Virchows Arch (1996) 429:159–164

ORIGINAL ARTICLE

Claus-Peter Adler · Hartmut Friedburg
Georg W. Herget · Michael Neuburger
Heinrich Schwalb

Variability of cardiomyocyte DNA content, ploidy level and nuclear number in mammalian hearts

Received: 18 March 1996 / Accepted: 5 July 1996

Abstract DNA content, ploidy level, cell size and nuclear number were investigated in 54 mammalian hearts from nine species. DNA content was determined biochemically and ploidy level of cells was studied by the means of Feulgen cytophotometry. Nuclear number was calculated by a new method, while cell size was determined by using ocular micrometry. In most mammals diploid cell nuclei predominate. Higher ploidy levels were found in the human and the pig hearts. The total amount of DNA correlated with the myocardial weight. Eight million heart muscle cell nuclei were found in mice (myocardial weight 160 mg), and 2600 million heart muscle cell nuclei in the human heart (myocardial weight 210 g), but in the hearts of horses up to 35 000 million heart muscle cell nuclei (myocardial weight 3.4 kg) were found. The number of heart muscle and connective tissue cell nuclei was correlated with myocardial weight. The ratio of connective tissue cell nuclei to heart muscle cell nuclei was between 2:1 and 3:1. In cardiac growth this ratio shifted towards connective tissue cell nuclei. Increased heart weight corresponds to an increase in cell size. Diameter between 11 μm and 18 μm may be an optimum for heart muscle cells of mammals.

Key words Heart muscle cell · Cytophotometry · Myocardial DNA content · Nuclear number · Mammals

Introduction

At the beginning of the 1950s several authors described the cellular structure of the human myocardium by using electron microscopy [7, 29, 33], and a first calculation of the absolute number of nuclei was possible using a combination of cytophotometric, histomorphological and biochemical methods of DNA measurement [1]. It was found that in contrast to most of the experimental animals studied (cat, dog, bull), in which diploid cell nuclei predominated, the heart muscle cells of human and primate hearts contained polyploid nuclei [4, 11, 31].

Normal heart size depends on myocyte volume and mass, and number of cells and nuclei. The aim of this study was to compare the degree of polyploidization, DNA content, cell size and nuclei number of the myocardium in different mammals.

Materials and methods

A total of 54 mammalian hearts (6 mice, 7 rats, 6 rabbits, 6 dogs, 5 sheep, 6 pigs, 6 humans, 5 oxen and 7 horses) were studied. Post-mortem examinations were carried out within 24 h of death. After weighing (autopsy weigh), the hearts were freed from vessels, atria, heart valves, tendinous fibres and subepicardial fatty tissue [26]. Samples of muscle tissue measuring 3 cm^3 were studied biochemically and cytophotometrically at different sites of both ventricles - anterior wall right ventricle (1), posterior wall right ventricle (2), pulmonary outflow tract (3), anterior (4) and posterior (5) wall of the left ventricle and the septum (6). Because of the size of the heart in the mouse, rat and rabbit a method adjusted to the heart weight was used. Ventricles of the rabbits were divided into the right and left parts. Small samples were taken from both ventricles for histological investigation; the rest of the right ventricle and the left ventricle, which was divided into three parts, was used for cytophotometric and biochemical investigation. Small samples of heart muscle of mouse and rat were taken for histological investigation, and the rest of the myocardium for biochemical and cytophotometric DNA measurement.

After the fresh weight had been determined, the tissue pieces, weighing approximately 1.5 g, were minced, mechanically homogenised and centrifuged. The sediment was washed three to five times in 0.25 mol perchloride acid and hydrolysated subsequently in 0.5 mol perchloride at 90°C. Following centrifugation of the hydrolysate, the total DNA was found in the precipitate. Quantitative DNA determination was done according to the diphenylamine reaction [12, 13]: 1 ml hydrolysate + 3 ml diphenylamine reagent (1.5 g diphenylamine in 100 ml glacial acetic acid + 1.5 ml concentrated sulphuric acid) were incubated for 17 h at room temperature. Before use, 20 ml of the diphenylamine reagent was added to 1 ml of a freshly prepared acetaldehyde solution (9.8 ml distilled water + 0.2 ml acetaldehyde). For controls we used Salm

C.-P. Adler (✉) · H. Friedburg · G.W. Herget · M. Neuburger
Institute of Pathology (Ludwig-Aschoff-Haus), Albertstrasse 19, D-79104 Freiburg i. Br., Germany
Tel.: (49) 761-203-6747, Fax: (49) 761-203-6790

H. Schwalb
Inselspital Bern, Klinik für Viszeral- und Transplantationsmedizin, CH-3010 Bern, Switzerland

DNA (Carl Roth, Karlsruhe, Germany) with a standard solution containing 10 mg DNA in 40 ml 0.5% perchloride acid. For extinction measurement of the DNA solutions a spectral photometer PQM II (Carl Zeiss, Oberkochen) was used at a wave length of 595 nm (filter 0).

