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Alteration of the Gross Dimensions of the Heart and its Structures by Formalin Fixation*

A Quantitative Study

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Veränderung der Größendimensionen des Herzens und seiner Strukturen durch Formolfixierung

Eine quantitative Studie

Zusammenfassung. Die Veränderungen von Herzgröße und Herzform durch Formolfixierung sind mit und ohne Vorbehandlung zum Zwecke der Lösung der Totenstarre untersucht worden. 21 äußere und innere Meßreihen wurden vorgenommen a) an Herzen, die vor Fixierung, b) an solchen, die nach Fixierung zergliedert worden waren und c) an durch kontrollierte Druckperfusion dargestellten Herzen. Die Totenstarre konnte *nicht* gelöst werden, weder durch Einlegung des Materiales in kalte Salzlösung, noch durch Einfrierung oder Auftauung. Die erwähnte Druckfixierung konnte durch Druckperfusion über die großen Arterien am untergetauchten Objekt vereinfacht werden. Diese Methode erbrachte einen besseren Erhaltungszustand des Materiales für quantitative und qualitative Meßwerte und zeigte sehr viel geringere Streuwerte als bei konventioneller Fixation.

Summary. The alteration of cardiac size and shape by formalinfixation have been investigated with and without pretreatment to lyse the rigor mortis. Twenty-one exterior and interior measurements were taken in hearts a) dissected before immersion fixation, b) dissected after immersion fixation c) fixed by controlled pressure perfusion. Rigor mortis could not be lysed by soaking in cold saline or freezing and thawing of the specimen. The previously recommended pressure fixation could be simplified to pressure perfusion of the great arteries in the submerged specimen. This method produced a better preservation of the specimen for quantitative and qualitative evaluation, with less variation than introduced by conventional methods of fixation.

With the advent of angiocardiography, cardiac catheterization and open heart surgery, interest in standardized and precise post mortem measurements of the heart has been revived; the physiologist, radiologist, cardiologist and surgeon seek the best possible correlation of their findings with those of the anatomist.

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Data on the dimensions of the human heart and its component structures were first published in the middle of the 19th century. None of the early authors, such as BIZOT (1837), REID (1843), or PEACOCK (1854), provided values based on measurements of large numbers of hearts. This led FRIEDREICH (1861) to observe that in the absence of adequate data, "experience and a well-trained eye could make up for the lack of precise objective criteria". By the turn of the century, however, useful tables of measurements of fresh hearts were published by BENEKE (1878) and VIERORDT (1898). KOCH (1922) compared cardiac dimensions before and after "contraction" by rigor mortis. More recently, systematic studies of the dimensions of both fresh and fixed children's hearts have been stimulated by advances in the diagnosis and surgery of congenital cardiac malformations (DE LA CRUZ, ANSELMI, ROMERO and MONROY, 1960; SCHULZ and GIORDANO, 1962; LEV, ROWLATT and RIMOLDI, 1961; ROWLATT, RIMOLDI and LEV, 1963).

Hearts obtained at autopsy are usually opened according to a standard procedure, weighed and measured. Diseased hearts are often immersed in a fixative for further study and subsequent additional measurements and observations are usually made. There is no evidence that either flaccid or contracted post mortem hearts are adequate as standard anatomic preparations; nor is there any reason to prefer a specimen fixed by immersion before opening. Fixation by coronary perfusion with chambers distended at controlled pressures provides specimens with remarkably well preserved anatomic relationships and details (GLAGOV, ECKNER and LEV, 1963). However, the dimensions of such preparations may not necessarily correspond to those in the living heart.

