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Estimation of the Cell Number of Heart Muscles in Normal Rats

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The total number of heart muscle cells of rats was estimated by a histometrical method. It was approximately 5 millions immediately after birth and rapidly increased to over 19 millions in the first month of life. Then it gradually increased to 20–22 millions by the age of 2 years. The total number of interstitial cells was approximately 3.5 millions immediately after birth, and increased progressively to 28 millions in a month, to 68 millions in a year and to 72 millions in 2 years. The number of heart muscle cells per 1 mm^3 of heart muscle was approximately 330,000 immediately after birth and decreased with growth to about 1/9 of the initial number in 2 years. It was concluded that the heart muscle cells as well as the interstitial cells increased by cell division even after birth, and that the heart muscle cells not only increased in number, but also grew in size during the normal growth of the organ.

It has been generally accepted that heart muscle cells do not proliferate after birth, and that the heart is completely provided with its constituent muscle cells by the time of several weeks of gestation. It has been also stated that no regeneration of heart muscle cells takes place after myocardial infarction, in contrast to appreciable regenerative ability of the heart muscle, which is observed particularly in diphtheria, although it is not so pronounced as that of skeletal muscle.[1–5] If no regeneration occurs, a certain definite number of heart muscle cells must function during the whole life, except when some of the heart muscle cells are lost by fibrosis, necrosis, myocardial infarction or other myocardial injuries.

Attempts to calculate the nuclear number of heart muscle cells have been made by several investigators.[6–10] However, none of them estimated the total number of heart muscle cells, or referred to the possibility of cell division of the heart muscle. The present study was made to clarify whether heart muscle cells are constant in number after birth in rats, and the results were evaluated in relation to division of heart muscle cells.

Materials and Methods

Male rats of Wistar strain ranging from 1 day to 2 years of life were used. The animals used had not been subjected to any treatment which might have influenced cardiac

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activity. They were killed by decapitation, and the hearts were fixed as a whole in 10% formalin solution. The heart was sliced by coronal sections at equal intervals of 1 mm as shown in Fig. 1. Histological sections of 5μ thickness were made in the same direction. Histological specimens were stained with hematoxylin and eosin. They were magnified 10 times and projected on a sheet of tracing paper. The surface area of the muscle layer delineated on the paper was measured by planimetry. Multiplying the sum of areas in mm^2 by the thickness of each slice in mm, an approximate value of the total heart muscle

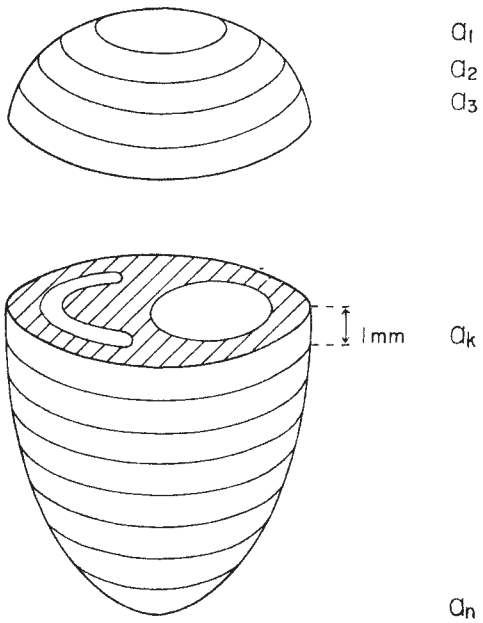

Fig. 1. Schematic illustration of making histological specimens to estimate heart volume.

volume V in mm^3 was obtained. This is shown in the following expression:

$$V = (a_1 + a_2 + a_3 + \ldots\ldots\ldots + a_n) \times 1 ,$$

where a_1, a_2, a_3,.... a_n are the surface areas occupied by myocardial tissues on individual slides. Using an ocular micrometer with lattice, all nuclei of heart muscle cells in a certain square ($50\mu \times 50\mu$) were counted under a microscope, and the procedure was repeated on 10 different places. The sum n of these counts can be regarded as the number of muscle cell nuclei in a heart muscle volume of $10 \times (50\mu \times 50\mu \times 5\,\mu)$, since the thickness of histological sections is 5μ. Thus, the total number N of the whole heart muscle cells can be calculated from the following expression on the assumption that the dimension of nuclei is negligible in comparison with the thickness of sections:

$$N = n \times \frac{V \times 10^9}{10 \times (50 \times 50 \times 5)}$$

The results were corrected by Abercrombie's method[11] to eliminate errors in cases when the nuclear diameter is not negligible in reference to sectional thickness. Abercrombie's formula is:

$$T = C \times \frac{s}{s + d} ,$$

where T is true count, C crude count, s thickness,

$$T = C \times s/(s+d),$$

where T is true count, C crude count, s thickness of specimen and d diameter of the nucleus. Since the nuclei of heart muscle cells are oval, the average length plus width of nucleus was

