Echocardiography a non invasive method for investigating preclinical drug toxicity and safety.

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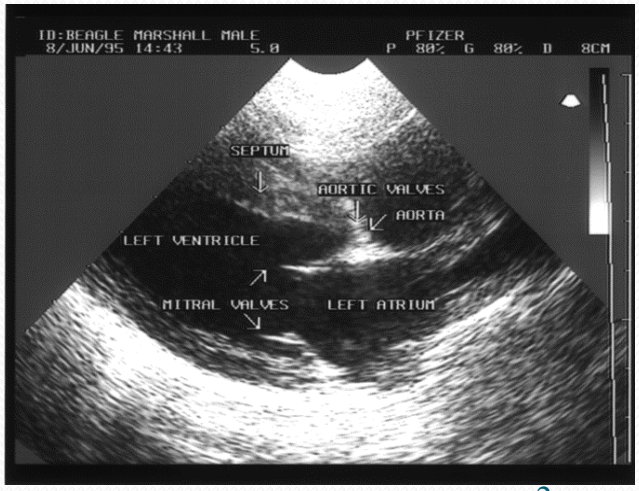
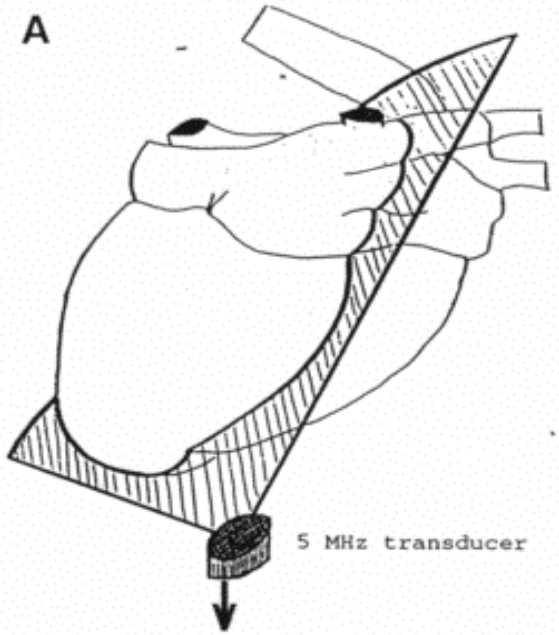
What is echocardiography (EC)

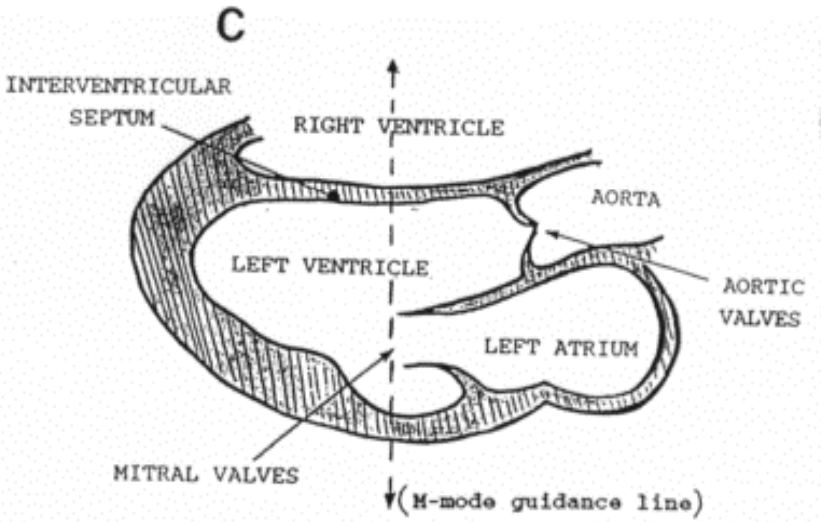
- Ultrasounds (US) are emitted by a transducer
- Reflection of US on tissues depends on their physical properties (echogenicity)
  - strong echogenicity : bones, air
  - weak echogenicity: liquids (blood, urine)
- Reception of reflected US by the transducer
- Processing of the information and image on the screen



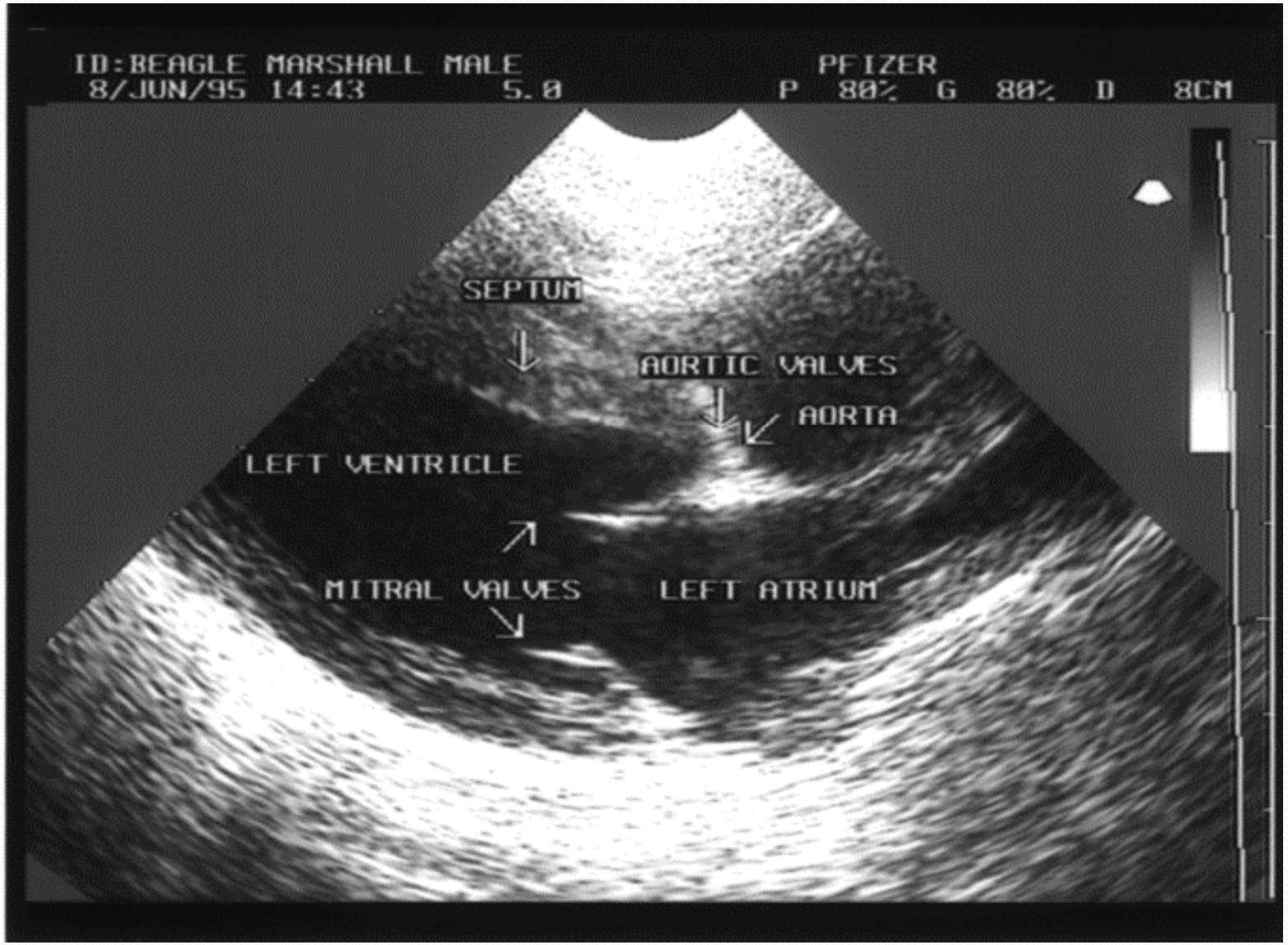
Bidimensional echocardiography (2-D EC) in right parasternal incidence

Visualisation of the heart structures in the plane of the ultrasounds beam: longitudinal section

