HYPERTROPHY OF THE HUMAN HEART AT THE LEVEL OF FINE STRUCTURE

An Analysis and Two Postulates

G. W. RICHTER, M.D. and A. KELLNER, M.D.

From the Department of Pathology, The New York Hospital—Cornell Medical Center, New York

ABSTRACT

Muscle cells in the left ventricular walls of four markedly hypertrophied human hearts (above 600 gm) were compared with muscle cells in four non-hypertrophied hearts (up to 310 gm). Blocks of tissue obtained postmortem within 6 hours were processed for light and electron microscopy under conditions suitable for good preservation of myofibrils. A lattice parameter, q_h, was defined as the number of myosin filaments per square micron in either H zones or A bands. By the use of methods of electron microscopy, q_h was determined for perpendicular cross-sections of A bands in a large number of well preserved myofibrils of muscle cells in both groups of hearts. Statistical evaluation of the distributions of values of q_h revealed no significant difference between the two groups. Thus, the myofilament lattices in hypertrophied cells were geometrically within normal limits. Planimetric measurements of cross-sectional areas of muscle fibers were made, using photomicrographs obtained from one representative hypertrophied heart and from one control. The size-frequency distribution of the measurements showed a marked difference between the two hearts, and confirmed the presence of hypertrophy of muscle cells. Counts of the number of myofibrils per muscle cell were determined for samples from the same two hearts, evaluated statistically, and found to be significantly higher for the hypertrophied heart. It is proposed (*a*) that myofibrils in hypertrophied heart muscle cells have filament lattices with geometrical arrangement and macromolecular parameters that are the same as those found in myofibrils of normal heart muscle cells; and (*b*) that in hypertrophy the number of myofilaments increases through formation of new myofibrils, and possibly also by addition of filaments to preexisting myofibrils.

INTRODUCTION

Careful postmortem studies of untold numbers of human hearts have served to emphasize that in myocardial hypertrophy the bulk of muscle cells is enlarged. The well known study of Karsner, Saphir, and Todd (1) provided quantitative evidence for an increase in size of muscle cells and for the concept that myocardial hypertrophy is due to an increase in size of cells rather than in their number.

Sections from hypertrophied left ventricular muscle characteristically reveal that the contractile substance itself occupies most of the available space in the enlarged muscle cells, though concomitant enlargement of cell nuclei is also frequent. How does the contractile substance in a hypertrophied heart muscle cell differ from that in a normal one in terms of fine structure? How can one explain the various degrees

of hypertrophy of different heart muscle cells and the variation in size of muscle cells in normal, non-hypertrophied myocardium?

The present report deals with an attempt to provide answers to these questions.

MATERIALS AND METHODS

Formal Considerations

The fine structure of contractile elements in mammalian cardiac and skeletal muscle cells has been described in detail by others (2–8). An essential attribute is the presence of assemblies of thick (H) and thin (I) filaments in geometrically specified order, each assembly forming a myofibril. The muscle cells contain varying numbers of myofibrils, and these, in turn, vary in size and reveal characteristic sarcomeres. A cross-sectional area in any part of a sarcomere can be defined by the number of myofilaments of specified type it contains, provided the characteristic geometrical pattern has been preserved. For example, if the lattice spacings of H filaments in A bands are approximately equal in myofibrils of different muscle cells, then the ratio *Number of* H *filaments/(micron)*^{2} should, within statistical limits, be the same in all comparable cross-sections. Because of the geometrical relationship of H and I bands, the same would apply to the ratio *Number of* I *filaments/(micron)*^{2}. From a technical point of view, the H filaments are more suitable for enumeration, and therefore our analysis was based on them.

In this report the symbol q_h is used for the ratio *Number of* H *filaments/(micron)*^{2}. The statistical distribution of the ratio q_h was determined for myofibrils in normal and in hypertrophied heart muscle cells with the aid of electron microscopy. In order to make a clear-cut comparison between greatly hypertrophied and non-hypertrophied human heart muscle, it was necessary to use hearts obtained postmortem, and to ensure, by traditional methods of pathologic anatomy, that these hearts were representative.

