiving dobutamine. Although the velocity time integrals were not significantly different during the dobutamine infusion, the median was increased (Figure 67). In addition the median aortic diameter measured by the leading edge method, although not significantly increased during the infusion of dobutamine, was larger (Table 32). These small but non significant increases in velocity time integral and vessel diameter may explain the significant increase in the calculated cardiac output during the infusion of dobutamine.

The increase in cardiac output recorded by Doppler echocardiography during the infusion of dopamine, appeared to result from an increase in heart rate. The administration of detomidine and butorphanol caused a significant decrease in cardiac output which was due to a decrease in the velocity time integral and a decrease in heart rate.

Cardiac output measured by Doppler echocardiography, was significantly different during different study periods. These same differences were also statistically significant when cardiac output was measured by thermodilution.

The velocity time integral has been shown to be strongly related to stroke volume in humans (Mehta and Bennett 1986). The present study showed a weak relationship between stroke volume measured by thermodilution, and the velocity time integral. This may be due to the small range of velocity time integrals studied. A larger range of velocity time integrals might have been obtained by altering the preload and afterload. Errors in the calculation of stroke volume by thermodilution occur due to the short time base over which thermodilution cardiac output is calculated. The average stroke volume over a minute is calculated by dividing the cardiac output by the heart rate. Inaccuracy may have occurred in the measurement of Doppler velocity time integrals due to distortion of the Doppler waveform during systole. Movement of the heart during systole can move the sample volume from the centre of the vessel. At the sides of the vessel velocities due to eddy formation may be recorded (Skjaerpe 1988). If the velocities are negative, this will cause the ejection time to appear shorter and the measured velocity time integral to be decreased. Movement of the heart was most apparent during the infusion of dobutamine.

Summary

The lack of agreement between the Doppler method of measuring cardiac output and thermodilution is to be expected, in view of the errors inherent in both techniques (Schuster and Nanda 1984). However this lack of agreement between the two techniques in horses appears to be no worse than has been demonstrated by

similar techniques in humans. It is likely that the Doppler method of measuring cardiac output in horses would be more useful for detecting changes in cardiac output than for recording absolute measurements. The Doppler technique may be useful in this species to monitor the response to drug therapy or to monitor cardiac output intraoperatively. However, the normal variability of this measurement in horses must be established before differences between measurements can be considered to be significant.

Doppler and conventional indices of ventricular function do not measure contractility directly, and therefore cannot be used to define the contractile state of the ventricle absolutely. However, this study suggests that Doppler indices are sensitive indicators of inotropic state in horses, and being noninvasive, the technique may be useful for serial evaluation of an individual animal. This study also demonstrates that Doppler echocardiography can provide information on the mechanism and haemodynamic effects of therapeutic agents with the horse in a resting physiological state.

GENERAL DISCUSSION

Echocardiography is shown here to be a valuable technique in the assessment of the equine heart. Despite advances in our knowledge of the pathology of valvular disease (Else and Holmes 1972) and the production of cardiac murmurs (Paterson, Detweiler and Glendinning 1965) there are few objective data on the progression of valvular disease and its relationship to athletic performance (Brown 1985). This paucity of data has been due to the lack of a suitable diagnostic technique in horses.

Colour flow Doppler echocardiography is a sensitive method for the detection of valvular regurgitation in horses. It can detect valvular dysfunction in its mildest form and is noninvasive, allowing horses to be investigated during periods of competitive work. In this study, horses with high grade murmurs of tricuspid regurgitation had longer colour flow jets, of larger area, than horses with lower grade murmurs of tricuspid regurgitation. This method therefore appears suitable for monitoring the progression of valvular regurgitation. However, repeatability studies are needed to examine day to day variations in jet dimensions, to determine the sensitivity of the technique to changes in valve function.

The colour Doppler flow patterns in a group of normal Thoroughbred and Thoroughbred cross horses are described in this work. Valvular regurgitation is present in a high percentage of clinically normal horses. It may be that the high incidence of valvular regurgitation detected in horses in this work is related to the type of horse studied. All of the horses in the present study were in some form of work. Human athletes have an increased incidence of valvular regurgitation compared to sedentary people (Douglas *et al.* 1989). It would be interesting to determine the incidence of valvular regurgitation in horses before and after the onset of athletic training.

The standard views described in the present study are repeatable, allow clear visualisation of intracardiac structures and are useful for guiding M-mode and

Doppler studies. The views allow more accurate alignment of the Doppler ultrasound transducer with blood flow than reported previously (Reef *et al.* 1989). Reference values for intracardiac measurements from M-mode and two-dimensional images, from a group of Thoroughbred and Thoroughbred cross horses, are recorded in Chapter 4. Chamber dimensions outwith the normal range were demonstrated in horses with high grade murmurs of aortic insufficiency. However, intracardiac dimensions were normal in horses with evidence of mild valvular regurgitation. The inability to measure atrial dimensions from two-dimensional images, is a limitation of the technique in horses. Such measurements would be of value, as atrioventricular valve regurgitation is a common condition in horses, from which dilation of the atria often develops. The horses studied in this work were not showing signs of cardiac failure, but were referred for the evaluation of cardiac murmurs. It is unlikely that two-dimensional and M-mode echocardiography will be sufficiently sensitive to detect changes in cardiac function in horses showing signs of poor athletic performance, but with no clinical evidence of cardiac dysfunction at rest.

The reference values reported here for selected Doppler measurements in a group of Thoroughbred and Thoroughbred cross horses may prove useful for the diagnosis of cardiomyopathies in horses. The results of the present study suggest that pulsed wave Doppler echocardiography is also useful for detecting the presence of volume overload at rest. Studies in humans have demonstrated that continuous wave Doppler echocardiography can be used to estimate transvalvular pressure gradients in patients with valvular regurgitation (Currie, Seward, Chan, Fyfe, Hagler, Mair, Reeder, Nishimura and Tajik 1985). High pulsed repetition frequency and continuous wave Doppler echocardiography can be used to record regurgitant signals in horses. The accuracy of the method is governed by the ability to align the ultrasound beam accurately with the regurgitant flow. Validation in horses will require comparative studies with invasive measurement techniques. Spectral Doppler recordings may prove useful in distinguishing horses showing reduced athletic ability as a result of

valvular disease from horses with cardiac murmurs showing reduced athletic ability of non cardiac origin.

Part 2 of this thesis shows that Doppler echocardiographic variables are sensitive to changes in inotropic state. Pulsed wave Doppler echocardiography is as sensitive as the invasive thermodilution technique for detecting drug-induced changes in cardiac output. Doppler echocardiography also gives further information on the pharmacodynamics of therapeutic agents in this species. This facility is valuable, as many drugs are developed in other species and applied to equine medicine with no previous knowledge of the heamodynamic effects in horses.

There is an unacceptably high incidence of post anaesthetic lameness in horses (Klein 1978), which is in part related to the decrease in cardiac function which occurs with various anaesthetic agents. Haemodynamic monitoring in veterinary anaesthesia is based on the mistaken assumption that an adequate blood pressure reflects an adequate cardiac output. Doppler echocardiography is a suitable technique for monitoring cardiac output in standing horses, and the provision of a transoesophageal ultrasound transducer will facilitate continuous monitoring of cardiac output in anaesthetised horses. Preliminary studies indicate that better alignment with aortic outflow is achieved from the transoesophageal route, than is achieved by transthoracic Doppler echocardiography.

Improvements in our monitoring capabilities will increase our understanding of the complex heamodynamic interactions which occur under general anaesthesia. This should promote the development of anaesthetic combinations which cause minimal depression of cardiac function in horses.