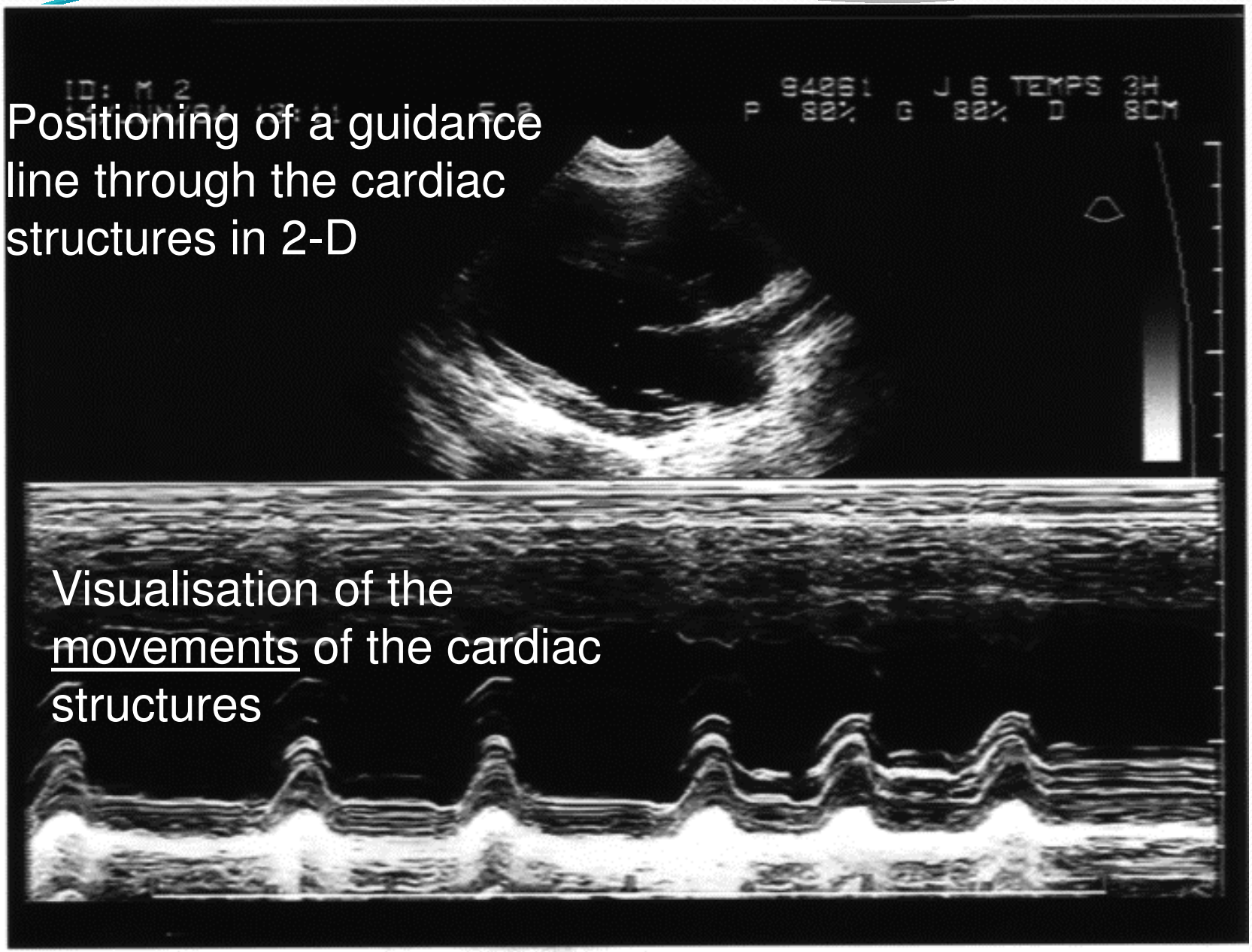




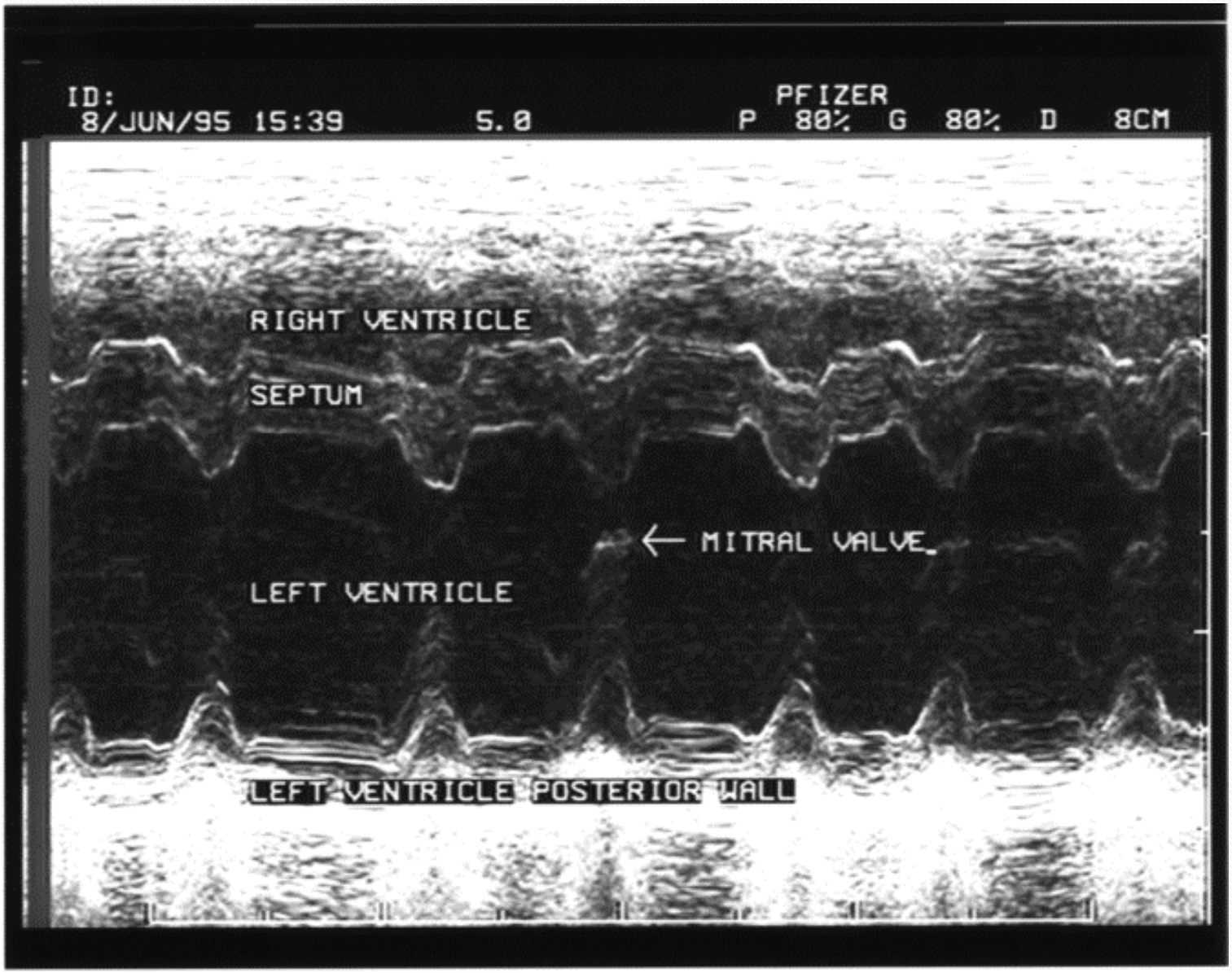
2-D EC: Longitudinal section



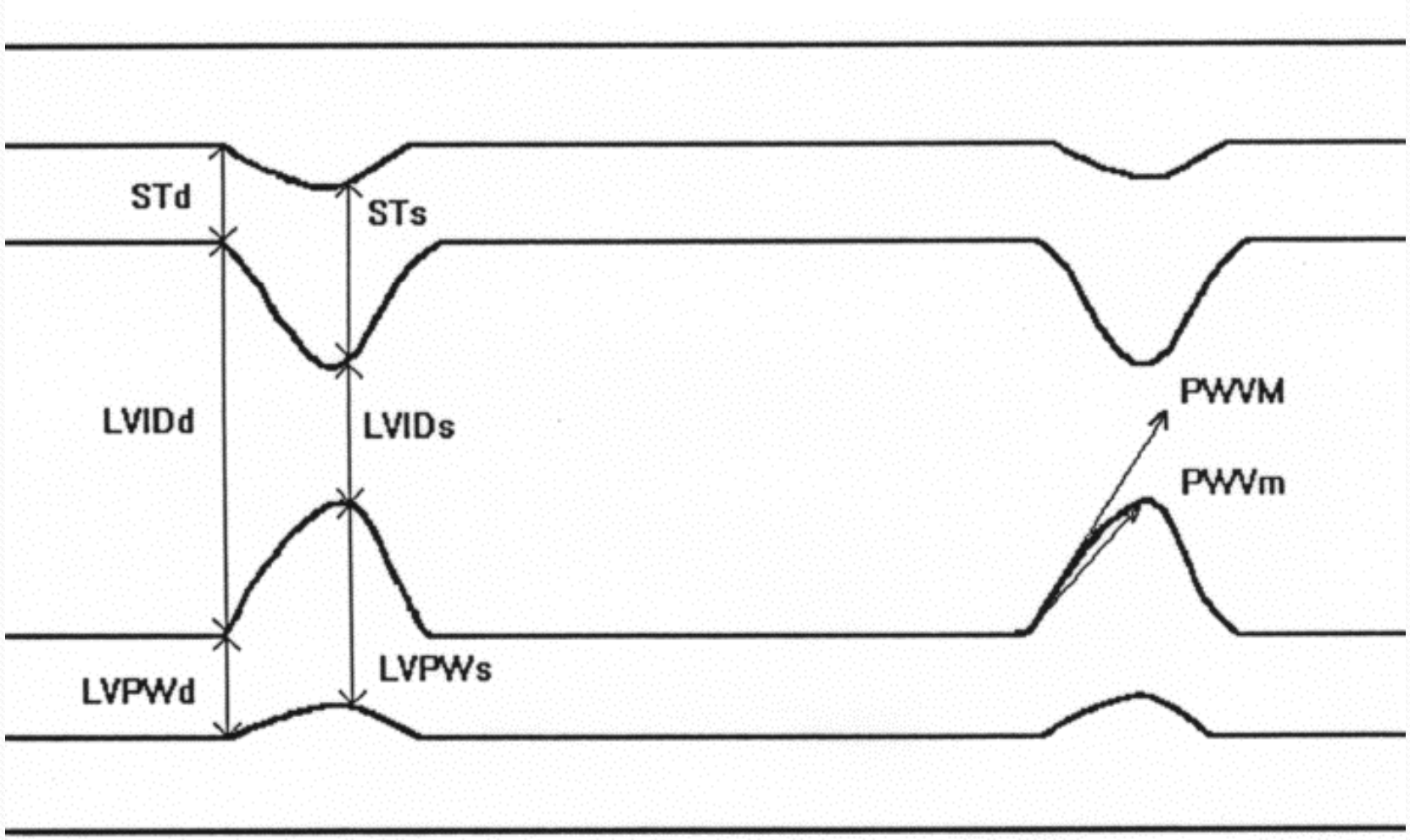
M-mode echocardiography



M-mode echocardiography



M-mode echocardiography
Schematic representation showing measured parameters



M-mode echocardiography calculated parameters

End diastolic volume	$EDV = \frac{7\ LVIDd^3}{2.4 + LVIDd}$
End systolic volume	$ESV = \frac{7\ LVIDs^3}{2.4 + LVIDs}$
Stroke volume	SV = EDV - ESV
Cardiac output	CO = SV x heart rate
Fractional shortening	$FS = \frac{LVIDd - LVIDs}{LVIDd}$
Ejection fraction	$EF = \frac{SV}{EDV}$
Percent of septum thickening	$PST = \frac{Std - Sts}{STd}$
Percent of posterior wall thickening	$PWT = \frac{LVPWd - LVPWs}{LVPWd}$