TABLE I

Summary of Cases

Group	Autopsy No.	Specimen No.	Age	Sex	Weight of Heart	Thickness of left ventricle at mitral ring	Relevant findings or history
					gm	*mm*	
Hypertrophied	19751	R-790	34	M	810	19	Cor atrioventriculare commune, bilateral superior venae cavae.
"	19780	R-791	73	M	610	18	Hypertensive and arteriosclerotic cardiovascular disease with nephrosclerosis and uremia.
"	19812	R-792	46	M	1070	19	Rheumatic heart disease with calcific aortic stenosis and mitral stenosis.
"	19965	R-802	54	M	680	20	Hypertensive cardiovascular disease, diabetes mellitus.
Control	19801	R-783	67	F	270	10	Glioblastoma multiforme, heart unremarkable.
"	19913	R-798	59	M	280	13	Carcinoma of stomach, heart unremarkable.
"	18869	R-799	84	F	280	14	Moderate mitral stenosis, calcification of annulus fibrosus.
"	19891	R-805	55	M	310	14	Carcinoma of lung, focal sclerosis of coronary arteries, but heart not otherwise remarkable.

Hearts

Hearts were obtained from cases that had come to autopsy within 5 hours after death, the bodies having been kept in a refrigerator in the interim. Eight hearts were chosen from a collection kept in 10 per cent neutral formalin. A summary of postmortem findings is given in Table I. In four hearts

there was marked gross hypertrophy of the left ventricle, and all of these hearts weighed more than 600 gm. The normal controls consisted of four non-hypertrophied hearts, three weighing less than 300 gm and one weighing 310 gm. It was obvious on gross inspection and by examination with the light microscope that, in the hearts weighing more than 600 gm, a great majority of muscle fibers in random sections from the left ventricles were markedly hypertrophied. Nevertheless, some size-frequency distributions of muscle fibers were determined by the method of Karsner *et al.* (1) and by planimetry, using photomicrographs of perpendicular cross-sections from blocks prepared for electron microscopy as described further on.

In the selection of controls, one heart with demonstrable valvular disease but no gross hypertrophy was included (Table I).

Preparation of Tissues

Although tissue taken at autopsy is generally not so satisfactory for electron microscopy as tissue obtained antemortem and fixed immediately, earlier work (9) indicated that under some circumstances postmortem material is suitable. In preliminary tests this proved to be true of blocks from the myocardium of about half the number of cases that were autopsied within 6 hours after death. Though preservation of cells was far from ideal, the filamentous lattices of sarcomeres were well preserved and were, in general, indistinguishable from those seen in fresh material (*e.g.*, biopsies).

In order to facilitate examination of a large number of cross-sectional profiles, blocks of myocardium were taken from trabeculae carneae, where many fibers run parallel. To maintain valid criteria for comparison, cylinders of muscular tissue were dissected from grossly hypertrophied trabeculae carneae in hypertrophied left ventricles. In the normal left ventricles, trabeculae of average thickness were sectioned.

The hearts had been fixed in 10 per cent neutral formalin for periods ranging from several weeks to 5 months. Blocks chosen were kept in Millonig's fixative (10) for 1½ hours, and were dehydrated in graded concentrations of ethyl alcohol on a 5-hour schedule. During dehydration the blocks were also treated with phosphotungstic acid. Thus, the 70 and 95 per cent alcohols contained 1 per cent phosphotungstic acid, and 2 per cent phosphotungstic acid was present in the first of two baths in 100 per cent alcohol. The blocks were embedded in Epon 812 Epoxy Resin according to Luft's method (16) with slight modifications. During embedding, the blocks were oriented so as to facilitate the cutting of sections normal to the longitudinal axis of most fibers. For electron microscopy, sections giving silver to gold interference colors were cut with a Porter-Blum microtome, mounted on carbon-coated copper mesh, and stained with lead hydroxide according to Millonig's modification of Watson's method (11). For light microscopy, sections 0.5μ in thickness were cut normal to the longitudinal fiber axes, and stained with phosphotungstic acid–hematoxylin.

Electron Microscopy. Standardization

Electron microscopy was done with a Siemens Elmiskop I. All pictures were taken at the same setting on the magnification scale; *i.e.*, 16,700. This setting was calibrated by taking pictures of monolayers of ferritin (12). Each time a series of pictures was taken, the magnification was recalibrated.