As part of an extensive investigation of factors which are likely to result in differences between clinical estimations of cardiac size and shape and post mortem appearances, we subjected pig hearts to several modes of fixation following various common pre-fixation conditions which may influence the degree of myocardial rigor mortis. Pig hearts were used because the range of their weights approximated that found in man and large numbers could be obtained easily shortly after slaughter. The data presented in the present communication indicate that 1. dimensions of preserved hearts fixed before chambers are opened differ greatly from those of hearts which were fixed after opening, 2. each of these procedures was associated with considerable variation in some of the dimensions, 3. fixation by controlled pressure perfusion of the coronary arteries and simultaneous controlled pressure pulmonary artery trunk distention yielded specimens with the least variation in dimensions, and 4. neither protracted immersion in isotonic saline solution nor freezing and thawing resulted in any significant lysis of rigor mortis. It is hoped that the data provided in this report may help to improve the correlation of post mortem with ante mortem measurements and provide a basis for reconciling discrepancies which may arise from differences in post mortem procedures.

Materials and Methods

Hearts and attached sets of hearts and lungs of pigs were obtained within 4 hours after slaughter from a local meat packing company and brought back to the laboratory. Organs damaged during evisceration were rejected. All of the hearts were contracted by rigor mortis to about the same degree. Each specimen was marked by a numbered metal staple attached to the cut edge of the aorta.

Relation of Prefixation Conditions and Degree of Dissection before Fixation to Cardiac Dimensions

Sixty hearts weighing 200 to 300 g each were used for this study; all were eventually fixed by immersion in buffered 10% formalin solution in individual containers. There were 3 prefixation treatment groups intended to simulate the usual range of conditions to which autopsied hearts may be subjected prior to fixation. Thus, 20 were fixed without any previous treatment (*fresh-fixed group*); 20 were stored in isotonic saline solution at 5 to 10° C for 24 hours and then fixed (*cold-saline group*); 20 were subjected to rapid freezing at —20° C, thawed to room temperature after 24 hours and then fixed (*freeze-thaw group*). Ten hearts in each of these prefixation treatment groups were opened by means of the usual incisions (Lev, Rowlatt and Rimoldi, 1961) before pretreatment and fixation. The others were fixed unopened after each of the pretreatments. The hearts were considered to be fixed completely after 7 days. On the 8th day, those which were immersed unopened were opened by the usual incisions.

Comparison of Several Modes of Controlled Pressure Perfusion-Fixation

A total of 52 heart and lung sets were used for this part of the study. All of the specimens were stored in cold isotonic saline at 5 to 10° C for 24 hours prior to fixation. The heart weights in this group ranged from 200 to 450 g. Three different modes of controlled pressure fixation were used: 20 hearts were fixed by simultaneous controlled pressure distention of the aorta at 100 mm Hg and the pulmonary artery at 25 mm Hg; 23 hearts were fixed by simultaneous controlled pressure distention of the aorta, the pulmonary artery and each ventricle at 5 mm Hg; 9 hearts were fixed by simultaneous controlled pressure distention of aorta, pulmonary artery, ventricles and atria at 2 mm Hg. The apparatus for fixing hearts under conditions of controlled pressure has been described in detail elsewhere (Glagov, Eckner and Lev, 1963). All of the hearts were immersed in a formalin bath during the distention and perfusion process; fixation by coronary artery perfusion was complete in two hours.

Measurements. The hearts were weighed on a Toledo spring balance to the closest 5 g interval. At the time of opening, blood clots were removed from the chambers, weighed and the original weights corrected. Weighings were repeated daily for the first 7 days after immersion in formalin, then weekly for 3 weeks and then monthly for 4 months.

Seven exterior and 14 interior dimensions were measured in each heart according to the method previously given by Lev, Rowlatt and Rimoldi (1961). The following lengths were measured, usually in the sequence indicated:

Exterior. 1. Anterior length from right base of the pulmonary trunk to apex (al).
2. Posterior length from midbase of ventricles to apex (pl).
3. Length of the acute margin from ventricular base to apex (am).
4. Length of the obtuse margin from ventricular base to apex (om).
5. Greatest transverse circumference of the heart standing on its apex (tc).
6. Right ventricular portion of the greatest transverse circumference (rvc).
7. Left ventricular portion of the greatest transverse circumference (lvc).

The *exterior* measurements were made on hearts before and after fixation.