The different modes Doppler

Assessment of

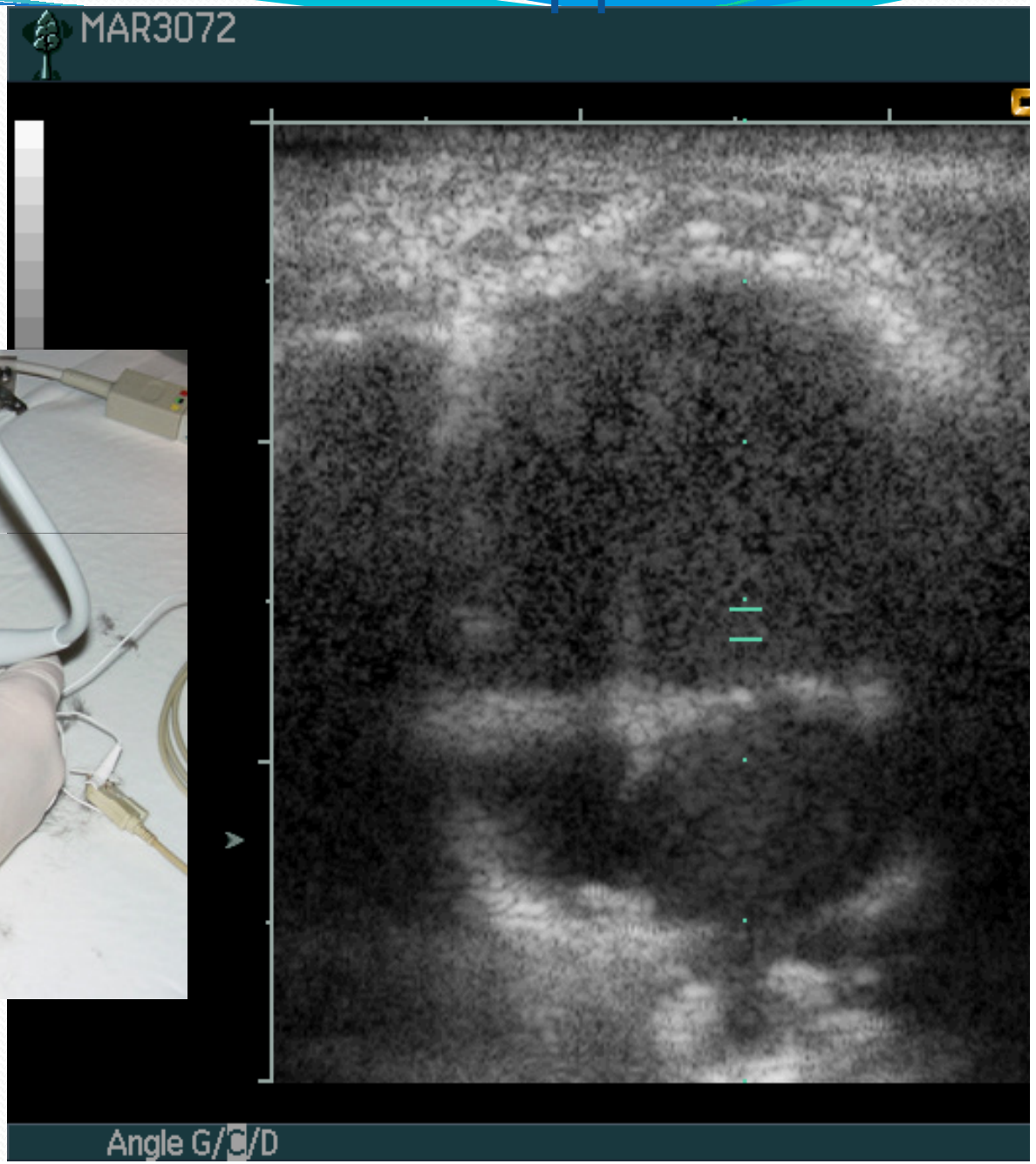
- Quantitative parameters of cardiovascular function
  - Flows: Stroke volume, cardiac or extra cardiac shunt flow, left coronary blood flow,
  - Pressure changes across valves and orifices or in cardiac chamber and great vessels
- Qualitative blood flow changes:
  - Laminar vs disturbed flow patterns

Doppler recording of intra-cardiac flows

- Visualisation of the heart structures in a 2-D mode section using apical incidence
- Positioning of the Doppler Window at the level of the aorta, pulmonary artery, mitral or tricuspid valves
- Recording of the changes in blood velocity over a few beats