Method of Determining q_h

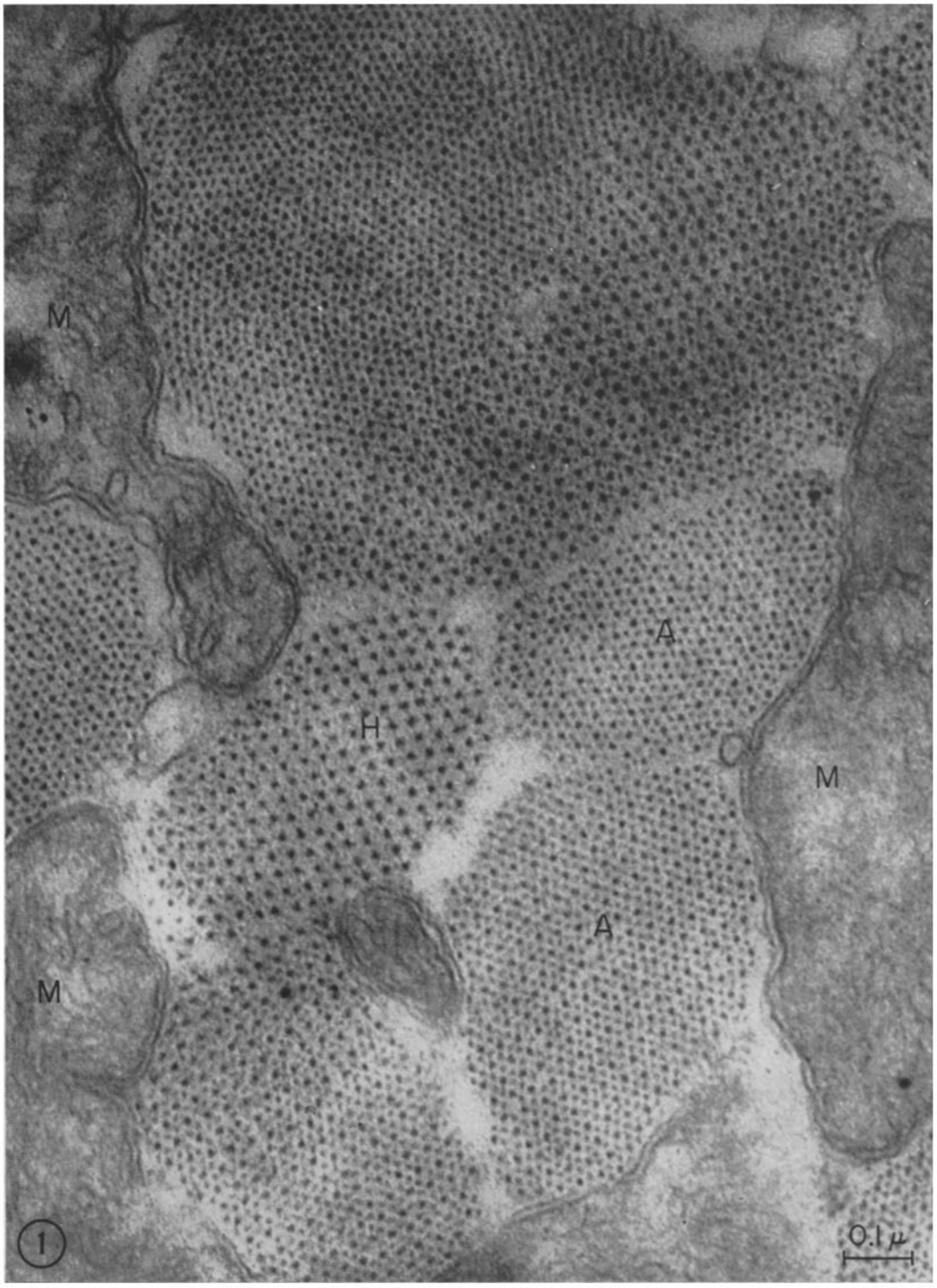
q_h was determined as follows. Suitable negatives were printed at predetermined enlargement. Only good cross-sectional profiles, normal to the longitudinal filament axes of A bands, and, occasionally, H zones, were evaluated. Circular areas of various sizes were cut out of old x-ray films and were used as templates. In general, a circular area most nearly fitting a given cross-section was chosen and superimposed upon the cross-sectional profile. The number of H filaments within the circle was counted, and q_h was then calculated, taking into account magnification of print and of original negative. q_h was expressed in filaments per square micron.

Since there is some variation in the diameter of each individual myofibril along the different segments of sarcomeres, even though the number of H filaments remains the same, q_h was determined for cross-sections through comparable regions of A bands.