TABLE 1. *Total number of heart muscle cells and of interstitial cells*

Age	Body weight g	Heart weight mg	Total heart volume mm³	Heart muscle cell			Interstitial cell		
				Number in unit vol.	Crude count ×10⁴	True count ×10⁴	Number in unit vol.	Crude count ×10⁴	True count ×10⁴
1 day	6	27	14.5	88	1,020	489	58	672	345
1 〃	5	25	14.4	90	1,036	493	59	679	348
1 〃	5	28	15.6	86	1,073	511	56	698	358
1 〃	6	28	16.0	85	1,088	518	60	768	393
1 week	12	55	25.8	80	1,651	786	63	1,300	666
1 〃	12	60	26.0	80	1,664	792	60	1,248	640
2 weeks	22	103	65.9	60	3,163	1,506	55	2,899	1,486
2 〃	22	121	67.6	59	3,190	1,519	55	2,974	1,525
3 〃	52	198	96.5	55	4,246	1,806	51	3,937	2,019
3 〃	50	197	95.4	57	4,350	1,851	54	4,121	2,113
3 〃	52	222	98.6	53	4,180	1,778	51	4,022	2,063
4 〃	66	253	128.5	45	4,626	1,968	56	5,756	2,952
4 〃	60	240	132.7	43	4,564	1,942	50	5,308	2,722
2 months	140	504	222.0	29	5,150	2,146	49	8,702	4,462
2 〃	112	470	214.6	30	5,150	2,146	49	8,412	4,314
3 〃	240	770	354.1	19	5,382	2,196	38	10,764	5,520
3 〃	268	880	382.1	18	5,502	2,245	34	10,393	5,329
6 〃	290	713	369.3	17	5,022	2,008	37	10,931	5,605
6 〃	250	783	395.8	17	5,382	2,153	37	11,715	6,008
8 〃	286	940	400.1	16	5,121	2,048	35	11,202	5,745
1 year	370	1,163	516.1	13	5,367	2,064	32	13,212	6,775
2 years	450	1,250	582.0	12	5,587	2,148	30	13,968	7,163

regarded as the 'diameter'. Because the nucleus of heart muscle cell increases in size with growth, the size was measured in each specimen.

Exactly in the same way, the total number of nuclei of interstitial cells comprising all cells other than heart muscle cells was calculated.

Results

The age, body weight, heart weight, total heart volume, number of cell nuclei counted in a certain muscle volume ($10\times50\mu\times50\mu\times5\mu$), crude counts, and true counts of heart muscle cells and of interstitial cells of rats are given in Table 1. The length and width of nuclei of heart muscle cells and of interstitial cells are also shown in Table 2.

Table 2. *The nuclear size of myocardial cells of rats*

Age	Heart muscle cell		Interstitial cell	
	Length μ (Mean)	Width μ (Mean)	Length μ (Mean)	Width μ (Mean)
1 day	12(7)	6(4)	13(7.5)	3(2)
1 week	13(7)	7(4)	13(7.5)	3(2)
2 weeks	12(7)	6(4)	13(7.5)	3(2)
3 〃	14(9)	7(4.5)	13(7.5)	3(2)
4 〃	14(9)	7(4.5)	13(7.5)	3(2)
2 months	16(9.5)	6(4.5)	13(7.5)	3(2)
3 〃	15(10)	7(4.5)	13(7.5)	3(2)
6 〃	17(10.5)	7(4.5)	13(7.5)	3(2)
8 〃	17(10.5)	7(4.5)	13(7.5)	3(2)
1 year	17(11)	7(5)	13(7.5)	3(2)
2 years	17(11)	7(5)	13(7.5)	3(2)

The total number of heart muscle cells is approximately 5 millions immediately after birth and increases to 8 millions a week later. It increases to about 15 millions in 2 weeks, 18 millions in 3 weeks and exceeds 19 millions a month after birth. Then it continues to increase very slowly to 20–22 millions by the age of 2 years. It is apparent that the number of heart muscle cells increases rapidly during a month after birth and increases gradually thereafter (Fig. 2).