Interior. 1. Right ventricular wall: 0.5—1.0 cm below pulmonic orifice (RV—P).
2. Right ventricular wall: 0.5—1.0 cm below tricuspid orifice (RV—T).
3. Left ventricular wall maximum thickness (LV—M).
4. Tricuspid valve circumference (TV).
5. Pulmonic valve circumference (PV).
6. Mitral valve circumference (MV).
7. Aortic valve circumference (AV).
8. Length of right ventricular inflow tract-tricuspid annulus to apex (TA).
9. Length of right ventricular outflow tract-pulmonic valve to apex (PA).
10. Perimeter of right ventricular inflow tract-midway between tricuspid valve and apex (PRV_i).
11. Perimeter of right ventricular outflow tract-level of the arch of the crista (PRV_o).
12. Length of left ventricular inflow tract-mitral annulus to apex (MA).
13. Length of left ventricular outflow tract-aortic valve to apex (AA).
14. Perimeter of left ventricle-midway between valve orifice and apex (PLV).

Interior measurements were obtained before and after fixation for those hearts which were opened before fixation, but could be obtained after fixation only for those which were fixed unopened.

In order to estimate the error inherent in identifying reference points and in making measurements of length, all 21 measurements were made 9 times each on each of 5 randomly chosen unfixed hearts by one of us (FAOE). The exterior measurements were made on each unopened heart in the sequence shown above. The hearts were offered to the observer at random for repetition of the sequence of measurements until 9 sets of measurements for each heart were obtained. The hearts were then opened and the same procedure repeated for the internal measurements.

Statistical Analyses. Probit plots of representative measurements indicated that the ratio *post-fixation measurement/fresh measurement* seemed to be more nearly normal in distribution than either the difference of the two or the logarithm of the ratio. Consequently, this statistic was the one used to measure the pretreatment-fixation effect. Confidence limits for the ratios of means were obtained by Feiller's method (FINNEY, 1952).

For the 52 hearts used to compare the modes of controlled pressure perfusion-fixation, data were analyzed as follows:

a) Analysis of variance (BMDOIV) for one-way design version of June 11, 1964, Health Sciences Computing Facility, UCLA for all 52 hearts using means of outside and inside measurements.

b) Analysis of ratio of the means fixed/fresh for hearts up to 300 g (as above) versus immersion fixed hearts of the saline pretreatment groups.

Results

Relation of Prefixation Conditions and Fixation to Heart Weight

Although formalin fixation altered heart weights, neither the mode of fixation nor the prefixation conditions had any demonstrable effect. The weights of all fixed hearts increased gradually during the first week, reaching a maximum at 7 days; at that time weights were about 10% greater than fresh weight. During the second and third weeks of fixation weights decreased; all hearts returned to the fresh weight by the end of three weeks. From then on a slight weight loss was found during the next 5 months. These findings are essentially the same as reported by HORT (1961).

Error of Measurements of Heart Dimensions: Reliability of Reference Points

Analysis of the data obtained by making all of the 21 length measurements on each of nine unfixed hearts revealed that the error of measurement was least for valve orifice circumferences and greatest for wall thicknesses. The mean standard deviations and the per cent deviation from the mean of the standard deviations for each of the measurements are given in Table 1. The per cent deviation of two standard deviations ranges from only 3 to 4 per cent for the valve circumferences and 3 to 7 per cent for inside ventricular measurements. However, outside measurement deviations range from 3 to 12 per cent of the mean and wall thicknesses from 8 to 13 per cent.

Relation of Prefixation Conditions and Degree of Dissection before Fixation to Cardiac Dimensions

One and two way analyses of variance were done in the hope of simplifying the data with respect to the prefixation conditions (*fresh*, *cold saline* or *freeze-thaw*)