RESULTS

Qualitative Findings

Fig. 1 shows a representative electron micrograph in which there are profiles of cross-sections of myofibrils from hypertrophied left ventricular muscle. It can be seen that the filament lattices are well preserved and have retained the characteristic geometrical relationship of H and I filaments. The cross-sectional diameters of H (myosin) and I (actin) filaments were measured. Calculations made, taking into account the variation due to the presence or absence, respectively, of cross-links, indicated that the diameters of H filaments were generally between 110 and 140 A, and those of I filaments between 50 and 100 A. Figs. 2 and 3 are photomicrographs of muscle cells in thick sections and at the same magnification.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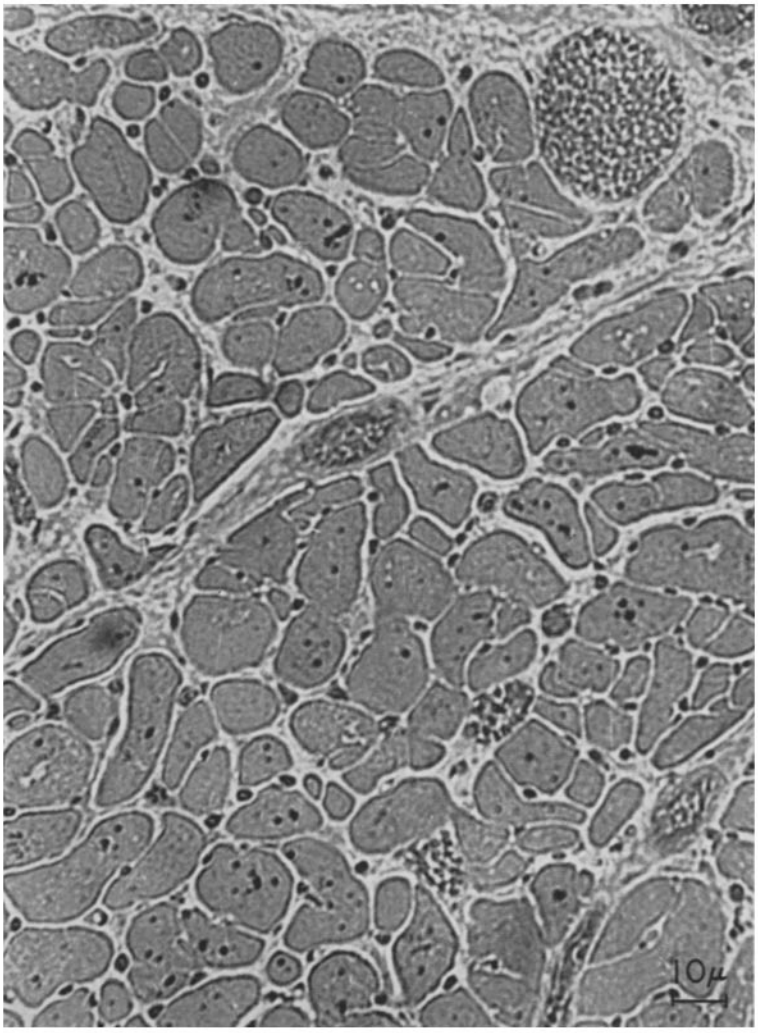

FIGURE 2

Photomicrograph of muscle cells from hypertrophied heart (R-791). Myofibrils are barely visible. Thick section from block embedded in Epon 812. Stained with phosphotungstic acid and eosin. × 700.

FIGURE 1

Electron micrograph showing cross-sections of myofibrils in muscle cell from a hypertrophied left ventricle. The heart was obtained 5 hours postmortem, kept in 10 per cent neutral formalin for several weeks, fixed in Millonig's fluid, and impregnated with alcoholic phosphotungstic acid. The characteristic myofibrillar structure is well preserved. H zones (*H*) and A bands (*A*) can be seen. *M*, mitochondria. × 100,000.

By comparison of Figs. 2 and 3 one can readily tell that Fig. 2 shows hypertrophied heart muscle, but quantitative confirmation was sought by further analysis.

Quantitative Evaluation

In Fig. 4 the size-frequency distribution of muscle cells in a trabecula carnea from one of the control hearts (R-783, 270 gm) is compared with that of muscle cells in a trabecula carnea from a hypertrophied heart (R-791, 610 gm). As given in Fig. 4 (legend), the results of this statistical comparison can leave no doubt about the fact that in the sample from the hypertrophied heart most muscle cells were significantly larger than were the cells in the control. This finding provided confirmation of the validity of the criteria used in selecting tissue from hypertrophied hearts and from controls.