On the other hand, the total number of interstitial cells is approximately 3.5 millions immediately after birth, and it increases progressively to 6.5 millions in 1 week, 15 millions in 2 weeks, 20 millions in 3 weeks, 28 millions in 4 weeks, 44 millions in 2 months, 55 millions in 3 months, 56 millions in 6 months, 58 millions in 8 months, 68 millions in 1 year, and 72 millions in 2 years. The linear increase in number of interstitial cells is well demonstrated in Fig. 3.

Approximate numbers of heart muscle cells and interstitial cells per 1 mm^3 of heart muscle in each animal are shown in Table 3. The number of heart muscle cells per 1 mm^3 is approximately 330,000 immediately after birth, and it decreases gradually with growth. The initial number falls to a half in 3–4 weeks, to about 1/8 in 1 year, and finally to about 1/9 in 2 years. The number of

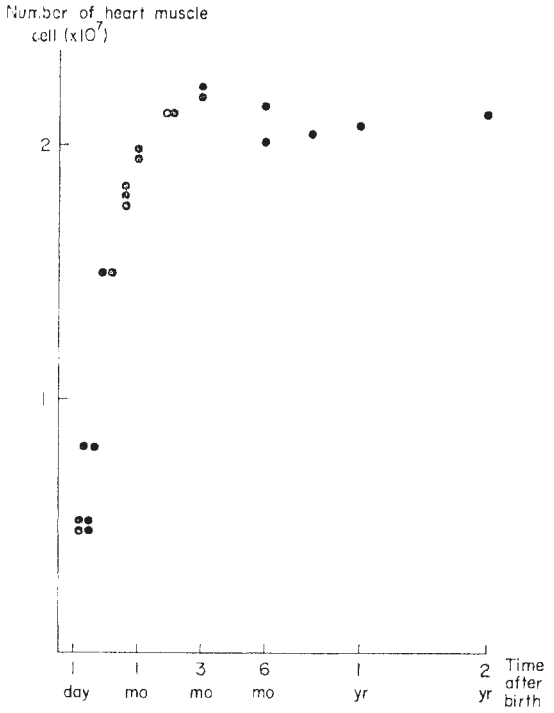

Fig. 2. Total number of heart muscle cells and age.

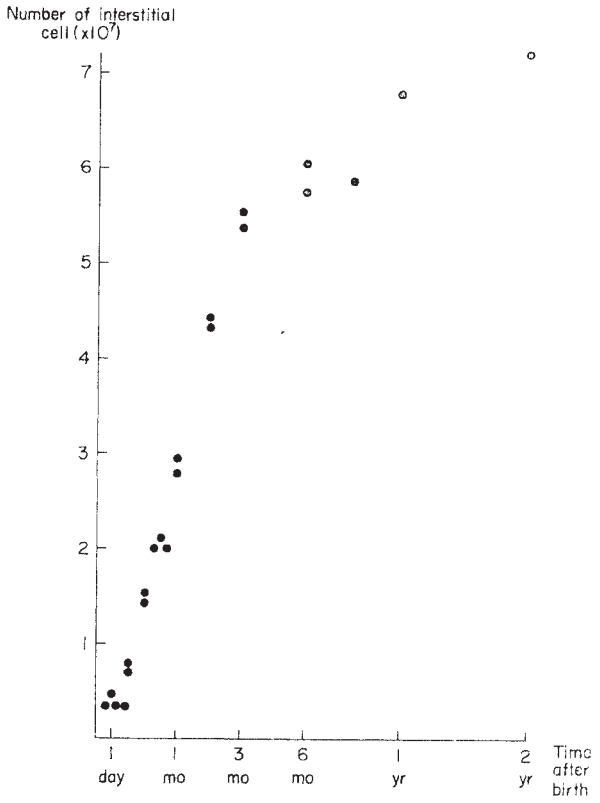

Fig. 3. Total number of interstitial cells and age.

