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Evaluation of myocardial fiber size and capillary supply in the rat

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BOSTON UNIVERSITY

GRADUATE SCHOOL

Thesis

EVALUATION

OF

MYOCARDIAL FIBER SIZE AND CAPILLARY SUPPLY

IN

THE RAT

by

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(B.S., Boston College, 1960)

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requirements for the degree of

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INTRODUCTION

Alterations in the myocardium present problems of both clinical and theoretical importance. Changes in the heart structure occur as physiological and anatomical reactions in response to changes in the environment. Such changes act as stimuli and may produce either excitation, an increased response, or depression, a decreased response. Cardiac muscle reacts quantitatively by hypertrophy or atrophy of the myocardial fibers. These quantitative changes can then be measured. However, before attempting to analyze myocardial alterations, it is necessary to obtain accurate information concerning the normal unaltered myocardium. Unless there is a fixed standard of comparison, a constant morphological pattern representing the normal myocardium, no exact comparison or analysis can be made. If there is consistency in the size of normal cardiac muscle fibers and in the ratio of capillaries to fibers then this will comprise a constant morphological pattern with which altered myocardia can be compared.

It is the purpose of this study to determine whether there is such a consistency of fiber diameters, fiber numbers, capillary numbers and capillary-fiber ratios in the ventricles of normal rats. This thesis will present the histological and statistical procedures that have proved most efficient, in terms of accuracy and simplicity, for this type of study. Comparison will be made between the normal hearts and hearts showing the effects of experimentally produced hypertrophy in order to determine whether the histological and statistical methods are

sufficiently sensitive to demonstrate alterations.

The animal investigated in this study is the rat. In addition, published data for man and other mammals will be tabulated for comparison.

I. HISTORICAL BACKGROUND

A. Normal Hearts

Although the literature pertaining to the histology of the mammalian myocardium is abundant, that concerned with the measurement of cardiac muscle fibers, the determination of capillary supply, and the quantitative relationship between capillaries and fibers is rare and scattered. The majority of articles which present measurements of myocardial fibers deal mainly with alteration in size as a result of such pathological conditions as hypertrophy and atrophy. Few of these contain significant data on the variation in width of normal fibers.

1. Fiber size

The earliest attempts at fiber measurements date back to the middle of the nineteenth century. The early researchers, however, were hindered by inadequate histological and statistical techniques. Zielonko (1874) and Tangl (1889) teased fibers apart before measuring them, thus introducing a significant error. Kölliker (1867), Stricker (1871), Letulle (1879), Toldt (1884), Palczewsha (1910), and Wemer (1910) measured fiber diameters, but did not treat their data statistically. Although the contribution of these researchers stimulated interest in this field, their statistical results are not acceptable by modern research standards and will not be quoted here.

The first work of significance with respect to the present investigation was that of Goldenberg (1886). He used techniques that were superior to those of his contemporaries and many of his successors.

Goldenberg studied three normal human hearts. The tissues were fixed in Müller's fluid, dehydrated in alcohol, embedded in celloidin, cut fifteen micra thick, and stained with alum carmalum. He measured microscopically one hundred fibers from each ventricle. As to the use of Müller's fluid, Guyer (1936) stated, "It is a hardening rather than a fixing agent," and this reagent no longer is used. Although modifications have been made on his technique, Goldenberg set the basic pattern for this type of study. Goldenberg's results, as well as the results of other investigators, are listed in Table I.

The first measurements of cardiac muscle fibers published in the United States were those of Karsner, Saphir, and Todd (1925) who, like Goldenberg, were interested mainly in the state of cardiac muscle in hypertrophy and atrophy. They measured 2153 fibers, all from the left ventricle of a thirty-two year old man. In each field, one large fiber and one small fiber were measured. The blocks were fixed in 4 per cent formaldehyde, embedded in paraffin, cut in serial sections five micra thick, and stained with hematoxylin and eosin. The width of longitudinally cut fibers were measured microscopically.

Harrison, Ashman, and Larson (1932) studied the relationship between the thickness of the cardiac muscle fibers and the optimum rate of the heart beat in humans, dogs, cows, sheep, rabbits, rats, and guinea pigs. From each heart they measured microscopically ten cross-sectional fibers at the level of the nucleus. The histological procedure was not described.