In further work, counts were done to determine the frequency distribution of the number of myofibrils per muscle cell in the same two hearts (R-783 and R-791). For this purpose, blocks of tissue that had been fixed in Zenker-formol solution and embedded in paraffin were used. Sections, averaging about 3μ in thickness, were

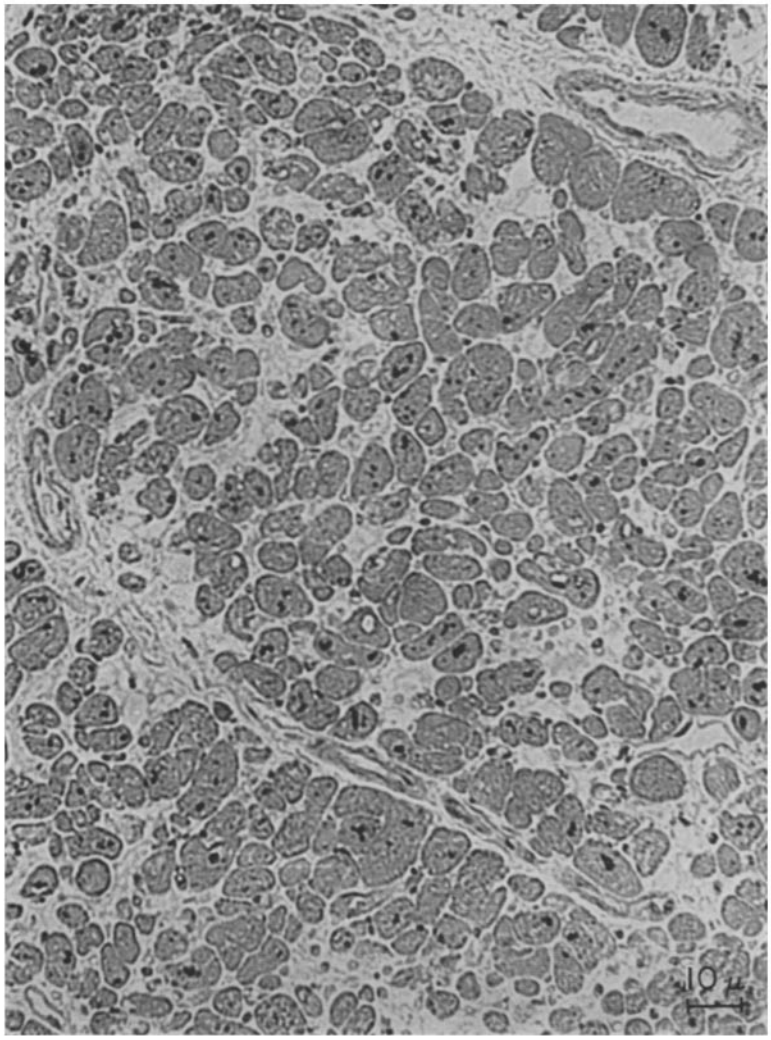

FIGURE 3

Photomicrograph of muscle cells from control (non-hypertrophied) heart (R-783). × 700.

stained with phosphotungstic acid–hematoxylin solution (13) so that myofibrils could be distinguished with certainty from other cell constituents. Photomicrographs were taken through an apochromatic objective with a numerical aperture of 1.4. Counts of the number of myofibrils per cell were made on suitable enlargements of cross-sectional images that were normal, or close to normal, to the fibrillar axes. The muscle cells on which counts were made were selected at random. The results are shown in Fig. 5 and in Table II, in which a statistical evaluation is given. Although the size of each sample (70 cells from each of the two hearts) was small, the counts of myofibrils (6,908 and 11,070) were large enough to permit extended analysis. The difference in the two distributions was highly significant, as is shown in Table II. One can infer, therefore, that hypertrophied heart muscle cells have more myofibrils than do normal heart muscle cells.

Values of q_h were obtained for perpendicular cross-sections through A bands and H zones of myofibrils in different cells. For the final statistical evaluation only q_h values for A bands were used, because preliminary graphic analysis showed that within individual myofibrils the q_h of H zones differed to a possibly significant degree from the q_h of adjacent A bands. This discrepancy applied equally to controls and to hypertrophied heart muscle and is therefore irrelevant to the problem under consideration. For general reference, a tabulation of unselected q_h values for A bands and H zones is given in Table III. In order to provide a valid comparison, the statistical analysis of the distribution of q_h given in this