TABLE 3. *Numbers of heart muscle cells and of interstitial cells per mm³ of heart volume*

Age	Heart muscle cell $\times 10^3$	Interstitial cell $\times 10^3$
1 day	335	237
1 〃	340	242
1 〃	327	229
1 〃	323	246
1 week	304	258
1 〃	304	246
2 weeks	228	225
2 〃	224	225
3 〃	187	209
3 〃	194	221
3 〃	180	209
4 〃	153	229
4 〃	146	205
2 months	96	201
2 〃	100	201
3 〃	62	155
3 〃	58	139
6 〃	54	151
6 〃	54	151
8 〃	51	141
1 year	40	131
2 years	36	123

interstitial cells per 1 mm³ is approximately 240,000 immediately after birth and remains fairly constant until 2 months. Then it gradually decreases to about a half of the initial number in 2 years.

DISCUSSION

The heart muscle has been long believed to be of syncytial structure. However, recent progress in studies of the ultrastructure of heart muscle cells by electron microscopy has elucidated that each heart muscle cell is definitely separated, and overlapping of cells gives only apparent syncytial appearance.[12,13] Although Henschel[7] states that there are many double nucleated muscle cells in the heart of a young man, one heart muscle cell harbors usually only one nucleus and seldom two nuclei. Therefore, there is no doubt that calculation of the nuclei is a reliable procedure to obtain an approximate number of heart muscle cells. Similar attempts to estimate the cell number of heart muscles have been made by several investigators.[6–10] Beginning with calculation of the number of muscle fibers, Linzbach[8–10] and Henschel[7] counted the number of papillary muscle cells of human hearts and of nuclei of ventricular muscle cells. Linzbach proposed a specific formula to calculate the number of cell nuclei per unit area of heart muscle. He stated that the number of muscle cell nuclei in a unit area of heart muscle was constant in both the right and left ventricles, and the number of cell nuclei in the ventricle is proportional to the heart weight, when it was below so-called critical heart weight. According to Linzbach, all human hearts have essentially the same number of myocardial fibers and myocardial nuclei. He has

also mentioned that human hearts below the critical heart weight of 500 g is a 'zellkonstantes' organ, because the relation of the number of heart muscle cell nuclei to that of muscle fibers is constant.

Hort[6] modified the method of Linzbach and found that the number of heart muscle cell nuclei in newborn rats was about a half of that in adult rats. In these previous investigations, however, neither the total heart muscle volume nor the total number of heart muscle cells was estimated. In order that the correct total number of heart muscle cells may be obtained by our method, the distribution of heart muscle cells must be uniform in the whole heart. In other words, the muscular architecture must be the same in both ventricles as well as in both atria. It has been reported that the cell number is exactly the same in a unit area of both ventricles.[8–10] It was also confirmed in our histological specimens that the number of muscle cells in the same surface area of histological sections was the same in the atria and ventricles. Since the muscular architecture seems to be the same in every part of the heart, it is reasonable to calculate the total number of heart muscle cells by our method.

Our results show that there is a definite and rapid increase in the total number of heart muscle cells from 1 day-old to 1 month-old and then a gradual increase up to 2 year-old animals. This suggests that heart muscle cells divide and increase in the early period of life. With the growth of the heart the cell number in a unit muscle volume appears to become progressively smaller, as is shown in Table 3. These figures indicate that individual heart muscle cells are enlarged to approximately 10 times the initial size in the course of 2 years of life. It is, therefore, evident that the heart muscle cells not only increase in number, but also grow in size during the normal growth of the heart.

Further evidence to support our view that the heart muscle cells increase in number by cell division has been presented. The chemical determination of DNA revealed that the total DNA content of the whole heart muscle of rats increased with the growth of the animals.[14,15] Microspectrophotometric determination of DNA content per each nucleus showed that the quantity was the same in both heart muscle cells and interstitial cells.[14] All of these reports indicate that the heart muscle cells divide and increase in number. Finally, autoradiographic studies of DNA synthesis by ^{3}H-thymidine on rats demonstrated a definite uptake of ^{3}H-thymidine into the nuclei of heart muscle cells as well as of interstitial cells.[16,17] It is thus concluded that the heart muscle cells increase by cell division even after birth.

It is interesting that although the number of interstitial cells is smaller than that of heart muscle cells immediately after birth, the former reaches the same level as that of the latter in 2 weeks and increases progressively thereafter. From the proportion of the number of interstitial cells to the number of heart muscle cells, approximate age of a rat may be estimated. This finding that the interstitial cells exceed the heart muscle cells in number with growth of the heart seems to be important. Since we did not discriminate the endothelial cells of capillary vessels

from the interstitial cells, quantitative correlations between endothelial and muscle cells as discussed in Linzbach's report[8] could not be examined.

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