To determine the numerical ratio of heart muscle cell nuclei/connective tissue cell nuclei to cell size, conventionally processed paraffin-embedded material in 5-µm sections was used. The number of connective tissue cell nuclei per 100 heart muscle cell nuclei was counted. Longitudinally orientated fibres were taken for analysis.

Ocular micrometry was used to determine the cell size of heart muscle cells at the focal plane of the nucleus.

Tissue samples with a volume of 0.5 cm³ were mechanically homogenised using an Ultra-Turrax (Janke and Kunkel, Staufen i. Br., Germany) in 3 ml of cooled physiological saline solution. A few drops of the homogenisate were spread on glass slides. After the material had been dried, it was fixed in a Carnoy solution for 1 h, put into 96% alcohol for 2×15 min and into distilled water for 5 min. The smear preparations were then stained according to Feulgen [15] (hydrolysis in HCl for 12 min at 60°C, Schiff's reagent according to Graumann 1953 [18]). Stained bull spermatozoa were used as control for haploid DNA content. Fifty nuclei per sampling area were then measured on a Deeley integrating microdensitometer (Barr and Stroud, Glasgow, Scotland), i.e. a total of 400 nuclei per heart. Mean values of myocyte ploidy classes differed by no more than 3%. To investigate reproducibility we performed repeated measurements. Five samples of the same site were studied (SE ± 0.05) [21].

The absolute number of heart muscle cell nuclei can be obtained from the values of the total DNA amount in the myocardium determined by biochemical measurements, the percentage proportion of the various ploidy classes in the muscle nuclei, the numerical ratio of connective tissue cell nuclei to heart muscle cell nuclei and the pure weight of the myocardium [1, 5]. First of all, mean DNA amount of heart cells was determined (taking into account the percentage proportion of the various ploidy classes):

$$2c_{nucleus} = N_{\mathrm{CTCN}} + N_{2c} + 1.5N_{3c} + 2N_{4c} + 3N_{6c} + 4N_{8c} + \ldots / (N_{\mathrm{CTCN}} + 100\ \mathrm{HMCN})$$

where CTCN = connective tissue cell nucleus; HMCN = heart muscle cell nuclei; $2c_{\mathrm{nucleus}}$ = mean DNA amount; N_{CTCN} = number of CTCN/100 HMCN = number of genomes of CTCN/100 HMCN, because CTCN are diploid; N_{2c} = number of genomes with regard to the percentage of diploid heart muscle cell nuclei; $1.5N_{3c}$ = number of genomes with regard to the percentage of triploid heart muscle cell nuclei, etc.

Since 6×10^{-12} g DNA are present within a diploid cell nucleus [36], it is possible to determine the median DNA amount found in the total genomes:

$$\text{DNA amount}_{\mathrm{nucleus}} = 2c_{\mathrm{nucleus}} \times 6\times10^{-12}\ \mathrm{g}$$

The total number of cell nuclei is calculated from the ratio of the biochemically estimated total amount of DNA in the myocardium and the total amount of the genome determined on the basis of cytophotometric measurements:

$$N_{\text{total number of nuclei}} = \text{total DNA amount}/(2c_{\mathrm{nucleus}} \times 6\times10^{-12}\ \mathrm{g})$$

and the ratio of the total number of connective tissue cell nuclei to total numer of cell nuclei correlates with the numerical ratio of

Table 1 Percentage distribution of the ploidy classes of heart muscle cell nuclei in hearts of the different species

Species	2c (%)	4c (%)	6c (%)	8c (%)	12c (%)	16c (%)
Mouse	89.3	10.3	0.6	–	–	–
Rat	97.1	2.6	–	–	–	–
Rabbit	96.3	3.6	–	–	–	–
Dog	98.1	1.9	–	–	–	–
Sheep	96.6	7.5	–	–	–	–
Pig	77.6	16.8	1.3	2.2	0.2	–
Human	20.5	56.7	1.1	20	0.7	1.1
Ox	97.1	2.7	0.2	–	–	–
Horse	98.8	1.2	–	–	–	–

Table 2 Total heart weight (*THW*), total myocardial weight (*TMW*), biochemically determined DNA concentration (*conc*) and total amount of DNA