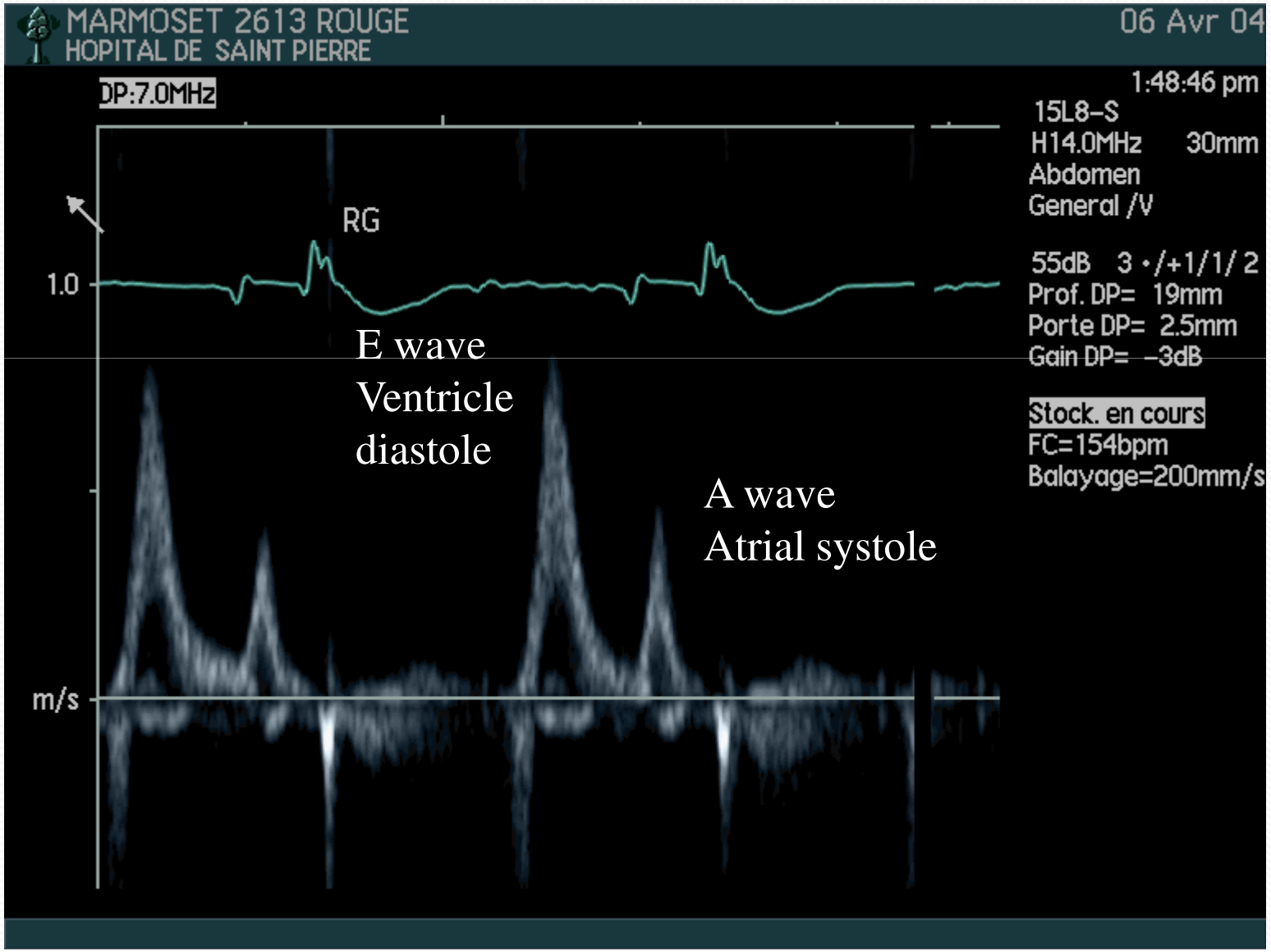
The different modes: Doppler

Four cavities view in apical incidence (marmosets)

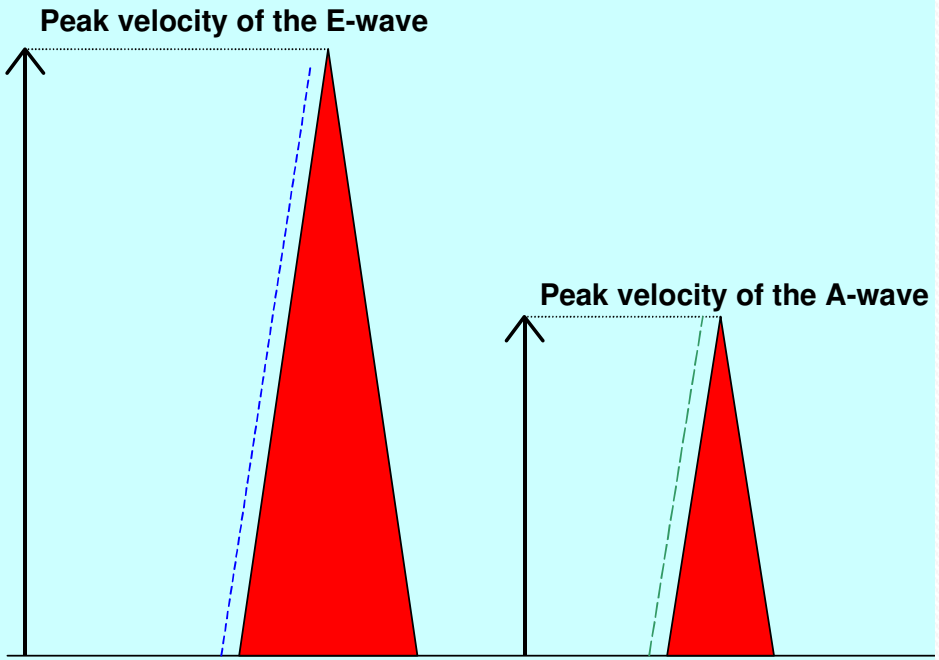


Echocardiography in marmosets: mitral flow

Spectrum of distribution of erythrocytes velocities

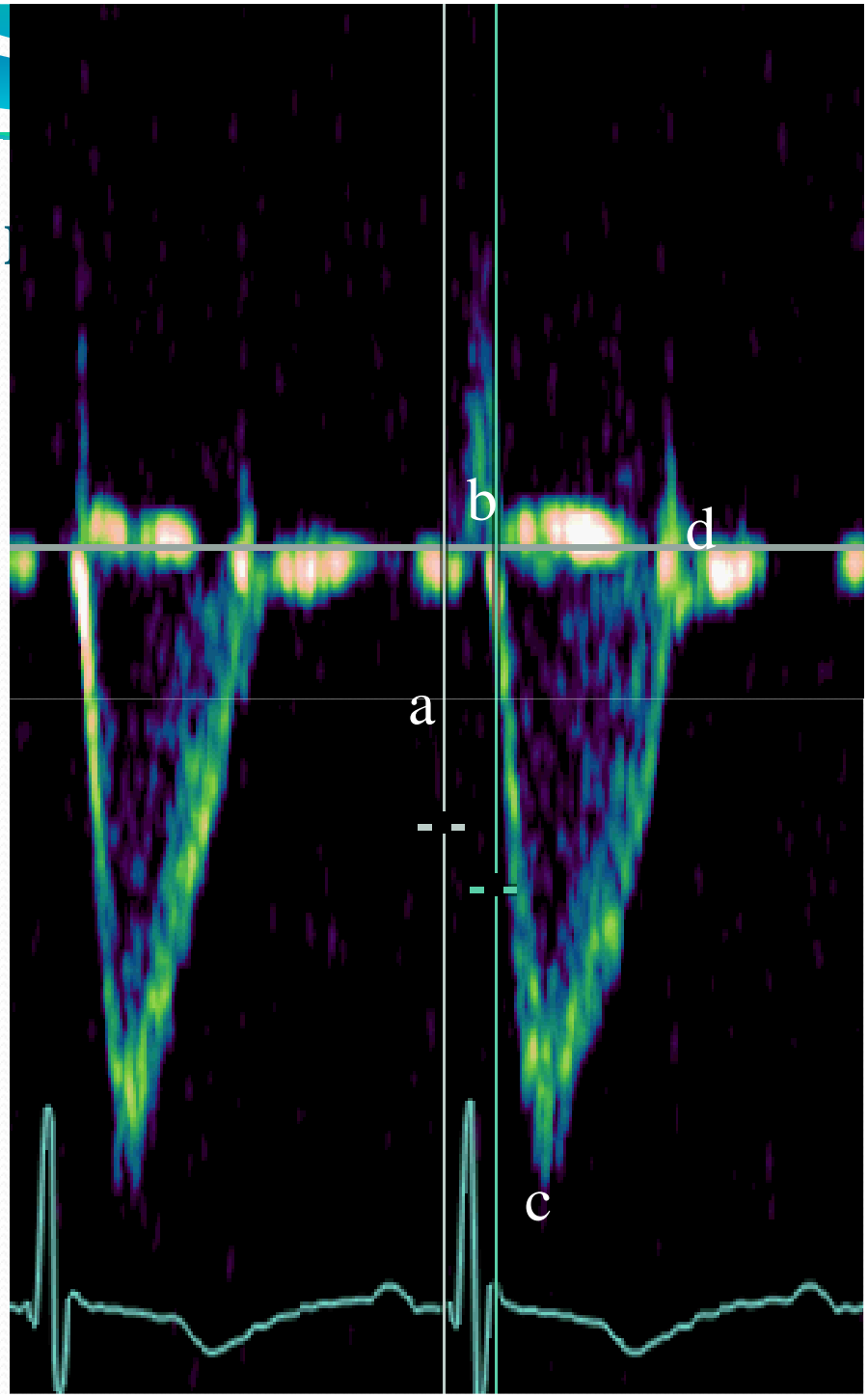


Schematic representation of measurements on a Doppler velocity spectrum of the mitral flow