Table 1. *Error of measurement of dimensions of 5 unfixed hearts*

Dimensions	Mean ± S. D. of 9 measurements of each dimension on each heart	Per cent deviation of 2 S. D.
Anterior length (al)	11.628 ± 0.222	3.78
Posterior length (pl)	7.860 ± 0.136	3.45
Acute margin length (am)	9.444 ± 0.308	6.52
Obtuse margin length (om)	9.192 ± 0.214	4.65
Transverse circumference (tc)	23.216 ± 0.377	3.25
Right ventricle circumference (rvc)	11.814 ± 0.447	7.57
Left ventricle circumference (lvc)	11.246 ± 0.669	11.89
Right ventricle wall[a] (RV-P)	0.630 ± 0.029	9.05
Right ventricle wall[b] (RV-T)	0.634 ± 0.040	12.62
Left ventricle wall (LV-M)	1.542 ± 0.065	8.43
Tricuspid valve (TV)	10.192 ± 0.212	4.16
Pulmonic valve (PV)	6.308 ± 0.100	3.17
Mitral valve (MV)	9.261 ± 0.139	3.00
Aortic valve (AV)	6.042 ± 0.122	4.04
Right ventricle inflow (TA)	5.806 ± 0.090	3.10
Right ventricle outflow (PA)	8.218 ± 0.279	6.79
Perimeter right ventricle[c] (PRV_i)	8.552 ± 0.314	7.34
Perimeter right ventricle[d] (PRV_o)	8.028 ± 0.231	5.75
Left ventricle inflow (MA)	7.566 ± 0.158	4.18
Left ventricle outflow (AA)	7.532 ± 0.219	5.82
Perimeter left ventricle (PLV)	10.310 ± 0.332	6.44

[a] Below pulmonic valve.
[b] Below tricuspid valve.
[c] At level of inflow tract-midway between apex and TV.
[d] At level of outflow tract-lower margin of crista supraventricularis.

and the degree of dissection before fixation (*opened before fixation* or *opened after fixation*). Since all effects and interactions were significant, this simplification could not be obtained. The effects of prefixation conditions and degree of dissection before fixation on cardiac dimensions are compared in Fig. 1. The ratio of the mean of the measurements on the *fixed* hearts to that of the mean of measurements of unfixed (*fresh*) hearts is shown for each dimension and prefixation condition (*fresh, cold saline* and *freeze-thaw*).

Hearts Opened before Fixation. In general, the outside measurements which include the right ventricle (al, am, tc and rvc) showed greater shrinkage after fixation than those involving the left ventricle (pl, om and lvc). The right ventricle wall (RV-P and RV-T) was 20 to 25 per cent thicker after fixation, while left ventricular wall thickness (LV-M) increased only 5 to 10 per cent. Valvular orifice circumferences (TV, PV, MV and AV) were between 6 and 20 per cent smaller than in fresh hearts. After fixation the right inflow tract was approximately 10 per cent shorter (TA) and its circumference PRV_i was 10 to 20 per cent less in circumference (PRV_i); the length of the right outflow tract (PA) was relatively unchanged but its circumference (PRV_o) reduced by 6 to 12 per cent. The length

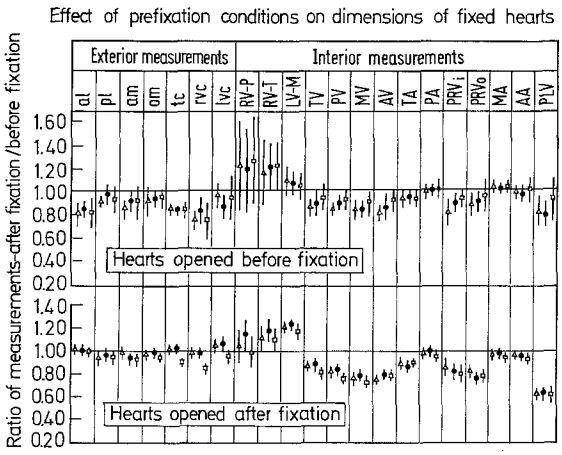

Fig. 1. Ratios of post fixation measurements to fresh measurements are computed for hearts opened before fixation with or without pretreatment. Similarly ratios of post fixation measurements to the previously used fresh mean measurements are computed for hearts opened after fixation. The graph shows the means of the ratios with the 95% population confidence intervals. △ No pretreatment before fixation. • Immersion in cold saline solution before fixation. □ Frozen and thawed before fixation

of the left ventricle (MA, AA) was not appreciably modified by fixation, but the circumference (PLV) decreased by 20 to 23 per cent. Variation within the sample obscured possibly present effects of prefixation conditions.

