Myocardial DNA and Protein in Maturing and Hypertrophied Human Hearts[1] (34434)

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There is general agreement that cardiac hypertrophy, as its name implies, is the result of cellular enlargement rather than increase in cell number. This concept was originally based on the fact that mitoses are unknown in adult cardiac muscle (1) and has gained support from radioautographic data in animals (2). One difficulty with this idea is that the DNA/protein ratio has not changed strikingly in almost every report in which it has been measured in experimental cardiac hypertrophy (3–5), despite repeated demonstrations that increased myocardial protein synthesis occurs (6–8). The obvious problems posed by these two sets of data, one based on studies of DNA synthesis or mitosis, the other on DNA/protein ratios, remain even after two recent significant types of studies. In man, Feulgen cytospectrophotometric data indicate that the normal adult human heart muscle cell is, on the average, tetraploid while in hypertrophy the incidence of ploidy of 8 or more is greatly increased (9). On the other hand, although increased DNA synthesis has been demonstrated in rat ventricular hypertrophy, radioautography has shown that the new DNA synthesis is virtually restricted to connective tissue cells (10). Nor is polyploidy striking in such rats (11).

Because of the paradox presented by these confusing sets of data, the problem was reinvestigated by measuring the DNA content of isolated left ventricular nuclei and DNA/protein ratios in whole muscle of normal and hypertrophied hearts of various ages in man. The collagen, water, and electolyte content was also determined.

Materials and Methods. A 2.5-cm cube of anterior-superior left ventricular muscle was obtained from adult autopsies performed within 18 hr after death. In infants, the upper one-half of the left ventricle was obtained. The endocardium, epicardium and all grossly visible scars were carefully dissected off. The specimen was then divided into three parts. The first portion was weighed, dried overnight at 104°, weighed again, and water content then was calculated from these two measurements. The dried tissue was then ashed in H_2SO_4 and Ca and Mg were measured with an atomic absorption spectrometer and Na and K by flame photometry. The second portion was homogenized in 0.25 *M* sucrose in a Waring Blendor and nuclei were isolated by a differential centrifugation procedure after layering the homogenate over 2.2 *M* sucrose (12). The resultant nuclear pellet was quite clean. In infant hearts, one size nucleus was identified. In adult hearts, small spindle-shaped nuclei, presumably from connective tissue cells, and large rectangular nuclei, presumably from muscle cells, were present in the pellet. The yield of nuclei from very large hearts was quite low, but could be quadrupled by adding 0.1% tetraphenylboron to the homogenate. Several trials showed that this procedure did not significantly alter the mean DNA content/nucleus. Nuclei were then counted in a hemocytometer and DNA was determined on an aliquot, containing a known number of nuclei, by the method of Giles and Myers (13).

The third portion was homogenized in a Waring Blendor in 0.9% NaCl and then pre-

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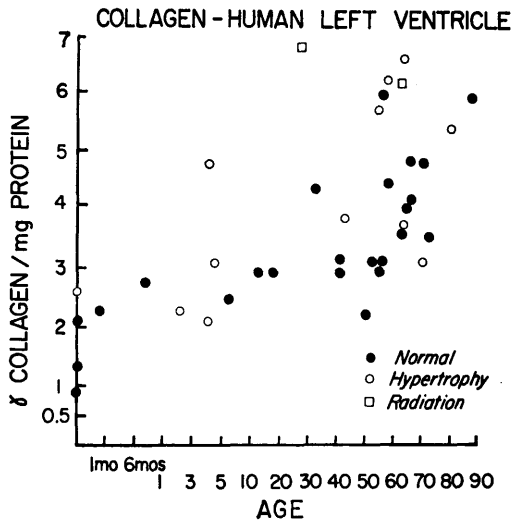

FIG. 1. Collagen content increases with age with a suggestion of increases in hypertrophied hearts. With the exception of two cases with extensive fine scarring, the highest collagen concentrations were in two patients who had received theapeutic mediastinal radiation.

cipitated with cold 5% trichloroacetic acid (TCA). The precipitate was washed twice with 5% TCA and extracted four times with hot (90°) 5% TCA. These extracts were pooled and DNA was measured in them by the method of Burton (14). The precipitate was washed with alcohol–ether, air dried, and weighed. In the precipitate, protein was determined by the Lowry method (15) and hydroxyproline by the method of Stegeman (16). Collagen was calculated by multiplying the hydroxyproline value by 7, since collagen is approximately 14% hydroxyproline.

