Relationships between Heart Weight, DNA Content, Cell Count and Thickness of Muscle Fibers in Human Heart Muscle

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SUMMARY

Chemical determination of DNA content and histometric estimation of the cell count and the thickness of muscle fibers in the human left ventricle were performed on 24 autopsy cases, in order to know relationships between heart weight, DNA content, cell count and thickness of muscle fibers with increasing heart weight. The following conclusions were obtained.

It is considered that DNA synthesis and the increase in cell count are attributable for the increase in heart weight. The mean thickness of muscle fibers in cardiovascular and renal disease group was bigger than that in malignant tumor and miscellaneous disease group. Then it is concluded that not only hypertrophy of muscle fibers but hyperplasia of heart muscle cells occur in cardiac hypertrophy.

Additional Indexing Words:

Autopsy Hypertrophy Cell division Hyperplasia Chemical
determination Histometric estimation Cardiovascular and renal
disease Malignant tumor

CARDIAC hypertrophy is said to be the result of overloading. There are, however, still unknown matters in not only its biochemistry but also its morphology. Furthermore, its definition and criterion are also obscure. Although the thought that no cell division occurs within heart muscle has persistently prevailed, recent studies have indicated that there is a possibility of cell division of heart muscle.¹⁾⁻⁴⁾ Beside a series of experimental studies on rats,⁵⁾⁻¹²⁾ the authors have reported that cellular hyperplasia rather than hypertrophy occurs in human hypertrophic hearts obtained at autopsy from chemical determination of DNA content and histometric estimation of cell count.¹³⁾

This paper is to report chemical determination of DNA content, histometric estimation of cell count and thickness of muscle fibers of human heart

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muscle and to discuss relationships between heart weight, DNA content, cell count and thickness of muscle fibers with increasing heart weight.

MATERIALS AND METHODS

Materials were obtained from autopsy cases at Department of Pathology, Tohoku University School of Medicine. Table I shows their age, sex, autopsy diagnosis, blood pressure and electrocardiographic findings. Total of 24 cases consist of 15 male and 9 female. Age ranged from 5 years to 77 years. Eleven died of cardiovascular and renal diseases, 9 of malignant tumor, including leukemia, 1 of combined diseases and 3 miscellaneous diseases. Heart weight ranged from 105 Gm. to 630 Gm.

Methods to determine DNA content of heart muscle, and methods to estimate cell count were described elsewhere.¹³⁾

The thickness of muscle fibers was estimated as follows. Histological specimens with vertically cut against the long axis of heart muscle cell were chosen. As shown

Table I. Date of Autopsy Materials

n	Age	Sex	Diagnosis	Heart weight Gm.	Blood pressure	ECG
1	5	M	Acute encephalopathy	105	N	N
2	37	F	Ca. of breast	170	N	N
3	13	M	Nephrosis, Uremia	180	N	N
4	14	M	Chr. myelocytic leukemia	230	N	N
5	65	M	Reticulosarcoma	260	N	N
6	73	F	Coronary sclerosis	260	N	N
7	47	F	Ca. of stomach	280	N	N
8	66	F	Ca. of tongue, Hypertension	290	180 ~ 90	N
9	36	M	Ca. of parathyroid	300	142 ~ 92	ST. T ↓
10	68	M	Ca. of stomach	330	N	N
11	43	F	Ca. of stomach	340	N	N
12	53	M	C V A	350	220 ~ 100	
13	39	M	Acute myelocytic leukemia	350	N	N
14	43	F	Ca. of breast	360	N	N
15	77	F	C V A	360	190 ~ 100	ST ↓ LVH
16	35	M	Adrenogenous collopse	360	N	
17	57	F	Fibrosis of liver	380	N	Atrial fib.
18	22	F	M S I	420	N	ST. T ↓ Atrial fib.
19	69	M	Acute hepatitis, Hypertension	450	174 ~ 82	
20	28	M	Chr. nephritis	490	150 ~ 80	N
21	72	M	C V A	540	160 ~ 100	LVH
22	48	M	Agranulocytosis, Hypertension	550	170 ~ 110	LVH
23	22	M	Myocarditis	610	136 ~ 90	AV Block II
24	55	M	Myocardial infarction	630	188 ~ 100	RVH

N: normal

A = Area of sampling plane

M = Total area of muscle bundles in A

Fig. 1. Schema of microscopic picture of transverse section of heart muscle fibers. Parts with oblique lines show transverse sections of muscle fibers, and the space between muscle fibers (E) is expressed by $A-M$.

in Fig. 1, when the thickness of muscle bundle is to be determined, the mean area of each muscle bundle (m) is expressed in the following way: $m = M/F$, where M is the total area of muscle bundles and F the number of muscle bundles. Provided the space E is artificially made and muscle bundles are similarly distributed in E as in M , the number of muscle cells (Z) in the unit muscle volume (V) should be calibrated in the following way: $Z = N A/M$, where N is the true cell count, A area of sampling plane, M total area of muscle bundles. Therefore, the theoretical length of muscle cell (L) is obtained in the following way: $L = V/Zm$.

