

Density and Water Content of Dog Ventricular Myocardium¹ (36927)

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(Introduced by D. E. Donald)

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There is little information on density of myocardial tissue; the value most commonly quoted is that of Creese (1) which was for the rat diaphragm. Herd *et al.* (2) provided a single value of 1.05 g/ml for the left ventricle of the dog; Allen, Krzywicki and Roberts (3) reported the density of fat-free myocardium of rats, guinea pigs or rabbits as 1.054 or 1.058 at 27 or 37°. In this laboratory, Richmond, La Force and Bassingthwaight (4) found values in cats of 1.057 g/ml (SD = 0.006, $N = 9$) for left ventricle and 1.053 g/ml (SD = 0.002, $N = 6$) for right ventricle at 23°, with a mean water content of 0.796 g/g (SD = 0.016, $N = 6$) for right ventricle.

In view of the widespread use of the dog as an experimental animal, particularly for studies on coronary flow, it seemed pertinent to provide data on densities of myocardium from various locations in canine ventricles. Since densities depend on the content of solid, fat, and water, we also estimated water content on myocardial pieces adjacent to those whose density was measured.

The appendix provides a general approach to the relationship between density and tissue composition.

Materials and Methods. Hearts were excised quickly from dogs killed by pentobarbital at the end of experiments done by our colleagues wherein the cardiovascular system was not excessively disturbed, or after exsanguination under the anesthesia (when only

the blood was used for other experiments). The hearts were not used if they showed macroscopic extravasation of blood or if the previous history of the dog suggested illness or dehydration.

The density was measured by the copper sulfate method of Phillips *et al.* (5) at 22.5 to 23.5° using a series of solutions with densities ranging from 1.048 to 1.070 at increments of 0.001. These solutions were replaced after about 20 determinations.

The hearts were sliced into portions containing anterior wall of right ventricle, anterior wall of left ventricle, apex of left ventricle, and septum. Each piece was washed in isotonic saline and lightly dabbed with absorbent paper so that the surface was devoid of blood. About 1–2 mm of the endocardial and epicardial surfaces were removed with a razor blade and the remainder cut into 2 to 4 mm cubes of tissue. The cubes were placed in the copper sulfate solutions, and the density of each taken to be that of the solution in which the cube neither sank nor floated to the surface.

For the water content, a larger piece, varying from 1 to 10 g, and also free of the epicardial and endocardial layers, was weighed (wet weight) and then dried at 70° for more than 48 hr. Previous study had shown that, after this interval, no further significant weight loss occurred with pieces of this size. From the dry weight, water content was calculated: $1 - \text{dry wt/wet wt} [(g/g) \text{ or } (ml/g) \text{ since the density } (0.9976 \text{ g/ml) of water at } 23^\circ \text{ is so close to unity}]$.

Results. In 41 hearts the regional variations were small and regional differences were not significant (see Table I). Densities averaged 1.063 g/ml (± 0.002 , $N = 160$; SD, no. of observations) and water contents, 0.777

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TABLE I. Myocardial Density (ρ) and Water Content (W).^a

Site	N	$\rho \pm \text{SD}$ (g/ml)	$W \pm \text{SD}$ (g/g)	Regression: $1/\rho = aW + 1/\rho_s$				
				$1/\rho_s$	a (slope)	$\text{SD}_{y \cdot x}$	$\text{SD}_{x \cdot y}$	r
RV	41	1.062 ± 0.003	0.775 ± 0.013	0.238	0.909	0.002	0.013	0.018
LV Ant. wall	40	1.063 ± 0.002	0.777 ± 0.014	0.823	0.151	0.001	0.012	0.504
LV apex	38	1.063 ± 0.002	0.777 ± 0.009	0.763	0.229	0.002	0.007	0.617
Septum	41	1.063 ± 0.002	0.779 ± 0.008	0.762	0.229	0.001	0.006	0.560
All samples	160	1.063 ± 0.002	0.777 ± 0.011	0.691	0.322	0.002	0.011	0.284

^a The linear correlation was calculated for the relationship between $1/\rho$ and W , this will result in a Y-intercept of $1/\rho_s$ and a slope of $(1/\rho_w - 1/\rho_s)$ where the subscripts refer to water (w) and solids (s), which is the type of equation shown in the inset in Fig. 1.

g/g (± 0.011 , $N = 160$). Expressions of the data in terms of linear regression equations of water contents and $1/\text{densities}$ are also provided. The widest scatter was seen with samples from the right ventricle (dots in Fig. 1), and these also showed the lowest correlation (r). Using the t distribution, in which t is obtained from $t = [r^2(n-2)/(1-r^2)]^{1/2}$, the correlation coefficient is significantly different from 0 ($p < .001$) in the left ventricular and septal samples (x in Fig. 1) and also for the whole group.