Species	No	THW (g)	TMW (g)	DNA conc (mg/g)	DNA (mg)
Mouse	1	0.26	0.16	0.91	0.15
	2	0.26	0.17	0.91	0.16
	3	0.26	0.19	0.91	0.17
	4	0.26	0.17	0.91	0.16
	5	0.32	0.20	1.10	0.22
	6	0.32	0.24	1.10	0.26
Rat	1	0.75	0.48	0.80	0.38
	2	0.76	0.65	1.10	0.71
	3	0.77	0.55	1.17	0.64
	4	0.84	0.60	1.09	0.65
	5	0.87	0.59	1.20	0.71
	6	0.94	0.62	1.11	0.69
	7	1.01	0.65	0.89	0.58
Rabbit	1	6.74	4.34	0.46	2.00
	2	6.95	5.41	0.47	2.53
	3	7.28	5.19	0.51	2.63
	4	8.00	6.23	0.36	2.25
	5	9.60	5.74	0.38	2.18
	6	11.1	6.44	0.41	2.61
Dog	1	58.3	41.1	0.53	21.93
	2	81.0	59.7	0.47	28.23
	3	106	83.9	0.41	34.26
	4	124	82.7	0.54	45.00
	5	141	91.0	0.47	42.69
	6	173	110	0.35	38.89
Sheep	1	158	112	0.34	38.53
	2	160	102	0.31	31.55
	3	185	115	0.33	37.49
	4	250	167	0.30	50.44
	5	257	184	0.35	63.46
Pig	1	38	24	1.48	35.39
	2	328	208	0.35	71.47
	3	350	205	0.27	54.52
	4	672	511	0.30	151.9
	5	647	455	0.31	138.8
	6	743	458	0.34	156.8
Human	1	250	143	0.29	41.46
	2	317	222	0.25	54.65
	3	330	150	0.35	51.81
	4	390	172	0.30	52.14
	5	450	212	0.40	85.17
	6	590	389	0.27	104.4
Ox	1	337	220	0.76	167.3
	2	1751	1196	0.30	355.3
	3	2113	1382	0.36	498.7
	4	2364	1712	0.22	380.9
	5	2743	1901	0.25	483.6
Horse	1	553	406	1.24	502.1
	2	1508	1270	0.30	382.6
	3	2040	1820	0.31	555.9
	4	2910	2267	0.33	748.2
	5	3232	2353	0.23	544.9
	6	4313	3304	0.24	804.0
	7	4900	3475	0.26	904.9

Table 3 Size of heart muscle cell (*LV* left ventricle, *RV* right ventricle, *sd* standard deviation, *m* mean value of cell size in heart, *M* mean value of cell size within the species (*V* variance, *SD* standard deviation)

Species			1	2	3	4	5	6	7	M	V	SD
Mice	LV	m	14.0	12.1	11.8	12.7	13.1	13.8		12.9	0.8	±0.8
		sd	±2.9	±2.5	±2.5	±2.6	±2.5	±3.1				
Rat	LV	m	10.7	11.5	12.5	12.6	12.5	12.3	12.5	12.1	0.5	±0.7
		sd	±2.9	±2.2	±2.3	±2.6	±2.3	±2.2	±2.6			
Rabbit	RV	m	10.9	7.4	13.6	13.6	12.2	12.9		11.7	5.6	±2.4
		sd	±2.9	±2.0	±3.4	±3.7	±3.8	±2.7				
	LV	m	10.9	7.3	12.6	13.6	12.1	13.0		11.6	5.3	±2.3
		sd	±3.0	±1.9	±3.4	±3.3	±3.7	±2.9				
Dog	RV	m	16.0	14.1	14.7	14.4	16.1	19.4		15.7	3.9	±1.9
		sd	±4.2	±3.2	±3.1	±3.7	±2.5	±3.6				
	LV	m	15.4	14.0	14.2	14.2	15.8	18.3		15.3	2.7	±1.6
		sd	±3.7	±3.5	±3.3	±3.8	±2.8	±3.7				
Sheep	RV	m	10.2	12.2	9.9	11.7	11.6			10.9	1.0	±1.0
		sd	±2.3	±2.1	±2.3	±3.0	±2.7					
	LV	m	12.0	11.5	12.1	12.0	11.5			11.8	0.1	±0.1
		sd	±2.8	±2.5	±2.3	±3.7	±2.6					
Pig	RV	m	5.2	12.5	12.9	15.0	16.0	14.9		14.3	2.3	±1.5
		sd	±1.3	±3.5	±3.3	±5.2	±6.6	±4.5				
	LV	m	6.0	13.0	15.7	19.6	16.8	13.0		15.6	7.7	±2.8
		sd	±1.3	±3.4	±4.7	±5.7	±4.3	±3.6				
Human	RV	m	12.1	12.5	13.7	14.3	18.0	17.2		14.6	6.0	±2.4
		sd	±1.5	±1.7	±1.2	±0.8	±1.9	±2.0				
	LV	m	13.6	13.3	14.7	16.1	18.8	19.5		16.0	6.7	±2.6
		sd	±1.2	±2.2	±1.4	±2.8	±0.8	±2.2				
Ox	RV	m	7.1	14.8	15.1	15.3	15.2			15.1	0.1	±0.2
		sd	±2.3	±4.1	±3.3	±3.1	±3.0					
	LV	m	6.7	17.7	15.9	17.7	17.1			17.1	0.7	±0.8
		sd	±2.5	±3.6	±3.5	±2.9	±3.2					
Horse	RV	m	8.0	14.8	14.9	14.5	12.7	17.7	15.7	14.1	2.6	±1.6
		sd	±1.8	±4.2	±4.2	±3.2	±3.1	±4.2	±3.3			
	LV	m	6.6	15.9	17.1	17.7	17.8	17.2	16.3	17.0	0.6	±0.7
		sd	±1.6	±4.9	±4.7	±4.2	±4.7	±4.7	±3.7			

