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MEASUREMENTS OF MASS, LENGTH AND VALVE DIAMETERS FROM NORMAL FORMALIN-FIXED OVINE HEARTS

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ABSTRACT

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Hearts from 60 Merino sheep of known age, sex and live mass and with no known history of disease were collected and fixed in buffered 10% formalin. Systematic light microscopical examination did not indicate any abnormality in hearts of any of the sheep. The mass of various parts of the hearts, the length of the hearts and the diameters of the heart valves were measured to establish a basis for quantitative assessment of possible pathological changes associated with the ingestion of cardiotoxic plants.

The mass measurements and, to a lesser degree, the lengths of the hearts varied considerably, but the ratio of the mass of the left ventricle plus ventricular septum divided by the right venticular free wall mass was remarkably stable, and is promising as an indicator of right ventricular hypertrophy.

INTRODUCTION

Despite an abundance and variety of cardiotoxic plants which affect domestic ruminants in southern Africa (Steyn, 1949; Vahrmeijer, 1981) and concerted research into the effects of such plants, assessment of gross pathological alterations in affected hearts has been limited to subjective impressions, based upon visual inspection. There has been no attempt to assess hypertrophy or atrophy quantitatively in these hearts.

The only convincing way to assess ventricular hypertrophy at necropsy, as discussed by Davies, Pomerance & Lamb (1975), is to determine the mass of the separated ventricles. The most appropriate method, however, of separating the ventricles has been a subject of controversy, since there is no universally accepted clear boundary within the ventricular septum between right and left ventricles. In a study of hypertrophy in human hearts, Herrman & Wilson (1922) cut along a white line within the septum which they regarded as the dividing line between right and left ventricles. A similar line of demarcation has been claimed and used for ventricular separation in a study of calf hearts (Groves, Greenberg, Rosenberg & McCrady, 1964) and of dog puppy hearts (Kirk, Smith, Hutcheson & Kirby, 1975). Fulton, Hutchinson & Jones (1952), on the other hand, doubted whether the white line in the septum of human hearts was a reliable guide for ventricular separation, and so they chose to separate right and left ventricular free walls from the septum and determined the mass of each part separately. Right ventricular free wall separation was found to be easy and accurate, whereas left ventricular free wall separation was difficult and subject to error. In view of this, Fulton *et al.* (1952), basing their proposal on their finding that the septum was never greatly increased in mass except in the presence of left ventricular hypertrophy, suggested that the left ventricular free wall and septum be measured together as one part of the heart. In studies of hypertrophy in bovine hearts (Alexander & Jensen, 1959; Hultgren, Marticorena & Miller, 1963; Blake, 1965) left and right ventricular free walls were also dissected from the septum and the mass of all 3 parts determined separately. The results obtained proved of value in assessing right ventricular hypertrophy in cattle kept at high altitudes. Hultgren *et al.* (1963) also included hearts from sheep and several other species in their study.

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The aim of this study was to establish a basis for quantitative assessment of possible pathological changes in the ovine heart associated with cardiotoxic agents.

MATERIALS AND METHODS

Animals and collection of hearts

Sixty (60) Merino sheep, consisting of ewes, rams and wethers were collected from various locations in South Africa and kept in concrete yards at the Veterinary Research Institute, Onderstepoort. Here they were fed chopped lucerne hay supplemented with cereal for a period of 1–3 months prior to slaughter. On the day of slaughter the masses of the sheep were determined and the sex of each sheep was recorded. The age of each sheep was known accurately from its records. The sheep were slaughtered by stunning and exsanguination. The hearts, attached to the roots of the great vessels, were removed and immersed in 10 % buffered formalin within 1 h of death, and were stored in the formalin for 2–3 weeks. The identity of the sheep of origin was recorded for each heart.

Measurements

The following measurements were made for each sheep:

1. Live mass of sheep (kg).
2. Mass of left ventricle plus septum (g) = LV+S.
3. Mass of right ventricle (g) = RV.
4. Mass of both atria (g).
5. The ratio, LV+S/RV.
6. Length of heart (cm).
7. Diameter of right atrioventricular valve ring (mm) = RAV.
8. Diameter of left atrioventricular valve ring (mm) = LAV.
9. Diameter of pulmonary valve ring (mm) = PV.
10. Diameter of aortic valve ring (mm) = AV.