For determination of area of M and E , microscopic pictures were made on histological specimens, and enlarged, delineated on tracing papers and measured by planimeter. The mean area of 50 muscle bundles was expressed as " m ". From this value, L was calculated.

RESULTS

The heart weight, DNA content per 100 mg., count of heart muscle cell and of interstitial cell in $4 \times 10^6 \mu^3$, thickness and length of heart muscle cell were shown in Table II.

The DNA content ranged from 57 $\mu g.$ to 160 $\mu g.$ There were no significant correlations between the DNA content per 100 mg. of heart muscle and heart weight. As shown in Table III, the mean DNA content was 83.5 $\mu g.$ in malignant tumor group and 108.9 $\mu g.$ in cardiovascular and renal disease group. There was a significant difference between these values ($p < 0.05$). There was similarly very significant correlation between the DNA content per 100 mg. of heart weight and the total cell count in $4 \times 10^6 \mu^3$ ($r = 0.85$, $p < 0.01$) (Fig. 2). Since it was considered that there was no difference in the distribution of cells in every part of the left ventricle, the weight of left ventricle was calculated according to Arai and his associates,³³⁾ and the total DNA content in the left ventricle was also calculated. There was very significant correlation between

Table II. DNA Content, Cell Count, Thickness of Muscle Bundle and Length of Muscle Cell

n	Heart weight Gm.	DNA $\mu\text{g.}/100\text{ mg.}$	Cell count / $4 \times 10^5 \mu.^3$			Thickness $\mu.^2$	Length $\mu.$
			Muscle	Interstitial	Total		
1	105	83	250	330	580	128	122
2	170	160	196	493	689	270	76
3	180	61	116	254	370	439	79
4	230	123	188	524	712	137	155
5	260	122	176	431	607	225	100
6	260	142	87	613	700	315	146
7	280	108	143	395	538	281	99
8	290	83	100	380	480	426	94
9	300	136	150	526	676	297	90
10	330	71	149	262	411	152	177
11	340	74	136	353	489	223	132
12	350	74	104	292	396	365	106
13	350	123	122	560	682	213	154
14	360	101	126	336	462	236	135
15	360	57	92	273	365	290	150
16	360	77	152	350	502	236	112
17	380	103	130	425	555	220	140
18	420	82	114	382	496	221	159
19	450	85	111	386	497	357	101
20	490	60	123	250	373	401	81
21	540	108	88	339	427	397	115
22	550	57	87	344	431	411	112
23	610	87	82	351	433	593	82
24	630	105	86	366	452	586	79

Table III. Mean Values of Heart Weight, DNA Content, Muscle Cell Count, and Thickness and Length of Muscle Cell in the Cardiovascular and Renal Disease Group and Malignant Tumor and Miscellaneous Diseases, and Statistical Differences Between These Values

	Cardiovascular and renal diseases	Malignant tumor and miscellaneous diseases	
Heart weight Gm.	432.7	305.5	$p < 0.02$
DNA $\mu\text{g.}/100\text{ mg.}$	83.5	108.9	$p < 0.05$
Muscle cell count/ $4 \times 10^5 \mu.^3$	99.1	151.6	$p < 0.01$
Thickness of muscle cell $\mu.^2$	397.7	226.4	$p < 0.01$
Length $\mu.$	110.0	124.5

Fig. 2. Correlations between DNA content per 100 mg. and total cell count.
 $\circ$ = cardiovascular and renal disease
 $\times$ = malignant tumor
 Δ = combined case with cardiovascular and renal disease and malignant tumor
 $\bullet$ = miscellaneous
 Same symbols were used thereafter.

Fig. 3. Correlations between total DNA content of the left ventricle and weight of the left ventricle.

Fig. 4. Correlations between muscle cell count in $4 \times 10^6 \mu^3$ and heart weight.

the total DNA content in the left ventricle and the the weight of left ventricle ($r=0.83$, $p<0.01$) (Fig. 3).

There were no significant correlations between the count of heart muscle cell in the unit muscle volume ($4 \times 10^6 \mu^3$) and heart weight. The cell count was relatively constant in hearts heavier than 400 Gm., but was not always constant in smaller hearts. As shown by the broken line in Fig. 4, there was a significant correlation between the cell count in the malignant tumor group (9 cases) and heart weight ($r=-0.93$, $p<0.01$). As shown in Table III, the mean value of cell count in the unit muscle volume was 99.1 in the cardiovascular and renal disease group and 151.6 in the malignant tumor group. There was a significant difference between these values ($p<0.01$).