(number of CTNC/100 HMCN) to ((number of CTNC/100 HMCN)+100 HMCN):

$N_{\text{total number of CTCN}}/N_{\text{total number of nuclei}} = N_{CTCN}/(N_{CTCN}+100\ \text{HMCN})$

The total number of connective tissue cell nuclei can be calculated from:

$N_{\text{total number of CTCN}} = N_{CTCN}/(N_{CTCN}+100_{HMCN}) \times N_{\text{total number of nuclei}}$

and the number of heart muscle cell nuclei, from:

$N_{\text{number of HMCN}} = N_{\text{total number of nuclei}} - N_{\text{total number of CTCN}}$

Results

Table 1 presents the distribution of the ploidy classes of the different species. Calculations were made on the basis of the average DNA content. In most of the species cell nuclei with 2c DNA predominate. 4c DNA nuclei were found in 0.33–4%, and sporadically in up to 10%. In human myocardium 20.5% 2c DNA, 56.7% 4c DNA and higher ploidy nuclei could be found. In hearts of pigs 77.6% 2c DNA and 17.8% 4c DNA nuclei were detected. As in the human myocardium, nuclei of higher ploidy were observed.

Autopsy weight, preparation weight, biochemically estimated DNA concentration (mean value of the six sites) and total amount of DNA (product of myocardial weight multiplied by the mean DNA concentration) are shown in Table 2.

We found a mean DNA concentration of 1 mg/g in mice; in the hearts of horses the mean DNA concentration was between 0.2 mg/g and 0.3 mg/g. For correlation analysis we excluded one heart each from the groups of pig, ox and horse hearts, as these were from neonates. As myocardial weight increased we found a decrease in DNA concentration [log(DNA concentration) = –0.15 log(myocardial weight)–0.12; $r = 0.9155$; $P < 0.001$]. The total amount of DNA correlates with myocardial weight [(log(DNA amount) = 0.85 log(myocardial weight)–0.12; $r = 0.997$; $P < 0.001$)].

Table 3 shows the cell size of heart muscle cells. Determination of the cell size in mice and rats was possible in heart muscle cells of the left ventricle and in all other species both ventricles were investigated. The cell size of the left ventricle was, on average, 12.9 µm (mouse), 16 µm (human) and 17 µm (horse). An increase in body weight from 0.02 kg (mouse) to 600 kg (horse) corresponds to an increase in cell size of 4–5 µm. The heart muscle cells of growing mammals were smaller. In contrast to the right ventricle, the cell size of the left ventricle is 10–20% bigger.

To calculate the absolute number of heart muscle cell nuclei the ratio of connective tissue cell nuclei/100 heart

Table 4 Numeric ratio of connective tissue cell nuclei (CTCN)/100 heart muscle cell nuclei (*HMCN*), total number of connective tissue cell nuclei, total number of heart muscle cell nuclei and total number of cell nuclei (*N*)