In a study of experimental renal insufficiency involving the relationship of left ventricular hypertrophy and hypertension in the

TABLE 1.-Summary of published data for adult, mammalian, ventricular, cardiac muscle fiber diameters (in micra) and capillary-fiber ratios

Author and Date	Species	L.V.*	R.V.	C-F	No. of Hearts	No. of Fibers Counted per Ventricle
Goldenberg(1886)	Human	12.4	12.7		3 (C)**	100
Karsner, Saphir, and Todd(1925)	Human	17.6			1 (P)	2153
Wearn(1928)	Human			1:1	4 (P)	
	Cat			1:1	3	
	Rabbit			1:1	5	
Harrison, Ashman, and Larson(1932)	Rat	11.2			10 (N)	10
	G. Pig	12.5			9	10
	Rabbit	14.5			9	10
	Sheep	14.8			6	10
	Human	16.2			12	10
	Dog	16.8			6	10
	Cow	17.6			6	10
Chanutin and Barksdale(1933)	Rat	14.5			1 (P)	70
	Rat		12.5		1	30
Shipley, Shipley, and Wearn(1937)	Rabbit			1:1	(G)	
Roberts, Wearn, and Badal(1938)	Human	14.0	14.0	1:1	10 (C)	
Roberts and Wearn(1941)	Human	13.9	13.9	1:1	26 (G)	
Ashley(1945)	Human	22.6	18.6		14 (G)	150
Lowe and Eate(1948)	Human	13.6(S.B.S.) 13.6(D.S.S.) 14.0(D.B.S.) 12.5(S.S.S.)			5 (P)	40

* L.V.-Left ventricular measurements; R.V.-Right ventricular measurements; C-F-Capillary-fiber ratio.

**Embedded in: (C)-celloidin; (P)-paraffin; (G)-gelatin; (N)-not listed.

rat, the mean diameters of myocardial fibers in the right ventricle of one control rat and the left ventricle of another control rat were determined by Chanutin and Barksdale (1933). The hearts were fixed in Helly's fluid, embedded in paraffin, and sectioned at six micra. Longitudinally cut fibers were projected upon a screen and the widths were measured at the level of the nucleus.

Roberts, Wearn, and Badal (1938) studied normal and hypertrophied hearts in man. Fibers from ten normal hearts were measured in cross-section. The tissues were fixed in 10 per cent formalin and embedded in gelatin. Frozen sections were cut at fifteen micra and stained with eosin. Arabic sugar was used as the mounting medium. Fiber diameters were measured microscopically and the measurements from the right and left ventricles were averaged. Roberts and Wearn made a more extensive study in 1941 and included twenty-six normal human hearts. The results were almost identical with the above. Again right and left ventricular sizes were averaged.

In 1945, Ashley studied fourteen normal hearts from humans between the ages of twenty-one and fifty-five years. All hearts were fixed in 10 per cent formalin and embedded in gelatin. Frozen sections were cut. The sections were projected upon paper screens; the cross-sectional fibers, at the level of the nucleus, were traced, cut out, and weighed. The size was calculated from the weights. The average number of fibers measured in each ventricle was approximately one hundred and fifty.

In 1948, Lowe and Bate studied the left ventricles of five human adults. Myocardial blocks were fixed in formalin, embedded in paraffin, and cut eight micra thick. Sections contained both cross and

longitudinally-cut fibers. Forty fibers were measured microscopically from each of the four major muscle bundles of the heart: the superficial bulbo-spiral muscle (S.B.S.); the deep sino-spiral muscle (D.S.S.); the deep bulbo-spiral muscle (D.B.S.); and the superficial sino-spiral muscle (S.S.S.).

2. Capillary-fiber ratio

The ratio of the number of capillaries to the number of myocardial fibers in a given sample of myocardium constitutes the capillary-fiber ratio. This ratio was determined for humans, cats, and rabbits by Wearn (1928). By perfusing the heart post mortem with oxygenated Locke-Rosenheim solution at body temperature, he was able to restore beating and to inject capillaries more completely than had previous investigators. For each animal, approximately a 1:1 ratio was obtained.

This 1:1 ratio was confirmed: in 1937 by Shipley, Shipley, and Wearn in a study on rabbits; in 1938 by Roberts, Wearn, and Eadal in a study of ten human hearts; and in 1941 by Roberts and Wearn using twenty-six human hearts.

E. Young Hearts

1. Fiber size

There is no multiplication of fibers after birth (Tangl, 1889). Growth takes place by enlargement of individual fibers. According to Shipley, Shipley, and Wearn (1937), the muscle fibers of the rabbit heart at the time of birth are very small, approximately seven micra in cross-sectional diameter; and since the circumference of the young

rabbit heart is small, the fibers are short. During growth, the length increases and the cross-sectional diameter becomes greater. This process continues until adult age is reached. Further evidence for this is presented by Letulle (1879), Goldenberg (1886), Dogliotti (1931), Harrison, Ashman, and Larson (1932), Roberts and Wearn (1941), Wearn (1941a, 1941b), Davies and Francis (1942), and Ashley (1947). Most of these authors present either information for the left ventricle only, or else they average measurements for both ventricles and present the averaged result. Only Goldenberg and Ashley follow the development of each ventricle separately. Both authors agree that the left ventricle grows faster than the right. They found that at each stage in development the left ventricular fibers are thicker than the right, at least until adulthood is reached. As may be seen in Table 1, these investigators disagree as to which ventricle has larger fibers in the adult.