Aortic flow recording (marmoset)

Measurements
•Vmax, VTI,
•Ejection time (ET): from the onset (b) to the end (d) of the velocity spectrum)
•Pre-ejection time from the Q wave of the ECG (a) to the onset of the Doppler velocity spectrum (b),
•Acceleration time from the onset to the peak of the velocity spectrum (c) and (d).



Doppler Echocardiography

Calculated parameters

- From tricuspid and mitral flow
  Ratio A/E waves for peak velocity or velocity-time integral :
  - *Relative contribution of atrial systole vs ventricle diastole to ventricle filling*

- From aortic flow
  Stroke volume = VTI x AA with

  VTI: velocity time integral
  AA: aortic diameter measured from M-mode trace

Application of echocardiography in preclinical safety assessment (1)

CONSEQUENCES of Cardiac toxicity

- Evaluation of morphological changes induced by test compounds (cardiac hypertrophy, dilation…)
- Measurement of functional consequences (changes in haemodynamic parameters and in contractility) of compound-induced cardiac lesions
- Measurement of haemodynamic changes associated with arrhythmias

Application of echocardiography in preclinical safety assessment (2)

CAUSE and MECHANISM of Cardiac toxicity

- Evaluation of pharmacological effects of cardiovascular drugs.
  - Measure of changes in cardiac contractility and in haemodynamic parameters
  - Clarification of the pathogenesis of cardiac lesions linked to exaggerated pharmacological effects: example of minoxidil

Value of echocardiography in toxicology as a method of refinement

- Non-invasive technique
  - No surgery
  - No pain or distress for the animal
  - Only a gentle restraint is needed
- No interference on cardiac function: measurement in normal situation
- No interference with the measurement of other parameters
- No influence on the results of the toxicity study
  - No medication
  - No effects of echography on the health status of the animal
- Measurements are easily repeatable and allow subsequent follow-up in the same animal

Minoxidil

- Potent vasodilator

- Cardiac toxicity of minoxidil in the dog
  - Produces necrosis of left ventricle at suprapharmacological doses (0.5-3 mg/kg)
  - Is due to the vasodilatory properties of the drug

Example of minoxidil

Experimental procedure

- Treatment with 0.5 or 2 mg/kg (single dose)
- 3 dogs/dose
- Measurement of echocardiographic parameters in M-mode and Doppler at different time points before and after dose

Minoxidil effects on parameters of left ventricle function evaluated by M-mode echocardiography

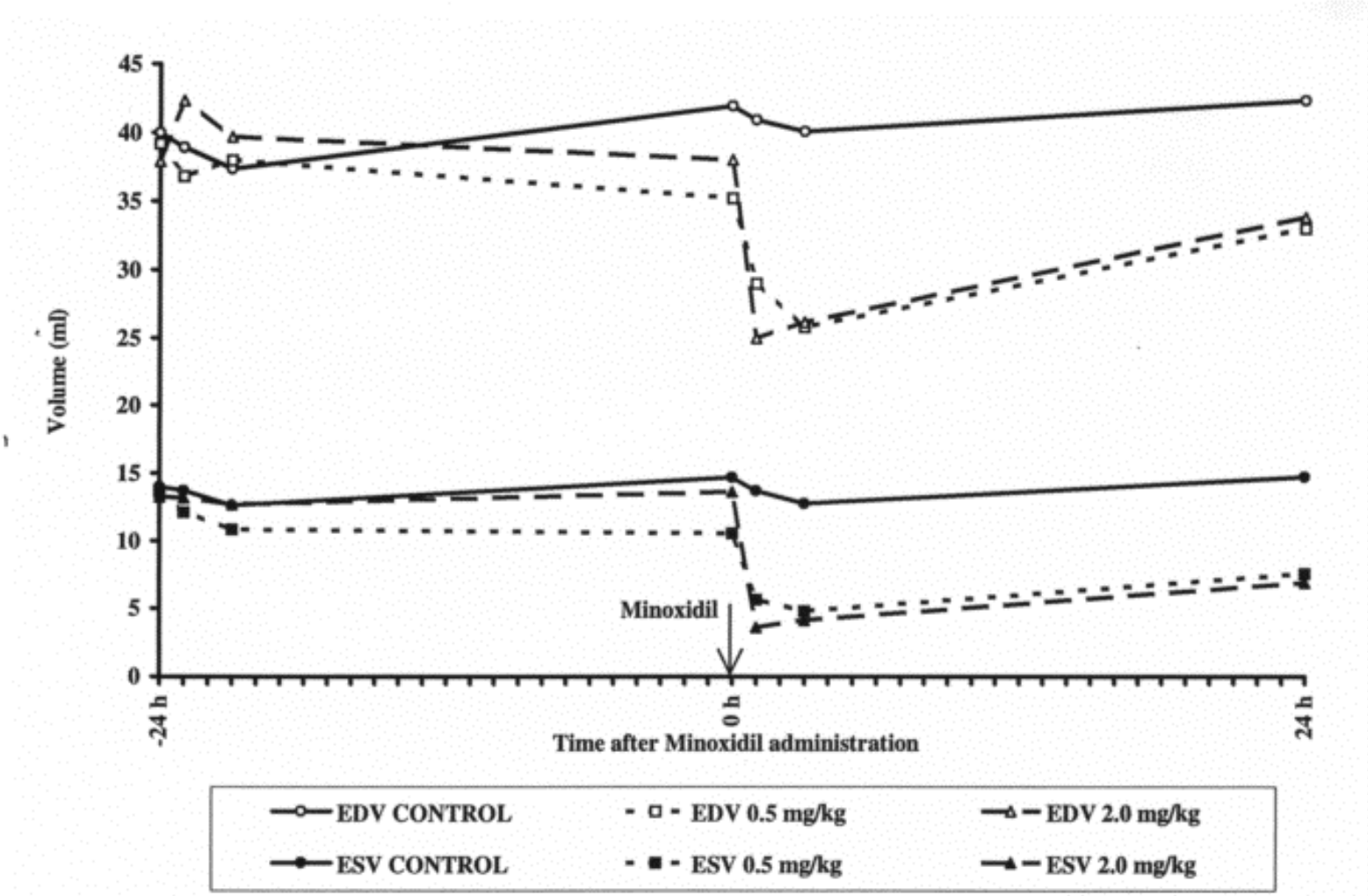
Change (%) in mean values recorded 1 hour after treatment compared to values recorded the day before treatment

	PST	PWT	EDV	ESV	EF	HR
Control	-14	- 17	-7	- 10	2	2
0.5 mg/kg	72	25	- 21	- 62	28	59
2 mg/kg	51	25	- 21	- 74	34	111