Hearts Opened after Fixation. Except for groups which were frozen and then thawed before fixation, outside measurements of hearts fixed before opening did not differ from those of fresh hearts. Right ventricular wall thickness (RV-P and RV-T) was increased by fixation depending on prefixation conditions; the increase was somewhat less in hearts opened after fixation than in hearts opened before fixation. However, left ventricular walls were somewhat thicker in hearts opened after fixation than in those opened before fixation. Valve orifices were smaller by 12 to 25 per cent. Fixation reduced ventricular chamber volumes much more when fixation preceded opening than when hearts were opened first. The circumference at the right ventricular conus (PRV_o), for example, was 20 per cent less than in fresh hearts; the left ventricle (MA and AA) was 5 per cent shorter and the circumference (PLV) 30 to 35 per cent less than in fresh hearts. In most instances the ventricular chamber was reduced to a cleft in hearts fixed before opening.

Fixation by Controlled Pressure Perfusion

Significant differences between the 3 procedures of fixing hearts by controlled pressure perfusion were found only in 2 of 21 measurements. One was the length of the obtuse margin of the heart. The second was the circumference of the pulmonic orifice. The latter could be explained by the fact that we were unable to cannulate all pulmonary arteries due to their shortness in some specimens. In Table 2 means and standard deviations are compared for the three procedures, i.e. controlled pressure in *pulmonary artery and aorta* (I), in *pulmonary artery*,

Table 2. *Effect of chamber distention during fixation by coronary perfusion*

Dimension	Distended during fixation			F
	Ao. and P. A.	Ao., P. A. and vent.	Ao., P. A., vent. and atria	
Anterior length (al)	12.500 ± 1.072	12.739 ± 0.988	12.222 ± 0.533	0.937
Posterior length (pl)	8.550 ± 0.820	8.978 ± 0.764	8.388 ± 0.808	2.327
Acute margin length (am)	9.925 ± 0.779	10.760 ± 0.689	10.444 ± 0.725	5.789
Obtuse margin length (om)	9.975 ± 0.798	10.043 ± 0.764	9.833 ± 0.334	0.258
Transverse circumference (tc)	25.700 ± 1.511	26.413 ± 1.666	25.500 ± 0.623	1.501
Right ventricle circumference (rvc)	13.225 ± 0.798	14.000 ± 1.197	13.833 ± 0.850	2.382
Left ventricle circumference (lvc)	12.475 ± 1.054	12.413 ± 0.941	11.555 ± 0.863	2.631
Right ventricle wall (RV-P)	0.750 ± 0.112	0.787 ± 0.080	0.777 ± 0.083	0.856
Right ventricle wall (RV-T)	0.860 ± 0.092	0.873 ± 0.096	0.833 ± 0.100	0.587
Left ventricle wall (LV-M)	1.857 ± 0.136	1.881 ± 0.166	1.855 ± 0.113	0.268
Tricuspid valve (TV)	9.400 ± 0.607	9.691 ± 0.890	9.555 ± 0.671	0.792
Pulmonic valve (PV)	5.570 ± 0.450	6.147 ± 0.780	6.022 ± 0.622	4.483
Mitral valve (MV)	8.460 ± 0.694	8.460 ± 0.463	8.166 ± 0.455	1.011
Aortic valve (AV)	6.035 ± 0.550	5.795 ± 0.437	5.622 ± 0.465	2.545
Right ventricle inflow (TA)	5.660 ± 0.389	5.465 ± 0.525	5.533 ± 0.070	1.116
Right ventricle outflow (PA)	8.837 ± 0.650	8.834 ± 0.583	8.533 ± 0.438	0.978
Perimeter right ventricle (PRV_i)	8.877 ± 1.031	8.500 ± 1.316	7.911 ± 0.940	2.200
Perimeter right ventricle (PRV_o)	7.000 ± 0.721	7.565 ± 1.163	7.166 ± 1.148	1.733
Left ventricle inflow (MA)	7.985 ± 0.550	7.860 ± 0.462	7.644 ± 1.269	1.606
Left ventricle outflow (AA)	7.670 ± 0.613	7.734 ± 0.512	7.544 ± 0.406	0.405
Perimeter left ventricle (PLV)	7.795 ± 1.059	7.500 ± 0.659	7.644 ± 0.517	0.691