Heart dissection and measurement procedure

The dissection method was based on that suggested by Fulton *et al.* (1952). For each heart the epicardial fat was removed as completely as possible by sharp dissection, care being taken that none of the underlying myocardium was removed. The atria with the roots of the great vessels were separated from the ventricles by cutting with scissors at the level of the atrioventricular ring. This exposed the openings of the pulmonary, aortic and atrioventricular valves within the base of the ventricles. The length of each heart was measured with a flexible steel rule from the base of the left ventricle along the caudal aspect of the left ventricle to the cardiac apex. The attached aorta and pulmonary artery were separated from the atria by blunt dissection and any remaining fat was dissected free from the atria. The cranial and caudal vena cava and pulmonary vein were transected at their point of junction with the atria. The ventricles were transected parallel to their base, halfway between base and apex. RAV, LAV, PV and AV were measured by inserting a jeweller's graduated ring-measuring stick, which was calibrated for diameter (mm), into each valve opening until gentle resistance was felt, and by reading the graduation nearest to the valve opening.

The free wall of the right ventricle was separated from the septum by cutting in the same plane as the right ventricular surface of the septum. The moderator band in the right ventricle was transected at its junction with the septum, and was left attached to the right ventricular free wall. The cusps of all the valves were cut free at their bases with scissors, and the *chordae tendineae* of the right and left atrioventricular valves were cut at their junctions with the papillary muscles. The dissected parts of the atria and ventricles were washed free of clotted blood, blotted dry with filter paper and the mass determined on a balance accurate to 0,5 g.

Light microscopy

Tissue blocks were collected from the apex, left and right ventricular free walls and ventricular septum of each heart. Paraffin sections were prepared from the blocks and stained with haematoxylin and eosin according to standard procedures for light microscopy.

RESULTS

Measurements

The frequencies for the different ages and sexes of the sheep are depicted in Fig. 1. It is clear that neither age nor the sexes are equally represented. The absence of data for months 10–18 is especially troublesome. The body mass versus age for all sheep is plotted in Fig. 2. A growth curve would normally be expected, but the variations between sheep were extensive, while no data are available for months 10–18. Near maximum body mass is probably reached at 18 months or shortly thereafter. Variations in the mass of the ventricles and the atria were also of the same magnitude.

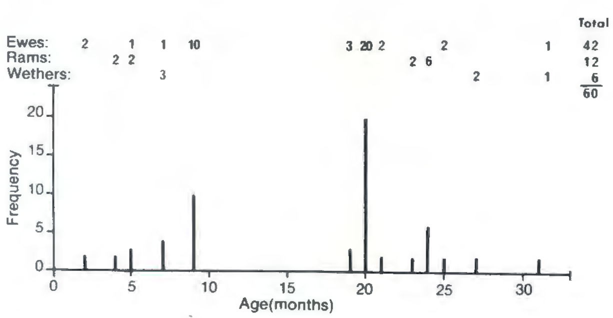

FIG. 1 Representativeness of sheep used

The means, standard deviations, coefficients of variation and the ranges for all measurements are given in Table 1. It is clear that the values for body mass, LV+S, RV and atria were considerably more variable than those for the other measurements. It must be remembered, however, that the values for the valve rings are not continuous, since they were measured by means of a graduated device.

Of real importance is the fact that the ratio, LV+S/RV, showed little variation over all ages and masses of sheep. Statistics relating to the normality of all