Discussion. The densities of canine myocardium were higher and the water content lower than those observed by Richmond, La Force and Bassingthwaighe (4) for cat right ventricular myocardium. In Richmond, La Force and Bassingthwaighe's study the cat

myocardium contained endocardium and epicardium, and had been immersed for a few hours in Tyrode's solution. Their observations are compatible with the regression relationship found for our data and illustrated in the inset in Fig. 1. Similarly, the values of Allen, Krzywicki and Roberts (3) fall close to our average regression line. Our values for water content are similar to those of Griggs, Tchokoev and Chen (6) for outer (0.779 ± 0.005), middle (0.782 ± 0.003) and inner (0.779 ± 0.004) portions of canine myocardium from 8 dogs, and to those of Yipintsoi and Bassingthwaighe (7) (0.76 ± 0.01 , $N = 10$).

The data in Fig. 1 show that the samples with above average water content have less than average densities. This suggests that the variation from sample to sample is predominantly in water content, and not in fat content. (With variation in fat content, and with constancy of the ratio of water to nonfatty solids, lower water contents associated with higher fat contents would give lower densities: see Appendix.)

The observed relationships summarized by the regression equations in the table and by the regression line for the whole data in Fig. 1 are similar to those calculated for fat-free soft tissues by Allen *et al.* (3, 8). Our data suggest that the density of solids contained in the heart (the ordinate intercept $1/\rho_s$ on the inset in Fig. 1 obtained by extrapolation from the water point, $1/0.9976$, through the mean ρ and W) averaged 1.38 ± 0.04 g/ml. This value is slightly different from that of Allen, Krzywicki and Roberts (3) for the heart

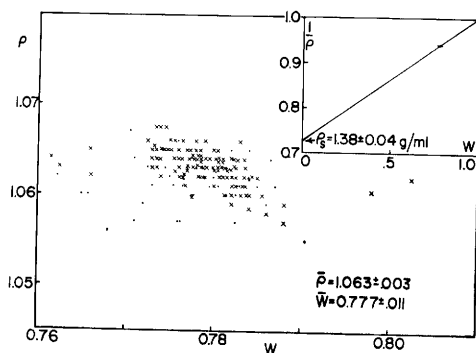


FIG. 1. The relationship between myocardial density (ρ) and water content (W). (•) right ventricle; (×) left ventricular wall and septum. The inset shows a plot of $1/\rho$ (ml/g) against W (g/g) where for $W = 1.0$, $1/\rho = 1/0.9976$ at 23° , and the present result gives the point for W of 0.777 and $1/\rho$ of 0.941.

(1.36 and 1.40) and for all the different organs (1.39). This difference might be in part explained by the fat content which we have not taken into account. If we assume a fat density of 0.901 (3, 8) and a density of fat free solid (8) of 1.40, our value of 1.38 g/ml would indicate a fat content of 2% of total solids or 0.6% of wet weight, which is quite compatible with the observations of Dible (9), and suggests that the value of Allen, Krzywicki and Roberts (3) of 1.40 at 37° for the density of fat-free solids is reasonable for the heart. The heart mineral content is fairly low, so both our 1.38 and their 1.40 are not much higher than the value of 1.34 g/ml found by Haurowitz (10) for the "apparent density" of protein (which is the reciprocal of the protein specific volume at the minimum level of hydration permitting solution).

Densities are most clearly interpreted when the water content is known. On the other hand, in tissues of constant fat and solid content, density may provide a good measure of water content. More importantly, accurate values for density are required for obtaining correct expressions for blood flow per unit mass or volume of tissue. This becomes quite obvious when one applies compartmental analysis to a tissue such as bone where the density is great (11) and is even more pertinent when the partition coefficients for the tissue are dependent on the tissue density and composition.

Appendix. The inset in Fig. 1 can be expressed in more general form to take into account water, fat, and nonfat solids, which we will call protein. The tissue density ρ is the mass m divided by the volume V :

$$\rho = \frac{m}{V} = \frac{m_w + m_p + m_f}{V_w + V_p + V_f}, \quad (\text{A1})$$

where the subscripts refer to water, protein, and fat. Substituting m_w/ρ_w for V_w , etc., in the denominator and denoting the fractional masses (g/g of tissue) as W , P , and F , (e.g., $W = m_w/(m_w + m_p + m_f)$) provides equations defining ρ for all tissue compositions:

$$W + P + F = 1, \quad (\text{A2})$$

$$\frac{W}{\rho_w} + \frac{P}{\rho_p} + \frac{F}{\rho_f} = \frac{1}{\rho}. \quad (\text{A3})$$