Analysis of variance of raw postfixation measurements. An F of 3.0 is significant at 95% confidence.

aorta and both ventricular chambers (II) and in *pulmonary artery, aorta and atrial and ventricular chambers* (III).

For purposes of chamber volume estimation, inside measurements were used and valve orifice circumferences did not enter into the calculation (Lev, Rowlatt and Rimoldi, 1961). The measurements from all three perfusion fixation procedures in hearts weighing 200—300 g could, therefore, be pooled in order to compare the dimensions of perfusion fixed hearts with those of fresh and other fixed hearts. In Fig. 2 dimensions of hearts fixed by controlled pressure perfusion are compared with those of hearts fixed by simple immersion in formalin. The data are expressed as the ratio of "fixed" to "fresh" measurements. Only those hearts subjected to one prefixation condition, that of storage in cold saline, were considered.

Pressure fixed hearts were about 4 per cent longer than fresh hearts, their greatest circumferences were 12 per cent larger. The right ventricular circumference was relatively larger than the left one. These changes are largely explained by the greater wall thicknesses found in pressure fixed hearts. Valvular orifices were generally 5—9 per cent smaller than in fresh hearts. The right inflow-tract was 6 per cent shorter, but the dimension apex to pulmonic orifice was 9 per cent longer. The circumferences in the right ventricle were each 13 per cent smaller than in fresh hearts. The left ventricular length was essentially unchanged, the circumference 1/5th smaller than in the fresh specimen. The variation (95% con-

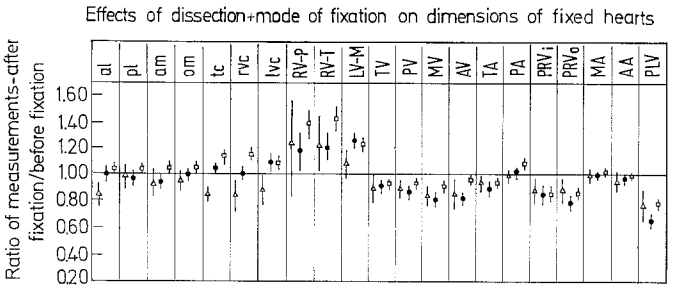

Fig. 2. Comparison of ratios of fixed to fresh mean measurements for same pretreatment. Graph shows means of the ratios with 95% population confidence intervals. △ Hearts opened before fixation by immersion. • Hearts opened after fixation by immersion. □ Hearts opened after fixation by controlled pressure distension und perfusion

fidance limit for the ratios) is much less for the pressure fixed hearts than for the 2 other methods used, therefore the pressure fixation yields a good standard preparation.

Estimation of Dimensions in Unfixed Hearts from the Fixed Specimen

Table 3 provides standardization factors for estimating dimensions of fresh pig hearts for fixed specimen. The estimated dimension is found by multiplying

Table 3. *Illustrative standardization factors (pig hearts): Effect of mode of fixation on dimensions of fixed hearts*