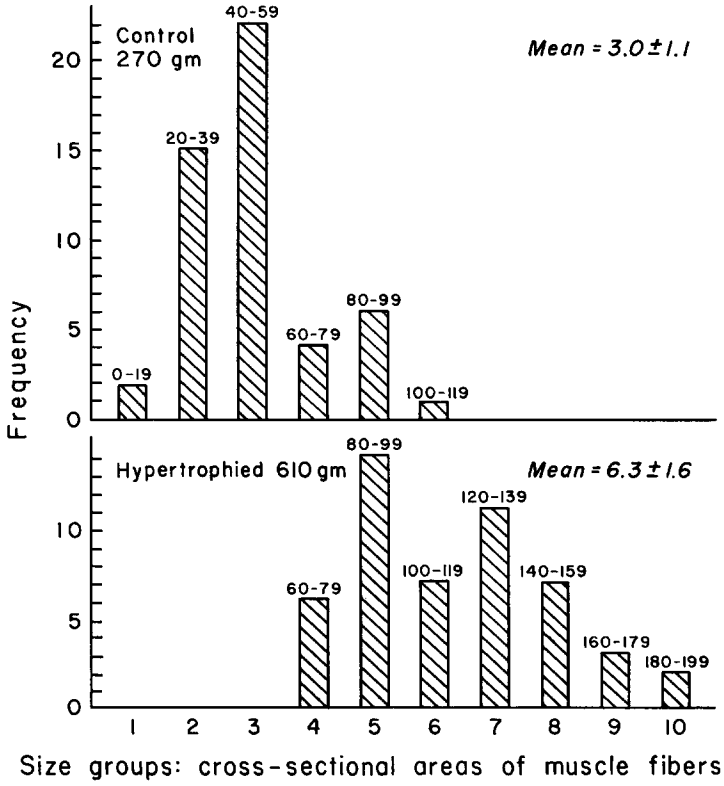

FIGURE 4

Size-frequency distributions of cross-sectional areas of muscle cells in a control and in a hypertrophied heart. The numerical ranges over the bars represent readings on the planimeter used to measure the areas. A t test of the two distributions shown gave the following results: t = 7.64; degrees of freedom: 98; $P \ll 0.001$, significant.

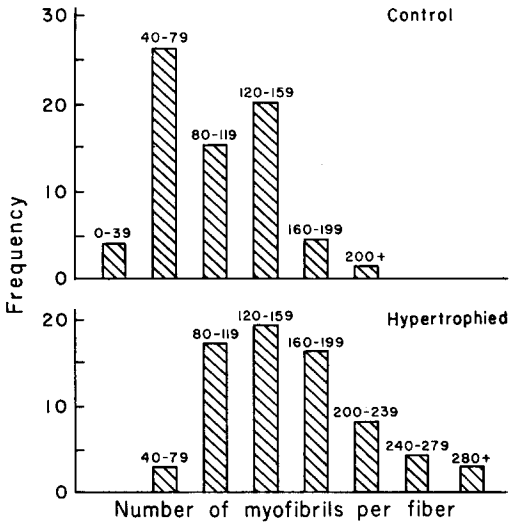

FIGURE 5

Frequency distribution of counts of myofibrils in heart muscle cells. A statistical evaluation is given in Table II.

report was based only on data derived from A bands.

Values of q_h, calculated from counts of H filaments in representative cross-sections through A bands, are given in Tables IV A and IV B. Also shown is "n," the number of myofilaments counted for each q_h. For a proper statistical evaluation of the data, it is necessary to know whether there is covariance of n and q_h. This test was applied as given by Snedecor (14), that is, by calculating the regression of n on q_h for each of the eight hearts. In this way coefficients of correlation were obtained which are shown in Table V. Also shown in Table V are the probabilities of significance of the coefficients of correlation. In studying these results, we recall that, if there is no covariance of n with q_h, the coefficient of correlation is zero, that is to say, the slope of the regression line is zero. Positive or negative deviation from zero may or may not be statistically significant, as determined for each instance. We note from the data in Table V that covariance is

TABLE II

Summary of Statistical Evaluation of Frequency Distributions Shown in Fig. 5

	Control	Hypertrophied heart	t	Degrees of freedom	P
Number of myofibrils counted	6,908	11,071			
Number of cells in which myofibrils were counted	70	70	6.37	138	$<<0.001$
Mean (myofibrils per cell)	99 ± 46	158 ± 62			significant

TABLE III

Summary of Distribution of q_h for Cross-Sections of A Bands and H Zones

Hearts	No. of q_h values	Range of q_h (H-filaments per μ^2) by groups: 1000–1199 No.	%	1200–1399 No.	%	1400–1599 No.	%	1600–1799 No.	%	1800–1999 No.	%	2000–2199 No.	%
Controls													
R-783	33	2	6	6	18	17	52	6	18	1	3	1	3
R-798	12	2	17	3	25	1	8	4	33	2	17		
R-799	45	1	2	20	45	11	24	7	16	5	11	1	2
R-805	18					2	11	7	39	8	44	1	6
Hypertrophied													
R-790	34	5	15	10	29	10	29	6	18	3	9		
R-791	19			7	37	8	42	1	5	3	16		
R-792	26	6	23	12	46	7	27	1	4				
R-802	22							7	32	9	41	6	27