Using Eq. (A2) to substitute for P , Eq. (A3) can be rewritten in a form that is appropriate for a constant F with variable water and solid content:

$$\frac{1}{\rho} = \left(\frac{1}{\rho_w} - \frac{1}{\rho_p} \right) W + \frac{1}{\rho_p} + \left(\frac{1}{\rho_f} - \frac{1}{\rho_p} \right) F, \quad (\text{A4})$$

where the slope of the W , $1/\rho$ relationship is $1/\rho_w - 1/\rho_p$ and the intercept is composed of the final two terms. Using the means of the data for ρ and W and taking $\rho_w = 0.9976$, $\rho_p = 1.400$, and $\rho_f = 0.901$ at 23°, Eqs. (A2) and (A3) can be solved to give $F = 0.006$ and $P = 0.217$ g/g, or, for all water contents, $F/P = 2.92\%$, and $F/(F + P) = 0.0286$ g fat/g total solids, which is comparable to Dible's data (9) on the human heart. Using $F = 0.006$, Eq. (A4) becomes:

$$\frac{1}{\rho} = 0.288W + 0.717. \quad (\text{A5})$$

The experimental line drawn in the inset of Fig. 1 through the mean of the data and the water point is:

$$\frac{1}{\rho} = 0.280W + 0.725, \quad (\text{A6})$$

which is very similar to the approximate equation for small constant F , Eq. (A5). The best fitting regression line for all samples, taken from Table I, does not take advantage of the information given by the known density of water, but is still quite similar:

$$\frac{1}{\rho} = 0.322W + 0.691. \quad (\text{A7})$$

A somewhat different, and in some ways simpler, point of view is obtained by considering not the fractional masses W , P , and F , but the fractional volumes W' , P' , and F' (ml/ml tissue). Substituting $\rho_w \cdot V_w$ for m_w , etc., in the numerator of Eq. (A1) provides a pair of equations in which ρ is linear rather

than reciprocal:

$$W' + P' + F' = 1, \quad (\text{A8})$$

$$\rho_w W' + \rho_p P' + \rho_f F' = \rho. \quad (\text{A9})$$

One will find that $W' = \rho \cdot W / \rho_w$, *etc.*, and substitution in Eq. (A8) yields Eq. (A3), in Eq. (A9) yields Eq. (A2). Now isodensity lines (constant ρ) are linear on a triangular plot with W' , P' , and F' as the axes. The data on ρ and W' can be plotted directly on such a graph. With density values chosen as given above, the data indicate the very low fat content directly, and a straight line from $W' = 1$ through the data, which is a line of constant F/P , intercepts the $W' = 0$ axis at $\rho = 1.38$. This value for ρ at $W' = 0$ is not independent of the value obtained in the inset in the figure but does show that Richmond's and our experimental data on ρ and W' , the values for ρ_w , ρ_f , and ρ_p , and Dible's value for F provide a self-consistent logical structure which is likely to be correct and is simply described by Eqs. (A8) and (A9).

1. Creese, R., Proc. Roy. Soc., Ser. B **142**, 497 (1954).
2. Herd, J. A., Hollenberg, M., Thorburn, G. D., Kopald, H. H., and Barger, A. C., Amer. J. Physiol. **203**, 122 (1962).
3. Allen, T. H., Krzywicki, H. J., and Roberts, J. E., J. Appl. Physiol. **14**, 1005 (1959).
4. Richmond, D. R., La Force, R. C., and Bassingthwaight, J. B., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 322, Abstr. (1970).
5. Phillips, R. A., Van Slyke, D. D., Dole, V. P., Emerson, K., Jr., Hamilton, P. B., and Archibald, R. M., Bull. U.S. Army Med. Dept. **71**, 66 (1943).
6. Griggs, D. M., Jr., Tchokoev, V. V., and Chen, C. C., Amer. J. Physiol. **222**, 705 (1972).
7. Yipintsoi, T., and Bassingthwaight, J. B., Circ. Res. **27**, 461 (1970).
8. Allen, T. H., Welch, B. E., Trujillo, T. T., and Roberts, J. E., J. Appl. Physiol. **14**, 1009 (1959).
9. Dible, J. H., J. Pathol. Bacteriol. **39**, 197 (1934).
10. Haurowitz, F., "Chemistry and Biology of Proteins," Academic Press, New York (1950).
11. Kelly, P. J., Yipintsoi, T., and Bassingthwaight, J. B., J. Appl. Physiol. **31**, 38 (1971).

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