No information has been published stating whether immature animals of the same species at a given age have the same fiber size or whether each individual animal grows at its own rate.

2. Capillary-fiber ratio

According to Wearn (1941a), the capillaries are distributed evenly throughout the muscle bundles, lying between and parallel to the fibers. In the newborn rabbit heart there are six muscle fibers to each capillary. In the seven-month human fetus there are six muscle fibers to each capillary, but shortly after birth (three weeks), the ratio becomes four fibers to one capillary. As the muscle fibers enlarge and the capillary bed increases, a change in ratio results, so that by the time the rabbit is approaching adult life and the child

is fifteen years old, there are two fibers to every capillary. When growth is completed the ratio is 1:1, and it remains at this figure throughout adult life. Thus during the normal growth period, the increase in muscle size is accompanied by capillary multiplication.

C. Hypertrophied Hearts

The work of Letulle (1879), Goldenberg (1885), Tangl (1889), Dehio (1899), Stadler (1907), Karsner, Saphir, and Todd (1925), and Lowe and Bate (1948) established the fact that gross hypertrophy of the heart was a result of enlargement of the individual muscle fibers rather than an increase in the number of fibers.

Until 1897, the hypertrophied heart was considered to be stronger than the normal heart. Horvath (1897) and Albrecht (1903) were among the first to associate cardiac failure with hypertrophy of the muscle fibers, but they offered no logical explanation as to why this was so.

It was not until 1927 that Christian suggested that failure might occur because of a decreased blood supply in the hypertrophied heart. Proof for this was furnished by Shipley, Shipley, and Wearn (1937) who determined the capillary-fiber ratio in hypertrophied rabbit hearts. They found no increase in capillary-fiber ratio from the 1:1 of the normal heart. Thus as a result of hypertrophy, each capillary had to supply blood to a considerably greater mass of muscle.

II. MATERIALS AND METHODS

A. Normal Hearts

1. Injection of capillaries

The normal hearts in this study were obtained from six, mature, male, Sprague Dawley rats, six to seven months old. The rats weighed four hundred and ninety-five to five hundred and sixty-two grams. The method of injecting capillaries was a modification of Wearn's method (1928). The animal was heavily etherized and the chest opened to expose the beating heart. A cannula was tied into the aorta at a sufficient distance from the heart to prevent damage to the openings of the coronary arteries and the aortic valves. The heart was perfused, in situ, with physiological saline injected through the rubber tubing above the aortic cannula by means of a needle and syringe. In the same way, 20 per cent Higgin's India ink in distilled water was injected until the heart became uniformly black. At this point, addition to the ink of two milliliters of a mixture of 10 per cent formalin in 95 per cent alcohol caused the heart to stop beating and to retain the injected dye. The hearts always stopped beating in a period of complete relaxation (Shipley, Shipley, and Wearn 1937).

2. Preparation of sections

The heart was removed from the rat and fixed in 10 per cent formalin for two days. It was washed thoroughly and cut in half transversely, midway between apex and base of the ventricles. The tissues were then dehydrated in 70, 95, and 100 per cent ethyl alcohol. They

celloidin

were cleared in methyl benzoate for forty eight hours and in benzol for ninety minutes. The tissues were infiltrated with three changes of melted paraffin and then blocked. Serial sections were cut at seven micra. This thickness permitted focusing in sufficient depth to determine whether the individual fibers were cut transversely or obliquely. All sections were taken from the middle third of the heart and included both ventricles. Sections were spread in distilled water containing a trace of Mayer's albumen fixative. The temperature was maintained constant in order to obtain uniform spread, and thereby counteract any wrinkling of the tissue that may have occurred when it was cut on the microtome.

3. Staining

The sections were hydrated and then stained with Delsfield's hematoxylin (Davenport, 1960) and Crossmon's (1937) modification of Mallory's connective tissue stain. They were then dehydrated, cleared, and mounted in Harleco synthetic resin. With this method nuclei are stained mauve; muscle, red; collagenous fibers, blue. These colors contrast with the India ink of the injected capillaries.

4. Counts and measurements

All counts and measurements were done with a four millimeter (15x) objective, a Howard ocular square, and a ruled micrometer disc. Calibration showed that each division of the micrometer was equal to 1.65 micra for this particular microscope. One square covered an area of 3136 square micra, and this is considered as one field. Since it was desired to determine the number of capillaries and fibers in "pure" muscle tissue, only intrafascicular fields were used. Fields containing

perivascular connective tissue, large blood vessels, or distorted tissue were avoided. Counts and measurements were made in areas in which the capillaries and fibers were cut in perfect cross-section and in which the capillaries were completely injected, as shown by their even spacing and regular distribution. Except for the restrictions mentioned, no deliberate selection of fields was made. For each rat, twenty-five fields were studied in each ventricle. In each field the capillaries were counted and the muscle fibers were counted and measured.