Species	No	CTCN/100 HMCN	CTCN $\times 10^9$	HMCN $\times 10^9$	N $\times 10^9$
Mouse	1	3.13	0.018	0.006	0.024
	2	2.68	0.019	0.007	0.026
	3	2.92	0.021	0.007	0.028
	4	2.73	0.018	0.007	0.025
	5	2.44	0.024	0.010	0.034
	6	3.21	0.033	0.010	0.043
Rat	1	3.57	0.050	0.014	0.064
	2	3.14	0.090	0.027	0.117
	3	2.97	0.081	0.027	0.108
	4	3.22	0.084	0.024	0.108
	5	3.24	0.086	0.027	0.113
	6	2.98	0.085	0.029	0.114
	7	3.35	0.074	0.022	0.096
Rabbit	1	2.38	0.231	0.099	0.330
	2	2.58	0.303	0.113	0.416
	3	2.39	0.313	0.120	0.433
	4	2.43	0.306	0.132	0.438
	5	2.66	0.264	0.097	0.361
	6	2.80	0.317	0.114	0.431
Dog	1	2.27	2.216	1.002	3.218
	2	1.94	3.113	1.554	4.667
	3	2.36	4.122	1.553	5.675
	4	2.61	5.384	2.050	7.434
	5	2.13	4.174	2.001	6.175
	6	2.57	4.309	1.655	5.964
Sheep	1	2.42	4.559	1.809	6.368
	2	2.10	3.583	1.609	5.192
	3	2.13	4.171	2.079	6.250
	4	2.08	5.538	2.784	8.322
	5	3.40	8.129	2.326	10.45
Pig	1	0.81	2.592	2.275	4.867
	2	2.82	8.058	1.153	9.211
	3	1.34	4.503	1.502	6.005
	4	1.3	12.96	3.832	16.79
	5	2.94	15.63	2.322	17.95
	6	2.41	15.12	2.262	17.38
Human	1	2.73	3.307	1.193	4.500
	2	2.04	3.786	1.826	5.612
	3	2.53	4.892	1.988	6.880
	4	1.93	3.915	2.010	5.925
	5	2.54	6.483	2.598	9.081
	6	1.76	6.160	3.301	9.461
Ox	1	1.64	16.13	9.909	26.04
	2	2.16	40.18	18.51	58.69
	3	2.39	57.38	24.61	81.99
	4	2.79	47.35	15.96	63.31
	5	2.42	57.41	22.01	79.43
Horse	1	0.77	33.24	4.942	38.18
	2	2.75	46.58	16.66	62.24
	3	2.9	86.66	23.65	110.3
	4	2.29	84.83	37.79	122.6
	5	2.72	67.25	23.28	90.53
	6	2.82	99.17	34.63	133.8
	7	3.16	113.3	34.93	148.2

muscle cell nuclei was used (Table 4). The ratio was between 2 and 3 (in most of the rats > 3), and no significant difference in the ratio was found between the left and right ventricles. In the hearts of neonatal mammals this numerical ratio was higher. The number of connective tissue cell nuclei was used as a reference point for capillary numbers, showing that the density of capillaries is increased 2–3 times in adult hearts.

The number of heart muscle cell nuclei, connective tissue cell nuclei and absolute number of cell nuclei are shown in Table 4. A correlation between number of heart muscle cell nuclei and myocardial weight was found [log(number of heart muscle cells) = 0.85 log(myocardial weight)–0.98; $r = 0.992$; $P < 0.001$] (Fig. 1). The number of connective tissue cell nuclei [log(number of connective tissue cell nuclei) = 0.84 log(myocardial weight)–1.5; $r = 0.992$; $P < 0.001$] and total number of cell nuclei [log(total number of cell nuclei) = 0.84 log (myocardial weight)–0.91; $r = 0.994$; $P < 0.001$] correlates with myocardial weight.

Discussion

We examined whether the myocardial DNA content and number of cell nuclei varies in different mammals. The main finding is the great variability of myocyte ploidy. In all species, nuclei with a DNA content higher than 2c were found, and on average in mouse hearts 90% of the cell nuclei were diploid and 10% tetraploid. In rats only 2–8% of cell nuclei were tetrapolid, in agreement with other investigations [19, 38]. In rabbits, dogs and oxen, hearts contain about 2–7% tetraploid cells. The percentage of tetraploid cell nuclei in the hearts of horses is low; we found 1–2% 4c DNA nuclei. There were different finding in the hearts of pigs, where besides the diploid nuclei (75%) and tetraploid nuclei (20%), 6c (1.6%), 8c (2.6%) and 12c DNA (1%) were found. The correlation between ploidisation and weight in the human myocardium agrees with the data of others [3, 10, 11, 16, 17, 32]. Up to the age of 7 years, diploid nuclei predominate in human myocardium [1, 2, 14, 28]. At the age of 8 years the extent of polyploidy increases, and tetraploid cell nuclei prevail in the myocardium. Similar findings were made in pigs. In the heart of the neonatal pig 95% diploid and 5% tetraploid cell nuclei were found. At 4–5 months after birth the percentage of 2c DNA decreases to 10–15% and cell nuclei with higher ploidy level increase.