	Mode of fixation		
	opened before fixation	opened after fixation	fixed by perfusion
Anterior length (al)	1.175	0.997	0.963
Posterior length (pl)	1.022	1.037	0.970
Acute margin length (am)	1.085	1.076	0.959
Obtuse margin length (om)	1.067	1.017	0.965
Transverse circumference (tc)	1.190	0.975	0.894
Right ventricle circumference (rvc)	1.211	1.009	0.883
Left ventricle circumference (lvc)	1.159	0.935	0.936
Right ventricle wall (RV-P)	0.850	0.872	0.743
Right ventricle wall (RV-T)	0.831	0.844	0.719
Left ventricle wall (LV-M)	0.917	0.933	0.971
Tricupid valve (TV)	1.126	1.105	1.089
Pulmonic valve (PV)	1.122	1.166	1.078
Mitral valve (MV)	1.190	1.236	1.094
Aortic valve (AV)	1.171	1.225	1.037
Right ventricle inflow (TA)	1.065	1.124	1.060
Right ventricle outflow (PA)	1.011	0.981	0.921
Perimeter right ventricle (PRV_i)	1.136	1.174	1.155
Perimeter right ventricle (PRV_o)	1.129	1.261	1.156
Left ventricle inflow (MA)	0.997	1.001	0.965
Left ventricle outflow (AA)	1.048	0.965	1.003
Perimeter left ventricle (PLV)	1.294	1.513	1.261

the value in the fixed specimen with the standardization factor (reciprocal of the mean ratio fixed/fresh) of this table. The table is given as an example for the comparison of pressure fixation, opening after fixation, and fixation after opening. All 3 methods were applied after identical treatment of storage in cold saline.

Comparison of Dimensions of Human and Porcine Hearts of Comparable Weight

Inside measurements of 22 normal human hearts weighing 200—300 g are compared with those of 27 pig hearts in the same weight range in Table 4. All were in cold saline before fixation. In contrast to the human hearts, the pig

Table 4. *Comparison of dimension of human hearts with pig hearts*[a]

Dimension[b]	Pig		Human	
	Mean ± S.D.	S.E.M.	Mean ± S.D.	S.E.M.
Right ventricle wall (RV-P)	0.781 ± 0.080	0.016	0.510 ± 0.165	0.045
Right ventricle wall (RV-T)	0.850 ± 0.086	0.017	0.455 ± 0.135	0.037
Left ventricle wall (LV-M)	1.792 ± 0.105	0.021	1.428 ± 0.140	0.039
Tricuspid valve (TV)	9.277 ± 0.754	0.148	10.719 ± 1.710	0.474
Pulmonic valve (PV)	5.688 ± 0.581	0.114	7.242 ± 0.759	0.210
Mitral valve (MV)	8.196 ± 0.481	0.094	8.777 ± 0.761	0.211
Aortic valve (AV)	5.692 ± 0.393	0.077	6.812 ± 0.511	0.142
Right ventricle inflow (TA)	5.396 ± 0.386	0.076	6.538 ± 0.703	0.195
Right ventricle outflow (PA)	8.550 ± 0.461	0.090	7.838 ± 0.817	0.227
Perimeter right ventricle (PRV_i)	7.912 ± 0.869	0.170	7.835 ± 1.843	0.511
Perimeter right ventricle (PRV_o)	6.969 ± 0.937	0.184	8.042 ± 1.462	0.405
Left ventricle inflow (MA)	7.623 ± 0.348	0.068	7.404 ± 0.500	0.139
Left ventricle outflow (AA)	7.388 ± 0.391	0.076	7.289 ± 0.436	0.121
Perimeter left ventricle (PLV)	7.554 ± 0.797	0.156	7.977 ± 1.462	0.405

[a] All hearts fixed by coronary perfusion: 27 pig hearts, 22 human hearts.
[b] All hearts weighed 200—300 g.

hearts were fixed after a uniform mode of death and a short post mortem interval; all of the porcine hearts were in comparable degrees of rigor at the time of fixation, the state of contraction of the human hearts was quite variable. The pig hearts had smaller cavities, thicker walls and smaller valve orifices than the human hearts. Considering the closeness of dimensions in pig hearts after pressure fixation to fresh pig hearts in rigor mortis, we feel it is highly probable that the pressure fixed human heart is a true representation of the heart in rigor mortis.