TABLE IV A

Values of q_h for A Bands in Hypertrophied Hearts

R-790		*R-791*		*R-792*		*R-802*	
n^*	q_h^*	n^*	q_h^*	n^*	q_h^*	n^*	q_h^*
391	1184	320	1310	66	1341	75	1760
103	1087	95	1352	85	1189	71	1666
185	1295	102	1452	168	1153	103	1663
98	1080	70	1448	198	1250	112	1809
285	1070	103	1468	188	1291	115	1856
227	1458	103	1468	269	1311	129	2082
192	1340	66	1803	92	1287	118	1904
106	1509	67	1827	84	1175	303	1703
95	1350	65	1774	92	1287	104	1678
		127	1808	59	1199	115	1856
		102	1455	88	1229	114	1840
		49	1337	85	1187	226	1791
		99	1358	90	1258	124	2002
		71	1469			124	2002
		371	1328			124	2002
		70	1449				
		65	1342				

* n, number of H filaments counted; q_h, number of H filaments per μ^2.

TABLE IV B

Values of q_h for A-Bands in Control Hearts

R-783		*R-798*		*R-799*		*R-805*	
n^*	q_h^*	n^*	q_h^*	n^*	q_h^*	n^*	q_h^*
108	1539	50	1321	240	1491	109	1906
120	1708	56	1122	244	1516	186	1596
56	1530	59	1182	428	1486	95	1661
83	1717	92	1842	271	1834	101	1765
89	1840	89	1782	91	1822	196	1683
51	1583	137	1888	208	1408	97	1695
56	1527			72	1442	230	1812
83	1711			66	1322		
78	1610			195	1320		
52	1615			70	1402		
53	1443			108	1488		
168	1076			95	1309		
105	1492			64	1282		
167	1165			93	1281		
178	1242			142	1685		
				110	1897		
				143	1696		
				81	1397		
				78	1346		
				79	1363		

* n, number of H filaments counted; q_h, number of H filaments per μ^2.

TABLE V

Coefficients of Correlation for n and q_h in Tables IV A and IV B

Group	No.	Coefficient of correlation	Degrees of freedom	P (Probability)	Significance
Hypertrophied	R-790	−0.1756	7	$P > 0.1$	−
"	R-791	−0.3472	15	$P > 0.1$	−
"	R-792	+0.2401	11	$P > 0.1$	−
"	R-802	−0.0770	13	$P > 0.1$	−
Control	R-783	−0.6686	13	$0.001 < P < 0.01$	+
"	R-798	+0.8617	4	$0.05 < P < 0.1$	−
"	R-799	+0.2369	18	$P > 0.1$	−
"	R-805	−0.1028	5	$P > 0.1$	−

TABLE VI

*Analysis of Variance on A Bands**

Control hearts

Heart	R-783	R-798	R-799	R-805	Totals
N	15	6	20	7	48
ΣX	22,798	9,317	29,787	12,118	73,840
ΣX^2	35,291,716	14,534,081	45,077,743	21,043,056	115,946,596
ΣcX^2	641,795.73333	619,952.83333	714,474.55000	65,066.85714	2,041,289.97381

Hypertrophied hearts

Heart	R-790	R-791	R-792	R-802	Totals
N	9	17	13	15	54
ΣX	11,373	25,448	16,157	27,614	80,592
ΣX^2	14,592,695	38,634,606	20,122,251	51,093,804	124,443,356
ΣcX^2	221,014.00	540,446.94912	41,585.69923	258,270.93333	1,061,317.58168

* The computations were done as given in Snedecor, G. W., Statistical Methods (see reference 14).

TABLE VII

Analysis of Variance on A Bands

Source of variation	df	Variation	Variance	F test	P value
Normal v. hypertrophied	1	53,511.843	53,511.843	df 1/94 = 1.62125	$0.20 < P$-non-sig.
Among normal and among hypertrophied	6	3,417,728.45245	569,621.48742	df 6/94 = 17.2579	$P < .001$-sig.
Box variation in Table VI	94	3,102,607.555	33,006.463		
Total	101	6,573,847.850			

suggested in only one of the hearts, a control (R-783), but not in any of the seven other cases. This result indicated that analysis of covariance was unnecessary for evaluation of data given in Tables IV A and IV B.