The factors that causes polyploidisation are unknown; various mechanisms have been discussed [30]. Why is polyploidisation only found in primates and pigs? Increased functional activity may induce an increase of polyploidisation. It has been shown that the intensity of metabolism and the rate of functioning of the cell is determined by the sum of its nuclei [8] and that replacement of cell divisions by polyploidisation may itself be favourable for permanently functioning cells, since polyploidisation does not require the reconstruction of cell junctions and extracellular structures [27]. However, in the horses studied, all but one of which were working animals, tetraploid cell nuclei were only 1–2% of the total. The ability to establish polyploidy may thus be genetically determined.

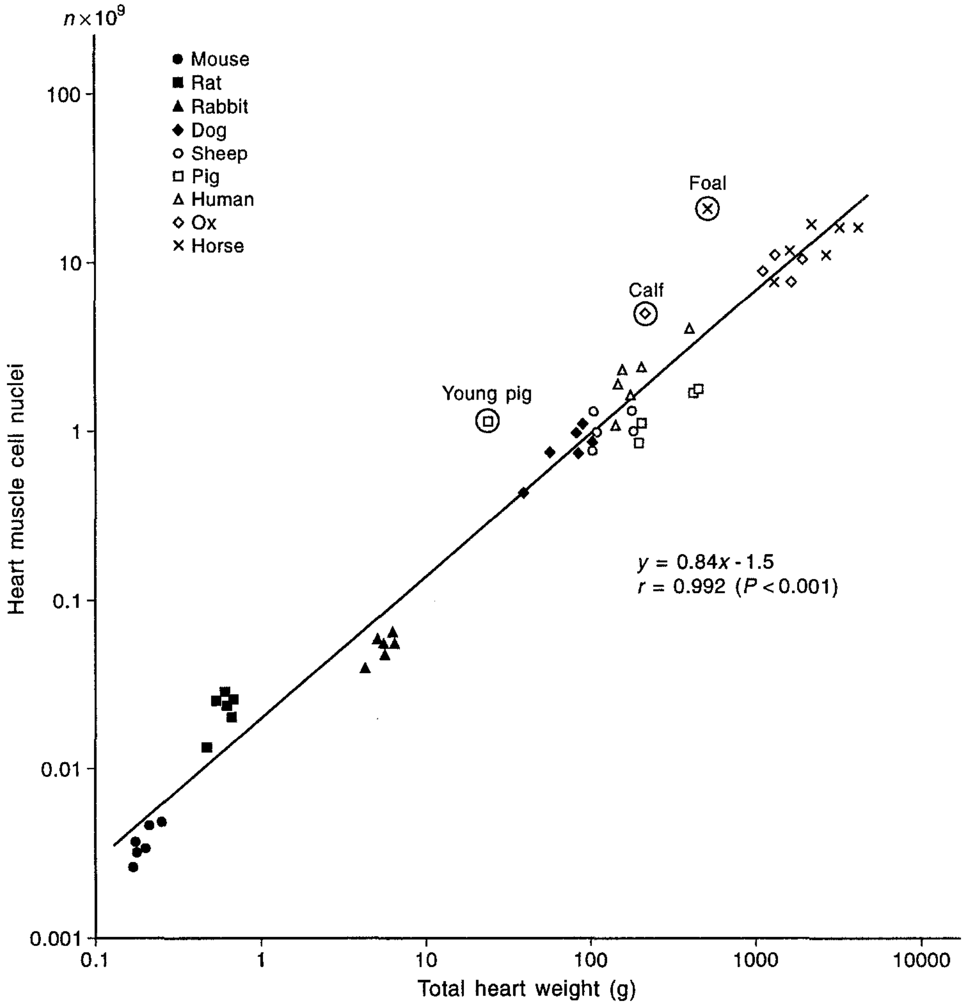

Fig. 1 Correlation between number of heart muscle cell nuclei and myocardial weight (9 species; hearts of neonatal mammals are marked with a *circle*)

Three percent aneuploid cell nuclei were found in hearts of pigs. The mechanisms of cell division and polyploidisation are basically the same in pigs, and incomplete (polyploidising) mitosis prevails in cardiac myocytes, as in many other mature cells (see [8, 35]).