There were no significant correlations between the count of interstitial cells in the unit muscle volume and heart weight.

Total heart muscle volume was obtained from the heart weight by assuming that the specific gravity of heart muscle was 1.0. And then, the total count of heart muscle cell and interstitial cell was calculated. There was very significant correlations between the total count of heart muscle cell and heart weight ($r=0.71$, $p<0.01$) (Fig. 5). There was also very significant correlation between the total count of interstitial cell and heart weight ($r=0.81$, $p<0.01$) (Fig. 6).

The mean thickness of muscle bundle was $397.7 \mu^2$ in the cardiovascular and renal disease group and $226.4 \mu^2$ in the malignant tumor group; and there was a significant difference between these values ($p<0.01$). However, there

were no significant correlations between the thickness of heart muscle bundle and heart weight.

The length of heart muscle cell ranged from 76 to 177 μ . There were no correlations between the length, heart weight, diseases and age.

Fig. 5. Correlations between total cell count of heart muscle cell and heart weight.

Fig. 6. Correlations between total cell count of interstitial cell and heart weight.

DISCUSSION

The purpose of this study is to know relationships between heart weight, DNA content, cell count and thickness of muscle fibers. However, hearts obtained from autopsy cases were not normal in the strict sense. So, cardiovascular and renal disease cases, most of which had hypertrophic hearts, were compared with malignant tumor cases, which had atrophic hearts, since there were not sufficient number of materials in our study. Reiner and his associates¹⁵⁾⁻¹⁷⁾ have reported an excellent study on the weight of human hearts. Most of cardiovascular and renal disease cases belong to the category of their hypertensive or ischemic heart disease cases and malignant tumor cases to the category of slightly emaciated normal cases. Case 1 was a 5-year-old child and this was excluded for the purpose in this study.

The findings that the total DNA content in the left ventricle increases with the increase in the weight of the left ventricle and that there are significant correlations between the total count of heart muscle cell and of interstitial cell and heart weight, indicate that the increase in the heart weight is related to the DNA synthesis and so to the cell division of heart muscle cells. This is rational because the DNA content per 100 mg. of heart weight was well correlated to the cell count in $4 \times 10^6 \mu^3$ of heart muscle volume.

The thickness of muscle fibers was also histometrically estimated in this study. As to the thickness of muscle fibers, Karsner et al.¹⁸⁾ in 1925 and Lowe and Bate¹⁹⁾ in 1948 measured the diameter of human heart muscle and reported that the diameter of muscle fibers in hypertrophic hearts was bigger than that in normal hearts, and that in atrophic hearts was smaller than that in normal ones. Roberts and Wearn²⁰⁾ mentioned there was a significant correlation between the diameter of muscle fibers and heart weight, and the mean diameter was approximately 14 μ . in the adult normal heart and 20 μ . in adult hypertrophied hearts. Suwa et al.²¹⁾ also reported similar results. In our study, the thickness was expressed as "area", but the values were also quite similar. Although there was a definite difference in the mean values of thickness in between malignant tumor group and cardiovascular and renal disease group, there were no significant correlations between the thickness of muscle fibers and heart weight in our study. It should be further evaluated that the thickness of muscle bundle was big enough in hearts of the cardiovascular and renal disease group even with less than 300 Gm. Together with statistical differences in the cell count in $4 \times 10^6 \mu^3$, DNA content in 100 mg. of heart muscle, it is evident that there is also hypertrophy of muscle fibers as well. The authors stressed rather too much that the hyperplasia of heart muscle cells rather than hypertrophy occurred in so-called hypertrophic hearts from studies on 10 cases

in our previous report.¹³⁾ Now it should be stated that the cardiac hypertrophy is associated with the hypertrophy of muscle fibers as well as hyperplasia of heart muscle cells.

Suwa et al.²¹⁾ mentioned that there was a significant correlation between the weight of the left ventricle and the total length of muscle fibers of the left ventricle. This finding suggests that there is cell division within heart muscle cells. The estimated theoretical length of heart muscle cell was 76–177 μ . in our study, and there were no significant correlations between the length and heart weight or diseases. The longest one was approximately 2.3 times of the shortest one. Suwa et al.²¹⁾ reported the longest one of the total length of the left ventricle was at least 5–7 times of the shortest one. To explain this, there are two thoughts: one is that the length of individual heart muscle cell has increased by 5–7 times and the other is that the total length of muscle fibers has increased due to cell division of heart muscle cells. It appears the latter is more natural.

Furthermore, the cell count of interstitial cells seems to increase by cell division proportionally with the increase of heart weight. This may suggest that the architecture of heart muscle is an important problem to discuss how the cardiac hypertrophy finally results in cardiac failure, beside problems on coronary insufficiency.

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