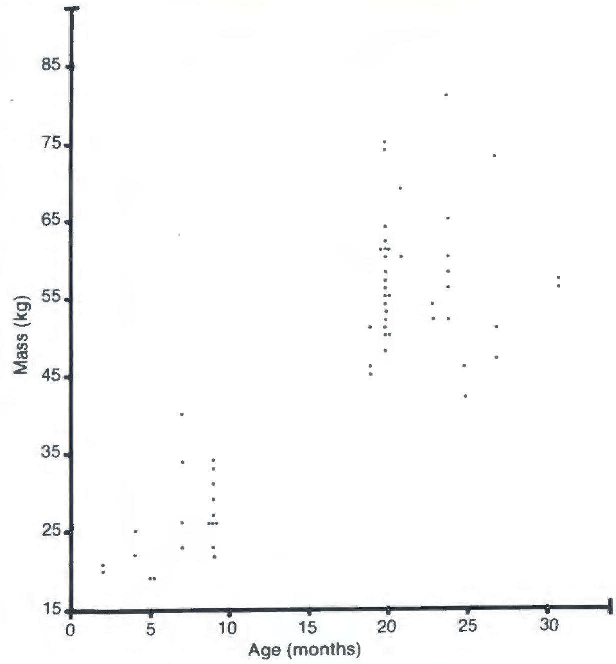

FIG. 2 Relation between mass and age of sheep used

TABLE 1 Statistics for all measurements

Measurement	Mean	Standard deviation	Coefficient of variation (%)	Range
Mass	46,73	16,500	35,3	19–81
LV+S	83,62	30,318	36,3	21–81
RV	24,78	8,834	35,6	7–45
Atria	15,18	5,318	35,0	4–26
LV+S/RV	3,37	0,274	8,1	2,85–4,13
Length	7,03	0,930	13,2	4,3–8,4
RAV	17,70	1,645	9,3	13,4–22,0
LAV	15,39	1,047	6,8	12,2–18,2
PV	13,03	0,595	4,6	12,0–14,2
AV	13,07	0,628	4,8	12,0–14,2

TABLE 2 Statistics relating to normality for all measurements

Measurements	Chi-squared	Degrees of freedom	Skewness	Kurtosis
Age	25,751**	4	−0,41	−1,06
Mass	15,789**	4	−0,20	−1,03
LV+S/RV	5,813	4	−0,37	−0,86
RV	4,517	3	−0,21	−0,77
Atria	4,564	4	−0,38	−0,77
LV+S/RV	2,663	3	0,61	0,36
Length	13,818**	3	−0,93	0,42
RAV	2,974	3	−0,08	−0,31
LAV	1,486	2	0,14	0,50
PV	51,578**	5	−0,17	−0,99
AV	41,137**	5	0,05	−1,25

** = Significant at 1 %

measurements are given in Table 2. Significant chi-squared values indicate non-normality. Skewness and kurtosis are also indicators of normality and these values should approach zero for a good approximation of the normal distribution. Positive skewness indicates bunching of values below the mean and a long tail above. Positive kurtosis again indicates a very peaked distribution. That non-normality was evident for age and mass was to be expected, since these 2 variables are correlated and because the sheep used, being randomly drawn from a large population, were not a normal sample. The non-normality for the length of the heart is somewhat unexpected. The valve measurements are not continuous variables and, especially for PV and AV, some measurements within the range did not occur. Possibly this may be due to the absence of sheep between the ages of 10–18 months.

Simple correlation coefficients between all measurements were calculated, and these are given in Table 3. As was expected, all measurements showed correlation to some degree or another, except for the ratio, LV+S/RV. The mass measurements and also the length of the hearts were more highly correlated than were the valve measurements.

The primary aim of this study was to describe the heart measurements which can be used as a reference base for good indicators of pathological changes in the ovine heart due to cardiotoxic agents. In such a context it is important to determine how many sheep must be present in a sample to distinguish a change in a measurement of a certain magnitude by means of a hypothesis test. The formula given by Southwood (1966) was used to determine this quantity for all heart measurements:

$$N = (t \cdot s/d \cdot \bar{x})^2$$

where t = the tabled t-value at $n-1$ degrees of freedom,
s = the standard deviation,
d = the change or level of accuracy as a decimal (e.g. 0,1 for 10 %)
and $\bar{x}$ = the mean.

Normality for all measurements was assumed.

The calculated numbers of sheep needed (N) are given in Table 4. For the measurements LV+S, RV and atria rather large samples are needed to distinguish small changes of the mean. For the ratio, LV+S/RV, a relatively very small sample is needed for similar changes of the mean, and the same is true for the valve measurements.