Discussion

For quantitative morphologic studies measurements have been taken from fresh and conventionally fixed hearts. After death cardiac size and shape is altered by rigor mortis, the degree of which will be influenced by postmortem interval and environment. Size and shape of heart will be further modified by the autopsy

procedure (handling and dissection) and measurements are usually taken during or at the end of this procedure. The drawbacks of measurements after additional modification by conventional (immersion) fixation have been recognized (ROWLATT, RIMOLDI and LEV, 1963). Therefore we devised a standard autopsy procedure with controlled pressure fixation after minimal handling of specimen and prior to dissection (GLAGOV, ECKNER and LEV, 1963). At the time we assumed that rigor mortis could be lysed by simple physical methods, (short of autolysis) and that standardization of the autopsy procedure would then yield specimen with chambers at their end diastolic volumes and shapes.

From our experience with about 500 pressure fixed hearts and the data presented here (Fig. 1) it became apparent that rigor mortis could not be abolished by the two methods used (soaking in saline, freezing and thawing). Thus the method of fixation could at best preserve size and shape of the heart in a state prior to handling. Since quantitative data regarding the gross morphological changes by fixation were lacking, we selected a model for this study which had uniform post mortem interval and environment as well as a uniform autopsy procedure.

If the heart was dissected prior to immersion in formalin exterior measurements of the fixed specimen were difficult to obtain because of differential shrinkage of the free walls towards the septum. With the increase in free wall thickness we observed greater shrinkage of exterior dimensions than of interior dimension of the right ventricle, whereas the interior dimensions of the left ventricle were smaller than the exterior dimensions. Left sided valvular orifices shrank more than right sided ones.

If the heart was dissected after fixation by immersion, the outside measurements were relatively unaltered, but interior dimensions were still smaller than in the previous group. The variation for each measurement in each group was rather large. Obviously structural rearrangement took place during fixation. Meaningfull comparisons of quantitative data and prediction of "fresh" values for a given specimen could only be made if the exact mode of fixation was known. The same would be true for microscopic quantitative studies with regard to fiber thickness and number of muscle fibers per cross section, for example.

The comparison of the 3 modes of pressure fixation did not show any difference between them. The size and shape of the pressure fixed heart with its well preserved anatomical relationships and detail is produced by the perfusion via the coronary arterial system. Due to the fact that rigor mortis could not be lysed under the circumstances described, infusion of fixative solution under pressure into the chambers did not alter size and shape of the specimen. The procedure could thus be simplified to pressure perfusion of aorta and coronary arteries as well as pulmonary artery. The resulting specimen has dimensions which approach the "fresh" one. Due to the filling of the coronary arterial and venous system with fluid, ventricle walls are thicker than in the fresh autopsy hearts. One of the main advantages of this preparation is the reproducibility and the small variation for any given dimension. We believe that, in human autopsy material with generally longer post mortem interval and less favorable environment (no or late refrigeration of the body), controlled pressure fixation will give interior dimensions of the "fresh" heart in rigor mortis before handling by the prosector. While an excellent pathological specimen is obtained this way, the relation of size and

shape of chambers to any one phase during life is in a sense an impossibility for two reasons:

1. The rigor mortis cannot be lysed by the employed physical means.

2. All coronaries are equally perfused at the same pressure and rate at a given time.

From the works of Puff (1960, 1965), we know that flow in coronaries depends on relaxation and contraction of in and outflow tracts of the ventricles. During contraction of the left ventricular inflow tract, the outflow portion will still be relaxed and the apex will be unfolded by the blood in the chamber. Similarly the subaortic wall will be still contracted at a time when blood "falls" from the left atrium into the unfolded and relaxed inflow tract. Therefore, the designation of any particular shape of ventricle as plain "systole" or "diastole" is unjustified. Furthermore, injection of fixative solutions prior to rigor mortis, by whatever means (Ross, Jr., Sonnenblick, Corell, Kaiser and Spiro, 1967), will produce contraction of the cardiac muscle before the muscle fibers become immobilized (Eckner, Thaemert, Moulder and Blackstone, 1967).

Controlled pressure fixation is a standardized autopsy procedure which allows a better specimen evaluation than the other conventionally employed modes of fixation.

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