For cytospectrophotometric study, smears were made of the nuclei on quartz slides. The slides were fixed and rinsed in ethanol and covered with n-1.459 glycerin. Quantitative absorption of individual nuclei was done with the Carl Zeiss Universal microspectrophotometer (UMSP-1) in the wavelength of 260 mμ with a measuring spot of the scan lines of 0.5μ.

Results. The content of water, Ca, Mg, Na, and K did not vary significantly among the hearts. The collagen content increased with advancing age and seemed to be greater in hypertrophied than nomal adult hearts. The amount of hydroxyproline present was surprisingly low, perhaps because great care was taken to dissect away all grossly visible connective tissue. Except for two instances with diffuse interstitial fibrosis, the two hearts which had the greatest collagen content were from patients who had received therapeutic doses of radiation to the mediastinum for carcinomas (Fig. 1).

The data on the DNA content of individual nuclei must be interpreted with caution. In contrast with cytospectrophotometric data, which measure the DNA content of individual nuclei, the chemical method only measures mean DNA content. In addition, the method of nuclear isolation does not separate connective tissue nuclei from those of muscle cells. This seemed to be much less of a problem in infants since the yield of nuclei was much greater than in adults. With these reservations in mind, Fig. 2 reveals essential agreement with published data based on cy-

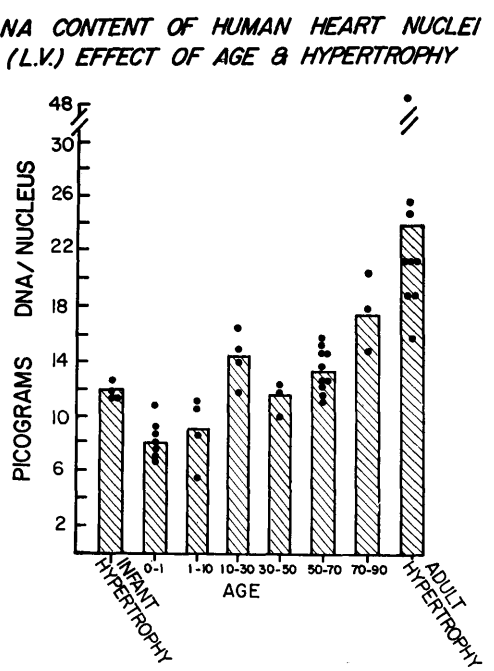

FIG. 2. By chemical measurement, infant heart nuclei appear to be diploid, rapidly becoming polyploid in childhood, and developing even greater polyploidy with advanced age or hypertrophy. Infantile hypertrophic hearts also appear to be tetraploid. Bars are of mean values, dots are individual ones.

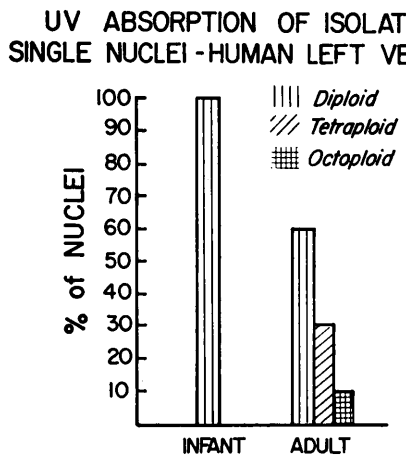

FIG. 3. Quantitative ultraviolet absorption studies individual nuclei from an infant heart show all the cells to be diploid, in agreement with the data in Fig. 2. In two adult hearts, the diploid nuclei had the morphology of connective tissue nuclei and the polyploid nuclei the morphology of muscle nuclei.

tospectrophotometry (19). Thus, normal adult hearts have DNA values greater than diploid, since the average human diploid cell contains about 7 pg of DNA/nucleus. Nuclei from hypertrophied hearts (<500 g), contained even more. Normal infant hearts averaged diploid values of DNA and became tetraploid in early childhood. The few available infant hypertrophied hearts appeared to be tetraploid.

As confirmary data, quantitative ultraviolet absorption studies of 40 nuclei each from one infant and two adult hearts were done. As shown in Fig. 3, all nuclei of the infant heart had a diploid DNA content. In the adults, those nuclei which were diploid by cytospectrophotometry were small and cigar-shaped and so considered to be of endothelial cells or fibroblasts. The other, muscle, nuclei were either tetraploid or octoploid. No intermediate values of absorption were found.