With increasing myocardial weight the DNA concentration decreases. The DNA concentration was higher in neonatal hearts, and a lack of proportionality between DNA content (cell ploidy) and protein content has been detected in neonatal human hearts [14]. However it is also found in adult human hearts [9, 10]. A decrease of the DNA content in the myocardium of growing children and, to a lesser extent, in the myocardium of adults up to the 5th decade of life has been reported [23]. Later in life the amount of DNA increases slightly and it appears to be produced mainly in the interstitium. In the hearts of oxen and pigs similar findings have been observed during cardiac growth [34]. In all species the total DNA content of the myocardium is closely correlated with the weight of the individual heart.

Many authors have studied the size of the heart muscle cell [6, 22, 37], the average cell size reported being between 11 μm and 18 μm. The structural changes can be explained by hypertrophy, and from an increased muscle mass in hypertrophied hearts enlargement of the individual heart muscle cells must be assumed. In the human heart (heart 4), the 20-μm mean cell size was calculated from a myocardial weight of 511 g and body weight of 116 kg. However, pigs with a weight of 200 kg did not have myocardial weights > 500 g. The size of heart muscle cells of one dog (6) exceeded the cell size of the other dogs; in this case the causes of the hypertrophy have to be discussed.

A similar structure of heart muscle cells is found in different species [20], and myocardial cell function depends on the distance of diffusion (critically between 11 μm and 18 μm). This does not differ between mice and whales, the smallest and the largest mammals [6].

An increased muscle mass in hypertrophied hearts is accompanied by enlargement of heart muscle cells [24, 25] and an increase in DNA content [1, 5]. This is brought about by increased DNA synthesis leading to polyploidisation of the nuclei [4]. Our morphological findings point to an increased number of heart muscle cell nuclei as myocardial weight increases in different species.

On average we found 8 million heart muscle cell nuclei in mice, 20 million in rats, 100 million in rabbits, 1500 million in dogs, 2000 million in sheep, 2200 in pigs, 2100 million in humans, 20 000 million in oxen and up to 40 000 million in horses.

The number of connective tissue cell nuclei that are diploid, was an average 2.5 times that of heart muscle cell nuclei. In the hearts of neonates the number of connective tissue cell nuclei was lower than in adult hearts. In hearts of neonatal human infants decreased number of connective tissue cell nuclei was also found [1, 2]. Later in life, the numerical ratio of heart muscle cell nuclei/connective tissue cell nuclei increases.

In the heart of horse (1), a foal, the number of heart muscle cell nuclei did not differ from that in adult animals. In the adult hearts of pigs and oxen the number of heart muscle cell nuclei double that in the neonatal hearts. It seems that the definitive number of cell nuclei is achieved later in life in these animals.

The cell size of heart muscle cells ranged on average between 11 µm and 18 µm. This is optimal for functional activity. With increasing myocardial weight we found decreasing DNA concentrations and a disproportionate increase in the total amount of DNA and in the number of cells.