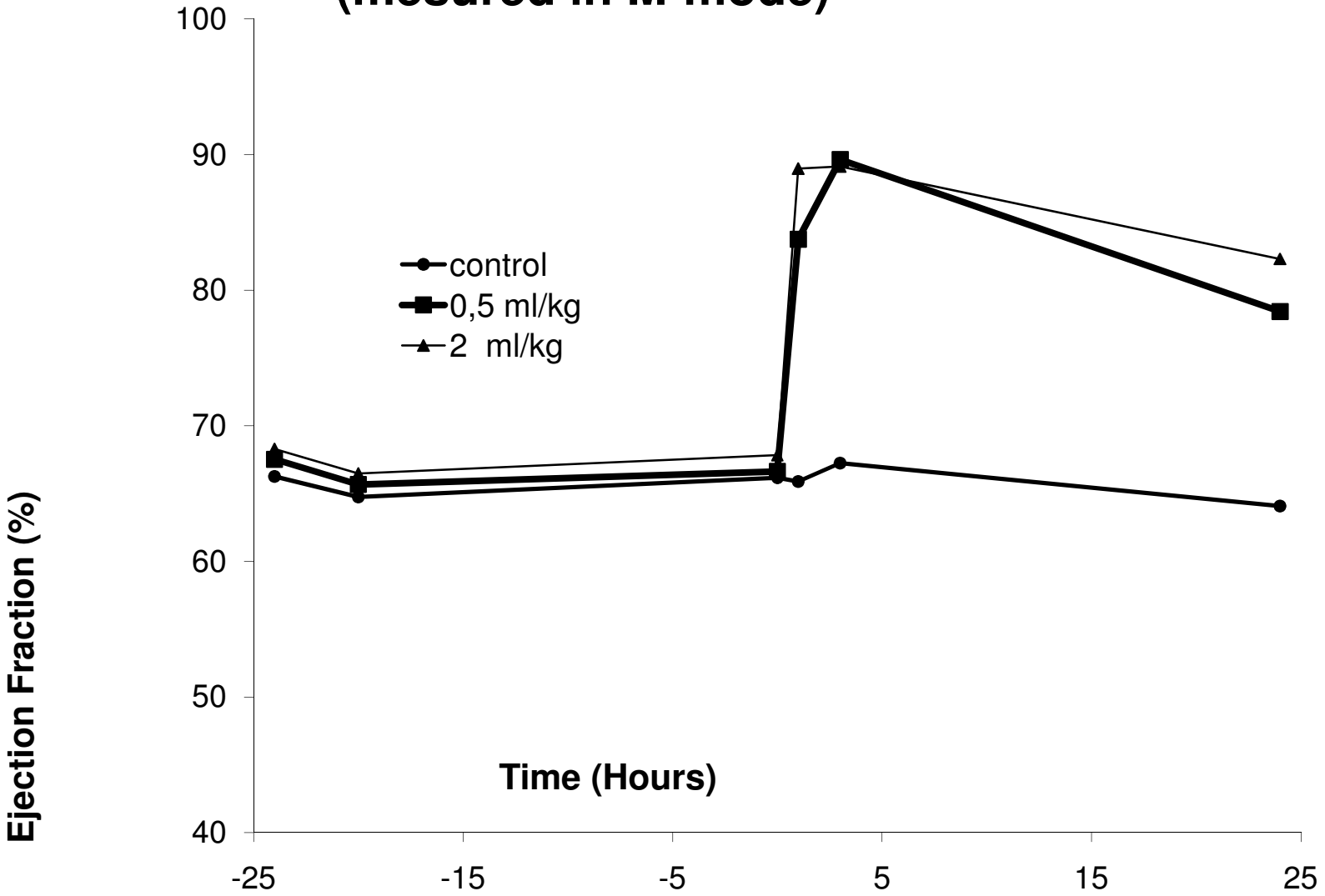
PST: Percent of septum thickening; PWT: Percent of left ventricle posterior wall thickening; HR: heart rate

EDV, ESV: end diastolic, end systolic volumes; EF: Ejection fraction

Effect of minoxidil on ventricular volumes



Effect of Minoxidil of Ejection Fraction (mesured in M-mode)



Minoxidil effects on aortic flow measured by Doppler echocardiography

Change (%) in mean values recorded 1 hour after treatment compared to values recorded the day before treatment

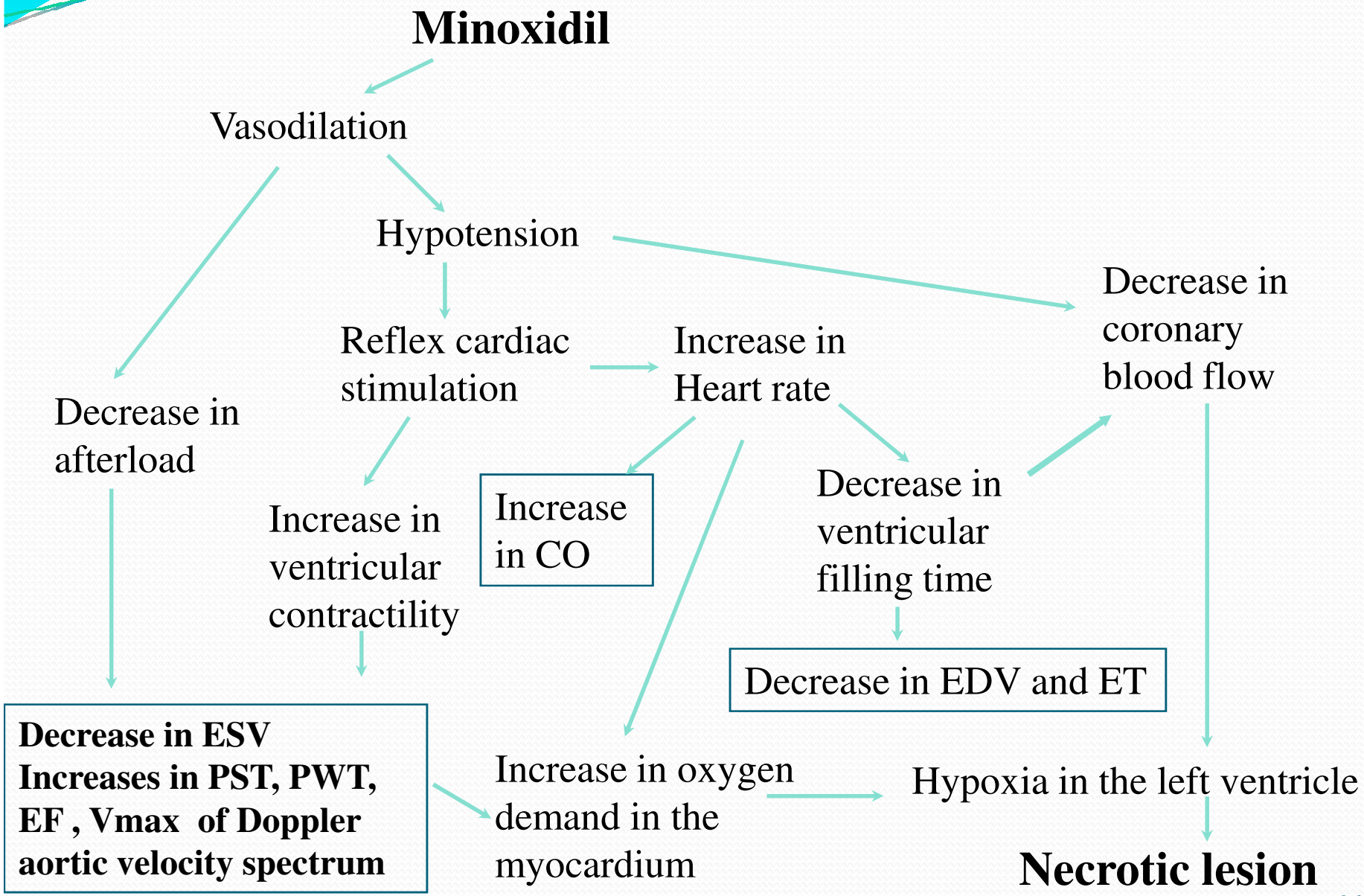
	Vmax	VTI	ET	Stroke Volume	Cardiac Output
Control	16	14	-2	8	10
0.5 mg/kg	29	18	-17	22	93
2 mg/kg	53	25	-18	33	181

Vmax: maximum velocity of the wave
VTI: velocity time integral
ET: ejection time

Minoxidil effects on parameters of left ventricle function evaluated by echocardiography

- Increase in contractility
  - Increase in ejection fraction and percent thickening of the left ventricle wall and septum
  - Decrease in end systolic volume
  - Increase in Vmax of aortic flow
- Mild increase VTI and consequently in stroke volume
- Marked tachycardia leading to
  - Decrease in ejection time
  - Decrease in end diastolic volume indicating decreased filling of the ventricle (decrease in inter-systolic time)
- Marked increase in cardiac output
  - Due mainly to tachycardia and to a lesser extent to increase in SV