The DNA/protein ratios (Fig. 4) were high at birth and decreased rapidly as the heart grew. The values in hypertrophied hearts, although more widely scattered, did not vary significantly from normal hearts of similar ages. It is of interest that there were two hearts with inordinately high values. The first, which was small (270 g), was from a patient with severe long-standing orthostatic hypotension. The other, which was large (750 g), was from a patient with idiopathic myocardiopathy. Recalculation of the data as DNA/noncollagen protein did not significantly alter the values.

Discussion. The data can be interpreted simply. At birth, heart muscle cells have a diploid complement of DNA. In early postnatal life, observations of mitoses (17) indicate that they do have at least a limited capacity to replicate DNA and mitose. The preponderance of tetraploid nuclei in older hearts and infant hypertrophies and of even greater polyploidy in adult hypertrophies as well as the failure to observe mitoses in adult hearts indicates that the ability to mitose is rapidly lost, but the ability to replicate DNA is not.

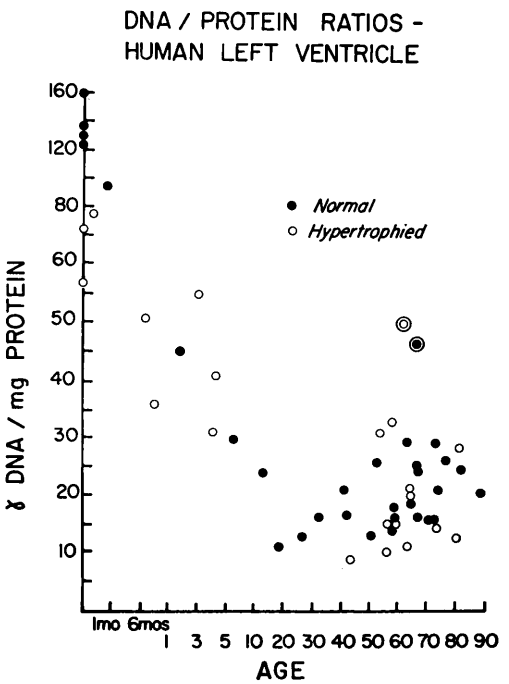

FIG. 4. DNA/protein ratios are very high at birth, rapidly decreasing with aging. In adults, although the scatter is substantial, there appears to be no change in this ratio in hypertrophied hearts. The high value represented by the double closed circle is from a patient with a small (270 g) heart and prolonged orthostatic hypotension. The open double circle is from a large (750 g) heart of a patient with idiopathic myocardiopathy. Recalculation of data as DNA/noncollagen protein did not significantly alter the graph.

So the heart, like other organs such as the liver, can replicate its DNA in response to functional demand, but cannot mitose. It is of interest that tumors of heart and neurons are virtually unknown and that, in the cat at least, hippocampal neurons are also tetraploid (18). The inability of these cells to produce tumors is thus related more to their inability to mitose than to make DNA.

The high DNA/protein ratios in early infancy reflect the fact that heart muscle cells are small at birth and the decline of the ratio in later life would be expected as the cell grew in size. The lack of change in hypertrophied hearts would be explained by the increased polyploidy. It also implies that there may be a maximal rate of protein synthesis for a given gene dose in a heart muscle cell and for excess protein to be synthesized above this limit, more DNA is required. This notion is supported by data that rat liver undergoes a spurt of mitosis before rapid synthesis of albumin begins in plasmapheresed rats (19) and similar data in other experimental situation (2). The two cases with high ratios are of some interest. One, from a patient with orthostatic hypertension, may be due to atrophy. The other, from a patient with idiopathic myocardiopathy, suggests that an inability of the nucleus to direct the synthesis of sufficient protein for the needs of the heart is part of this disease.

The increase in collagen in aging hearts is in agreement with data in the literature. The high collagen values in the two radiated hearts would be expected. The lack of the striking increases described in experimental hypertrophy (21) may be because such increases were masked by fibrosis due to coronary sclerosis in human hearts, or, more likely, because great care was taken to dissect away connective tissue before chemical measurements were made.

Summary. Human heart muscle cells are diploid at birth. In agreement with other data, they become tetraploid in normal adults and develop greater polyploidy in adult hypertrophy. Some cases of infantile hypertrophy appear to have mostly tetraploid nuclei. In keeping with the enlargement of muscle cells which occurs during growth, the DNA/protein ratio is high at birth, progressively decreasing until adulthood, after which it does not change in hypertrophied hearts except in clinically unusual conditions. This stability of the DNA/protein ratio in hypertrophy thus appears due to a concomitant increase in DNA and protein in the muscle cells.

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