In an analysis of variance, data derived from the group of four hypertrophied hearts were compared with the data obtained from the four controls. The calculated variances of q_h values obtained from both groups of hearts were compared in an F test (14). The essential intermediate steps and the final results obtained in this analysis are given in Tables VI and VII. The results show clearly that the differences between the two distributions of q_h, control and hypertrophied, were not significant. On the other hand, as shown in Table VII, there was considerable variation within each of the two groups, which, on purely statistical grounds, appeared to be due to factors other than random variation ($P < 0.001$). Such intragroup differences may have been caused by extraneous factors, such as variation in effects of fixative, shrinkage during the processing of the tissues. Possibly, they might have been caused by postmortem changes in the hearts. At any rate, this source of variation was distributed through both groups of samples, without reference to the presence or absence of hypertrophy.

The evidence presented indicates that the myofibrils in hypertrophied muscle cells have "normal" lattice parameters. The implications of the findings will now be considered.

DISCUSSION

The evidence obtained thus far provides a strong argument in favor of the inference that enlargement of heart muscle cells in hypertrophy is due principally to an increase in the number of myofilaments of normal variety, through formation of new myofibrils. If there were significant differences between the q_h values of filament lattices in normal and hypertrophied muscle fibers, analysis of our data should have revealed them. The lack of such differences warrants the assumption that hypertrophied fibers contain myofibrils with the same filament lattice geometry possessed by normal myofibrils. Moreover, hypertrophy was accounted for by the presence of additional myofibrils within muscle cells. However, the number of myofibrils need not always be increased during hypertrophy if one postulates that those myofibrils which are already present can become enlarged by addition of myofilaments, normal geometrical relationships being maintained.

The results of the foregoing analysis have led us to propose a hypothesis in the form of two postulates: (*a*) conservation of myofilament lattice parameters in the myofibrils of hypertrophied cardiac muscle cells; (*b*) increase in number of myofilaments through formation of new myofibrils and possibly also by addition of filaments to preexisting myofibrils.

An alternative, *viz.*, that filaments in existing myofibrils might increase in thickness without expansion of the filament lattice, and therefore without change in q_h, seems remote for several reasons. For example, the physicochemical requirements for changing two sets of macromolecular polymers in a given paracrystalline lattice are such that the resulting lattice spacings would be most unlikely to remain unaltered. One might also suppose that new myofibrils could be built of myofilaments which differ from those normally present. But chemical and statistical considerations would make it appear unlikely that in such a case lattice parameters (and therefore q_h) could be conserved.

The proposed hypothesis has certain features that recommend it. Chief amongst these is that only one assumption has to be made to explain various degrees of hypertrophy of muscle cells in different hearts, as well as variation in size of muscle cells in individual hearts, whether normal or hypertrophied. This is consistent with the theory of muscular contraction proposed by Huxley and Hanson (2, 3). No postulate implying the presence of a spectrum of different species of actin and myosin is necessary. Moreover, if size, shape, or geometrical relationships of myofilaments in different myofibrils could vary, the molecular mechanism whereby myofibrils contract would also vary, not only in different hearts, but in different cells of the same heart; but this is improbable. The hypothesis is also in harmony with some well known biological phenomena. For example, secretory cells (pancreatic acini, mucous glands) respond to increased demand for their products of secretion, or to stimulation, by producing more proteins of the kind they are already synthesizing. This applies also to cells of the liver parenchyma after starvation-refeeding, after massive loss of plasma proteins, or during regeneration.

Neither theoretical considerations nor evidence presented in this report preclude the occurrence of specific chemical abnormalities in heart muscle cells undergoing hypertrophy. Nor have we considered the possibility that *minor* fractions of the cellular myosin or actin may be polymerized in abnormal fashion in the course of hypertrophy, or when heart failure takes place (15). It may be recalled here that there was significant, but unexplained, variation of q_h *within* each of the two groups. Though we cannot eliminate the possibility that a small proportion of muscle cells contained myofibrils with "abnormal" actin and myosin filaments, our results indicate that, in its major aspect, the increase of contractile substance which occurs in hypertrophy cannot be due to production of abnormal forms of actin and myosin.

If the proposed hypothesis is true for hypertrophy of heart muscle cells, we can expect it to hold equally for skeletal muscle cells. Thus, without reference to any particular cause of hypertrophy, one can envisage one basic phenomenon to account for increase in contractile substance in all striated muscle cells.

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