Light microscopy

In most of the hearts, scattered, small, subepicardial haemorrhages were present. In many of the hearts, occasional small focal accumulations of lymphocytes were observed in the epicardium, endocardium and myocardial interstitium.

TABLE 4 Number of sheep needed per sample for hypothesis tests

Measurement	Percentage change of the mean				
	25	20	15	10	5
LV+S	9	14	24	52	210
RV	9	13	23	51	204
Atria	8	13	22	50	197
LV+S/RV	1	1	2	3	11
Length	2	2	4	8	16
RAV	1	1	2	4	14
LAV	1	1	1	2	8
PV	1	1	1	1	4
AV	1	1	1	1	4

DISCUSSION

The hearts were accepted as normal since there was no history of previous disease in the sheep from which they were collected, and light microscopical pathological alterations in the hearts were absent or minimal.

We believe that the method of separating and determining the mass of the ventricles, based on that suggested by Fulton *et al.* (1952), was appropriate to this study of ovine hearts. In these sheep hearts and in others examined previously there was no evidence of any line of demarcation by which to separate the septum into left and right ventricular portions (Newsholme & Howerth, 1982, unpublished observations). Separation of the right ventricular free wall from the septum was easy and clearly defined. Clear evidence of the occurrence of disproportionate ventricular septal hypertrophy without left ventricular hypertrophy has not been reported in acquired cardiac disease in sheep.

It is unlikely that the period of immersion in 10 % formalin (2–3 weeks) would have had any important effect on the heart masses. Hultgren *et al.* (1963) recorded a mass loss of less than 5 % in hearts fixed in formalin for up to 6 weeks, and Fulton *et al.* (1952) found the variation in heart mass to be less than 2 % after 4 days in this fixative.

Although good correlations between the mass measurements of the various parts of the heart are evident, the variation is still considerable. It seems unlikely, therefore, that these measurements will be good indicators of pathological changes due to cardiotoxic agents. Correlation between the cardiac mass measurements and live mass might have been improved if variations in certain factors which affect live mass, such as the mass of the fleece and of the digestive tract contents, had been minimized. The ratio, LV+S/RV, on the other hand, is

TABLE 3 Correlation matrix for all measurements

Measurement	Age	Mass	LV+S	RV	Atria	LV+S/RV	Length	RAV	LAV	PV
Mass	0,835**	—								
LV+S	0,732**	0,920**	—							
RV	0,707**	0,897**	0,975**	—						
Atria	0,696**	0,871**	0,954**	0,948**	—					
LV+S/RV	0,234	0,240	0,268*	0,057	0,197	—				
Length	0,751**	0,792**	0,872**	0,840**	0,857**	0,285*	—			
RAV	0,544**	0,564**	0,602**	0,574**	0,585**	0,218	0,509**	—		
LAV	0,235	0,454**	0,531**	0,519**	0,535**	0,159	0,415**	0,573**	—	
PV	0,206	0,295*	0,394**	0,403**	0,444**	0,058	0,417**	0,332**	0,279*	—
AV	0,378**	0,538**	0,591**	0,558**	0,618**	0,234	0,507**	0,583**	0,447**	0,606*

** = Significant at 1 %
* = Significant at 5 %
— = Relation above 80 %
—– = Relation between 49 and 80 %

very stable and uncorrelated with the other measurements. This ratio might well be most useful to assess right ventricular hypertrophy. Hultgren *et al*. (1963) expressed the mass of the right ventricular free wall as a percentage of total ventricular mass in normal sheep kept at sea-level and at moderate altitude. The mean value they obtained was 21,7 % for the 41 hearts studied. When our results are expressed in this way (RV × 100/RV+LV+S), the mean (21,6 %) is very close to this, indicating good conformity between the results of the 2 studies. (The elevation of Onderstepoort is approximately 1200 m above sea level).

The possible usefulness of the valve measurements will become apparent only when hearts of sheep affected by cardiotoxic agents are compared with those with normal hearts. Although the ratio, LV+S/RV, alone might be sufficient to indicate certain cardiotoxic effects, the use of discriminated analysis, incorporating all or several of the measurements for separating hearts of affected sheep from normal ones, should not be disregarded.

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