Relationship between changes produced by minoxidil on cardiac function and the development of cardiac lesions



Conclusion of minoxidil study

- Echocardiography allows the non-invasive investigation of changes in the cardiac function produced by a vasodilator known to play a critical role in the pathogenesis of cardiac lesions.
- In the past, these functional changes were assessed using highly invasive methods

CONCLUSION

Echocardiography has potentially a great value as a method for investigation of cardiovascular effects of drugs in toxicology and safety pharmacology

Acknowledgments

Establisment echocardiography in dogs and marmosets

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Minoxidil study, Scientific collaboration

M. Gautier, PhD student

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H. Petinay, N. Mauclair and O. Christin

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THANK YOU

for your attention

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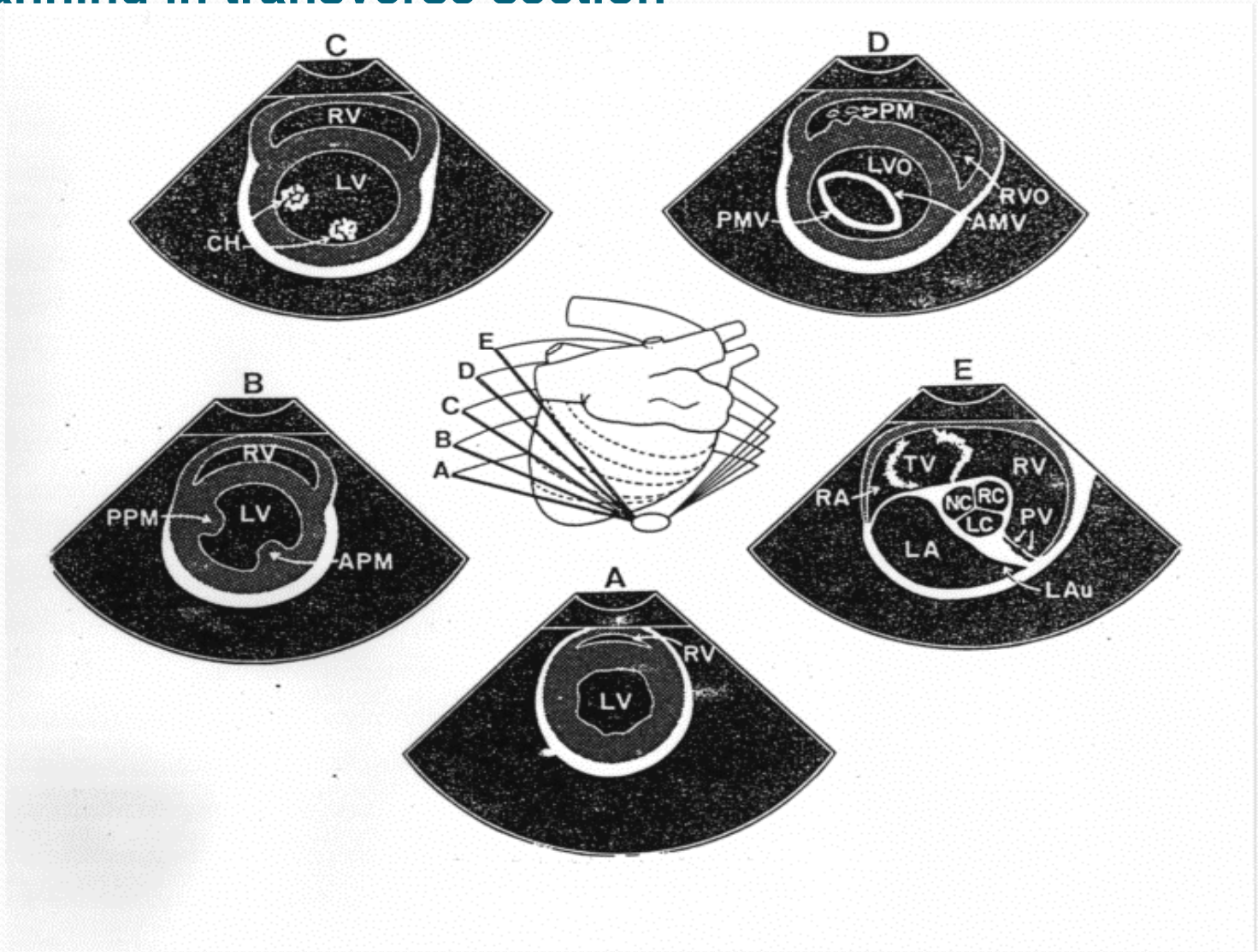
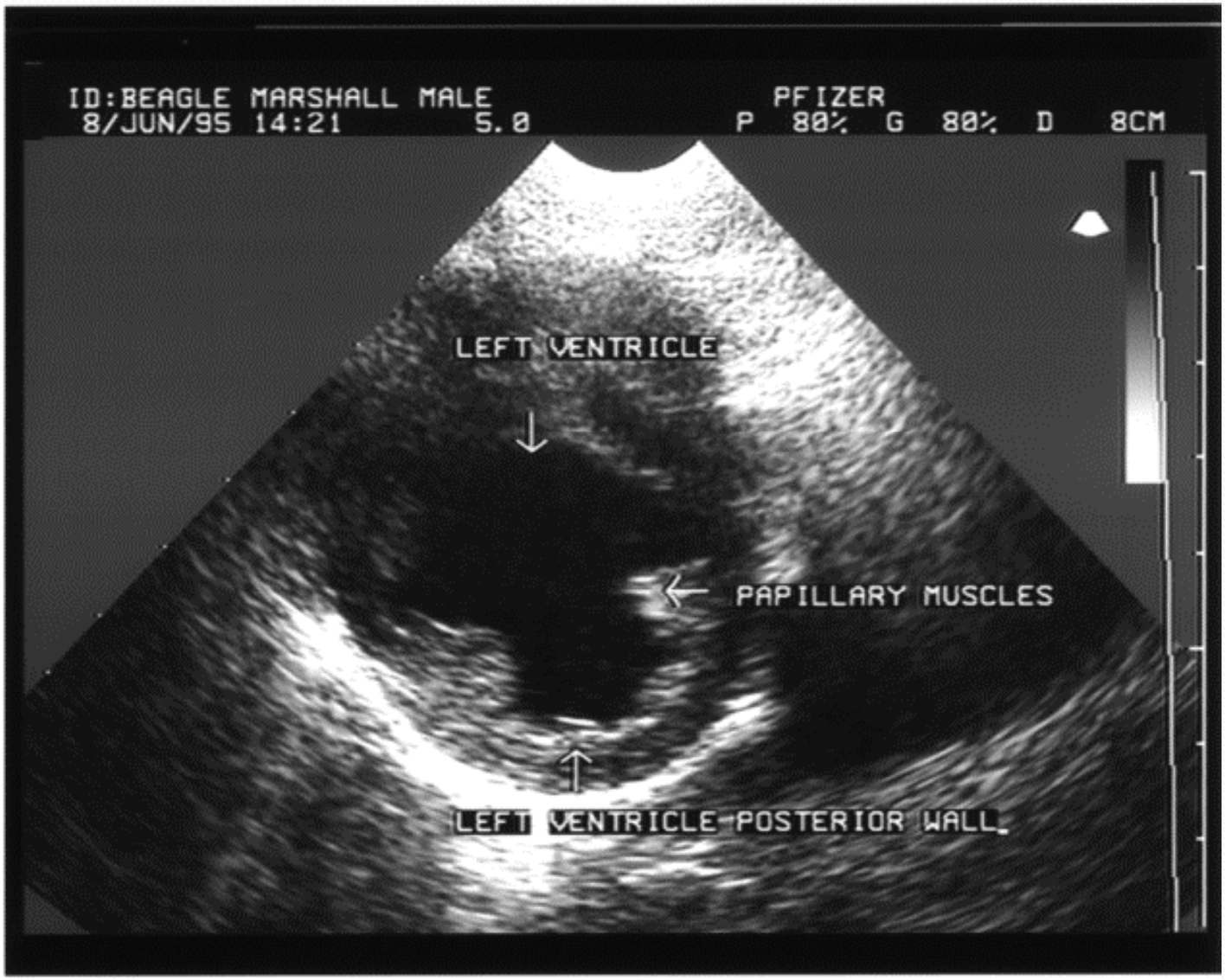
- Back Up slides