References

1. Adler CP (1971) Polyploidisierung und Zellzahl im menschlichen Herzen. Habilitationsschrift, University of Freiburg
2. Adler CP (1991) Polyploidization and augmentation of heart muscle cells during normal cardiac growth and in cardiac hypertrophy. In: Oberpriller JO, Oberpriller JC, Mauro A (eds) The development and regenerative potential of cardiac muscle. Harwood, New York, pp 227–252
3. Adler CP, Friedburg H (1978) DNS-Gehalt und Zellzahl in insuffizienten Menschenherzen. Verh Dtsch Ges Pathol 62:517
4. Adler CP, Friedburg H (1986) Myocardial DNA content, ploidy level and cell number in geriatric hearts: post-mortem examinations of human myocardium in old age. J Mol Cell Cardiol 18:39–53
5. Adler CP, Sandritter W (1971) Numerische Hyperplasie der Herzmuskelzellen bei Hypertrophie. Dtsch Med Wochenschr 48:1895–1897
6. Black-Schaffer B, Gristead CE, Braunstein JN (1965) Endocardial fibroelastosis of large mammals. Circ Res 16:383–390
7. Breemen VL van (1953) Intercalated discs in heart muscle studied with the electron microscope. Anat Res 117:49
8. Brodsky VY, Uryvaeva IV (1985) Genome multiplication in growth and development. Cambridge University Press, Cambridge
9. Brodsky VY, Chernyaev AL, Vasilyeva IA (1991) Variability of the cardiomyocyte ploidy in normal human hearts. Virchows Arch [B] 61:289–294
10. Brodsky VY, Sarkisov DS, Arefyeva AM, Panova NW (1993) DNA and protein relations in cardiomyocytes. Growth reserve in cardiac muscle. Eur J Histochem 37:199–206
11. Brodsky VY, Sarkisov DS, Arefyeva AM, Panova NW, Gvasava IG (1994) Polyploidy in cardiac myocytes of normal and hypertrophic human hearts; range of values. Virchows Arch 424:429–435
12. Burton K (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of desoxyribonucleid acid. Biochem J 62:315–323
13. Dische Z (1955) Color reactions of nucleic acid components. In: Chargaff E, Davidson JN (eds) The nucleic acids, vol 1. Academic Press, New York, pp 285–305
14. Eisenstein R, Wied GL (1970) Myocardial DNA and protein in maturing and hypertrophied hearts. Proc Soc Exp Biol 133:176
15. Feulgen R, Rosenbeck H (1924) Nachweis einer Nukleinsäure vom Typus der Thymonukleinsäure und die darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten. Z Phys Chem 135:203–248
16. Fischer B, Schlüter G, Adler CP, Sandritter W (1970) Cytophotometrische DNS-, Histon- und Nicht-Histonprotein-Bestimmung an Zellkernen von menschlichen Herzen. Beitr Pathol 141:238–260
17. Goodman LC, Epling S, Kelly S, Lee S, Fishbein MC (1990) DNA flow cytometry of myocardial cell nuclei in paraffin-embedded, human autopsy, cardiac tissue. Am J Cardiovasc Pathol 3:55–59
18. Graumann W (1953) Zur Standardisierung des Schiffschen Reagenz. Z Wiss Mikrosk 61:225–226
19. Grimm AF, Torre L de la, Porta M la(1970) Ventricular nuclei-DNA relationships with myocardial growth and hypertrophy in the rat. Circ Res 26:45–52
20. Hort W (1953) Quantitative histologische Untersuchungen an wachsenden Herzen. Virchows Arch 323:223–242
21. Immich H (1974) Medizinische Statistik. Thieme, Stuttgart New York
22. Korecky B, Rakusan K (1978) Normal and hypertrophic growth of the rat heart: changes in cell dimensions and number. Am J Physiol 234:H123–H128
23. Lindner J (1983) Age dependent biochemical changes of connective tissue. In: Platt D (ed) Cardiology and aging. Schattauer, Stuttgart New York, pp 37–55
24. Linzbach AJ (1947) Mikrometrische und histologische Analyse hypertrophischer menschlicher Herzen. Virchows Arch 314:534–594
25. Linzbach AJ (1955) Quantitative Biologie und Morphologie des Wachstums einschließlich Hypertrophie und Riesenzellen. (Handbuch der Allgemeinen Pathologie VI/1) Springer, Berlin Göttingen Heidelberg, pp 181–306
26. Müller W (1883) Die Massenverhältnisse des menschlichen Herzens. Voss, Hamburg Leipzig
27. Oberpriller JO, Oberpriller JC (1985) Cell divisions in cardiac myocytes. In: Ferrans VJ, Rosenquist G, Weinstein C (eds) Cardiac morphogenesis. Elsevier, New York, pp 12–23
28. Pfizer P (1971) Nuclear DNA content of human myocardial cells. Curr Top Pathol 54:1–30
29. Poche R, Lindner E (1955) Untersuchungen zur Frage der Glanzstreifen des Herzmuskelgewebes beim Warmblütler und Kaltblütler. Z Zellforsch 43:104–120
30. Rosenberg B, Pfitzer P (1983) Ploidy in hearts of elderly patients. Virchows Arch [B] 42:19–24
31. Rumyantsev PP (1982) Cardiomyocytes in processes of reproduction, differentiation and regeneration (in Russian). Nauka, Leningrad
32. Sandritter W, Scomazzoni G (1964) Desoxyribonucleid acid content (Feulgen photometry) and dry weight (interference microscopy) of normal and hypertrophic heart muscle fibres. Nature 202:100–101
33. Sjöstrand FS, Andersson E (1954) Electron microscopy of the intercalated discs of cardiac muscle tissue. Experientia 10: 369–370
34. Strunz K, Herrmann H (1967) Die Massenzunahmen von N und DNS in vier Geweben beim wachsenden Rind. Z Tierphysiol 22:286–290
35. Tschermak-Woess E (1970) Endomitose. (Handbuch der allgemeinen Pathologie II/2) Springer, Berlin Heidelberg New York
36. Vendrely R, Vendrely C (1949) La teneur du noyau cellulaire en acide deoxiribonucleique a travers les organes, les invidus et les épèces animales. Etude particulière des mammifères. Experientia 5:327–329
37. Zak R (1973) Cell proliferation during cardiac growth. Am J Cardiol 31:212–219
38. Zak R (1974) Development and proliferative capacity of cardiac muscle cell. Circ Res 34:17