2-D echocardiography in right parasternal incidence

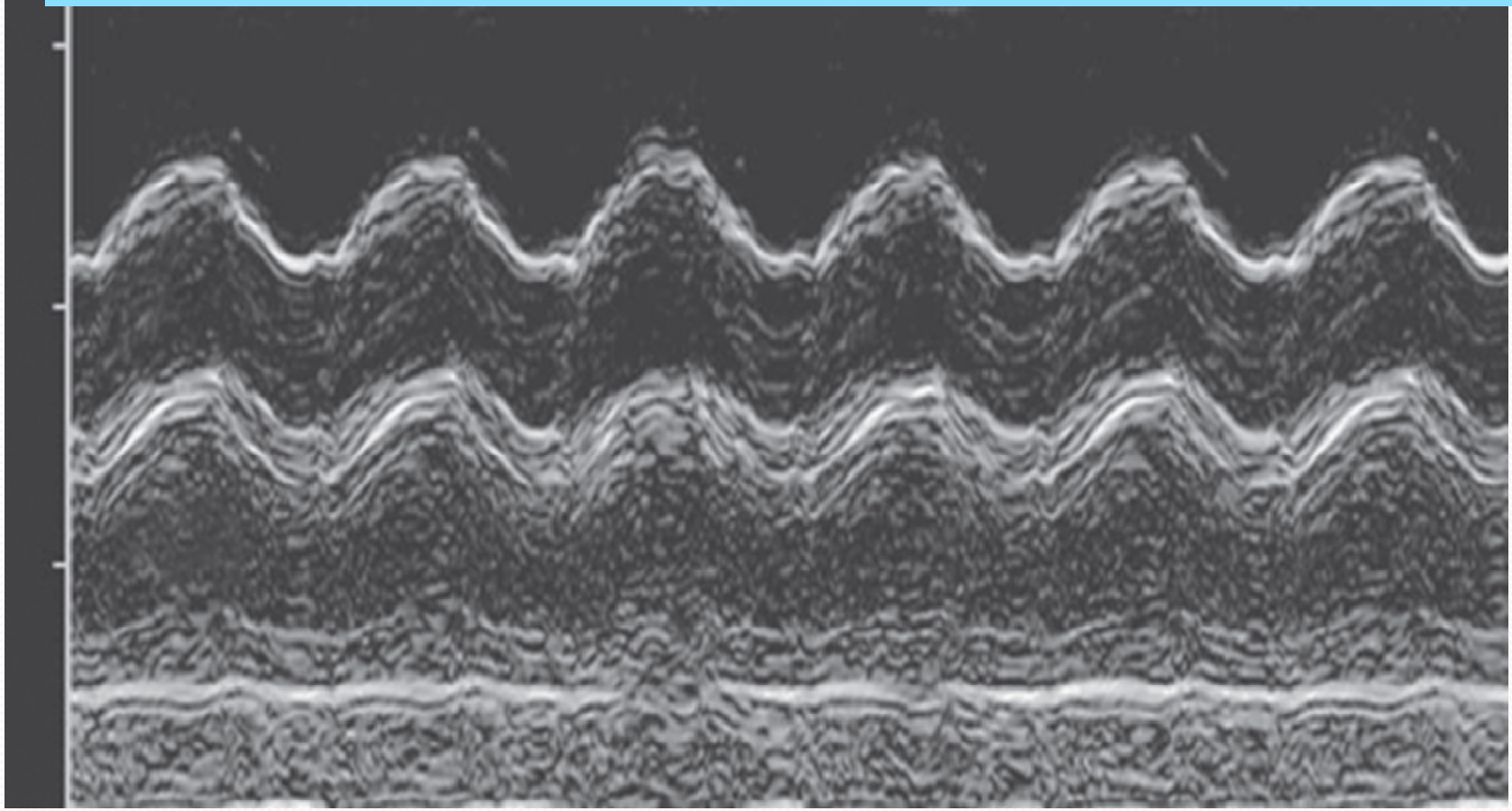
Scanning in transverse section



2-D EC: transverse section

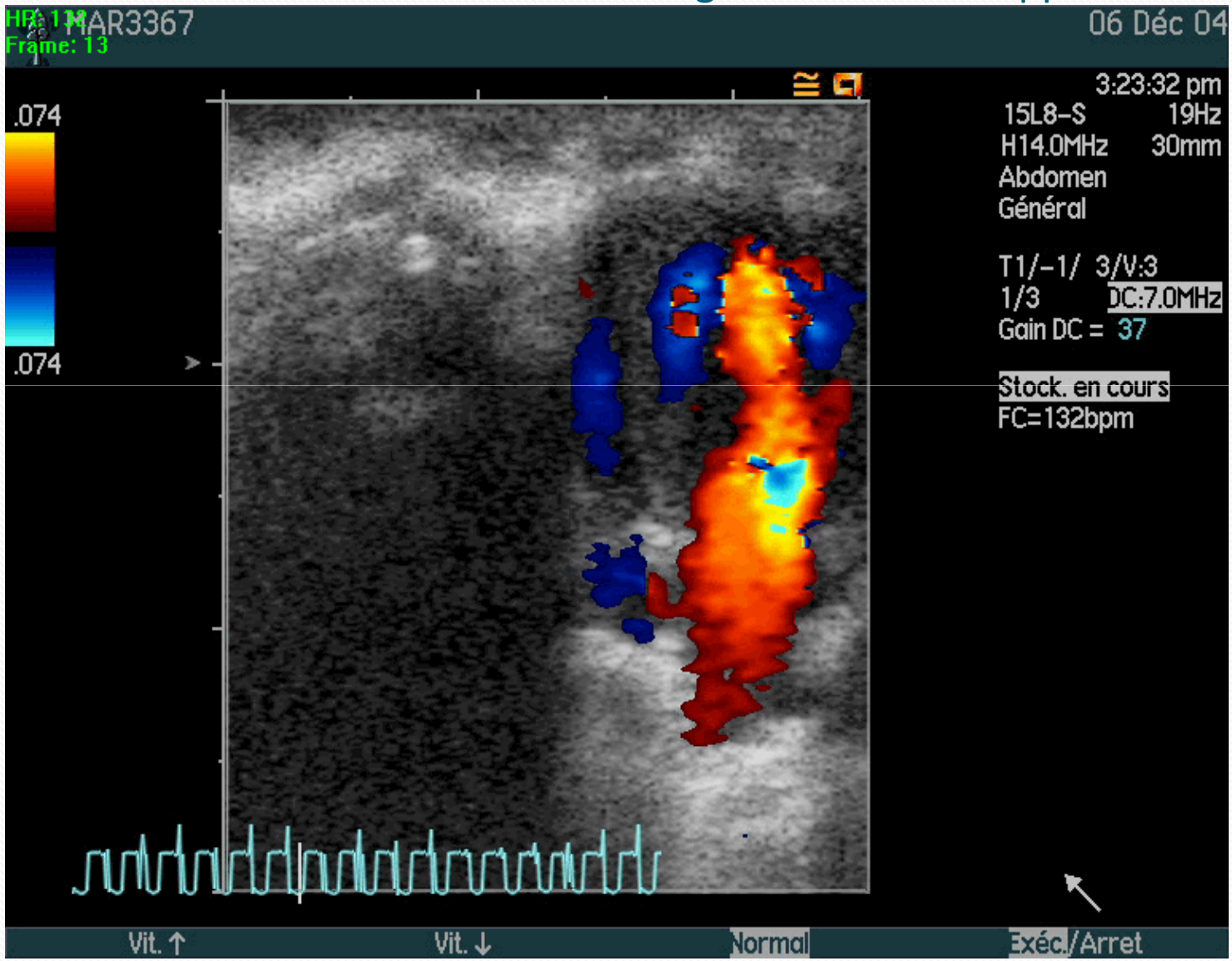


M-mode echocardiography of the upper part of the heart in a marmoset. The guidance line is positioned across the aorta and left atrium .The movements of aorta (AO) and left atrium (LA) marmoset, are recorded over time.



Color Doppler of intra-cardiac flows: ventricular diastole (marmoset).

The flow from left atrium to left ventricle trough mitral valves appears in red.



Color Doppler of intra-cardiac flows: ventricular systole (marmoset)

The aortic flow appears in blue