5. Statistical procedure

The number of fiber measurements per ventricle was over two hundred. This sample was large enough so that the error of random sampling was small; the results would not have been significantly affected by a larger number of measurements, i.e., within reasonable limits.

Frequency and cumulative frequency distributions were tabulated and plotted; and the mean, standard deviation, mode, median, and coefficient of variation were calculated for each left ventricle. Frequency and cumulative frequency distributions were then plotted from data which included fibers from all six left ventricles. Again mean, standard deviation, mode, median, and coefficient of variation were determined. This process was repeated for the right ventricles individually and then collectively. The fiber and capillary counts were compared to determine the capillary-fiber ratio. The data were then analyzed to determine whether there was any consistent morphological pattern within any individual ventricle, between right and left ventricles of each rat, and between ventricles of various rats.

Statistical procedures were those outlined by Snedecor (1956).

B. Young Hearts

Four Sprague Dawley rats, six weeks old, weighing approximately one hundred grams were studied. Comparison with the adult hearts was made for the purpose of determining changes that occur with growth in the normal non-pathological myocardium. Also, it was of interest to determine if young rats of the same breed, age, weight, and sex had a consistent morphological pattern that would present good control values for experimental work on the heart.

C. Hypertrophied Hearts

Four experimental rats, six to seven months old, weighing from five hundred and five grams to six hundred and three grams, were also studied using the procedures outlined above. These animals were treated two to six weeks with 1 mg/day/rat of a long-acting preparation of corticosterone acetate (DOCA) and given saline instead of drinking water. This treatment is known to produce hypertension and left ventricular hypertrophy (Selye, Hall, and Rawley, 1943). It was expected that the cardiac muscle fibers of the left ventricle would show evidence of hypertrophy, as a result of the hypertension. Comparison with the normal adult hearts was made to determine whether this had occurred.

III. RESULTS

A. Normal Hearts

1. Fiber diameters

a. left ventricle

More than two hundred fibers were measured in each left ventricle. These measurements were grouped according to fiber size and the distributions were tabulated for each of the six left ventricles individually (Table 2). The frequencies of fiber sizes in the six left ventricles collectively were then determined and this distribution also was tabulated (Table 2).

Diameters in divisions refer to the divisions of the micrometer scale with which the measurements were made. Calibration of the micrometer showed each division to be equal to 1.65 micra. The conversion of divisions to micra is stated in this table but all further analysis is given only in terms of micra.

As may be seen from this table, the fibers are compactly grouped within a twenty micra range, from five to twenty-five micra. Only a few extreme sized fibers extend beyond these limits.

Figures 1, 3, 5, 7, 9, and 11 show the plotted frequency distributions for the left ventricles of Rats 1 through 6 respectively. Figure 13 is the frequency distribution of fiber sizes in the six left ventricles grouped together.

The curves are unimodal, fairly symmetrical, and relatively compact, showing that in the normal heart there is only a small

variation of fiber sizes.

TABLE 2.-Frequency distributions of fiber diameters in the left ventricles of six normal rats

Diameters in Divisions	Diameters in Micra	Number of Fibers						Total
		R1	R2	R3	R4	R5	R6	
1	1.65	0	0	0	0	0	0	0
2	3.30	2	0	0	0	0	0	2
3	4.95	16	16	7	20	7	1	67
4	6.60	28	37	16	38	18	6	143
5	8.25	27	47	31	30	38	19	192
6	9.90	32	32	31	41	31	40	213
7	11.55	40	42	37	39	45	31	234
8	13.20	42	42	45	47	36	52	265
9	14.85	35	28	42	44	40	32	221
10	16.50	35	24	22	18	16	30	145
11	18.15	14	17	25	14	20	30	120
12	19.80	13	13	13	13	10	11	81
13	21.45	7	5	11	4	8	15	49
14	23.10	4	3	9	4	3	11	34
15	24.75	8	2	4	0	3	3	20
16	26.40	0	1	2	2	1	4	10
17	28.05	1	0	2	0	1	0	4
18	29.70	0	0	0	0	1	0	1
19	31.35	0	0	0	0	0	0	0
20	33.00	1	0	0	0	0	0	1
21	34.65	0	0	0	0	0	0	0
Totals		306	310	297	319	286	284	1802

Figures 2, 4, 6, 8, 10, and 12 are graphs of the cumulative frequencies of the six left ventricles individually; figure 14 is the cumulative frequency distribution of the six left ventricles collectively.

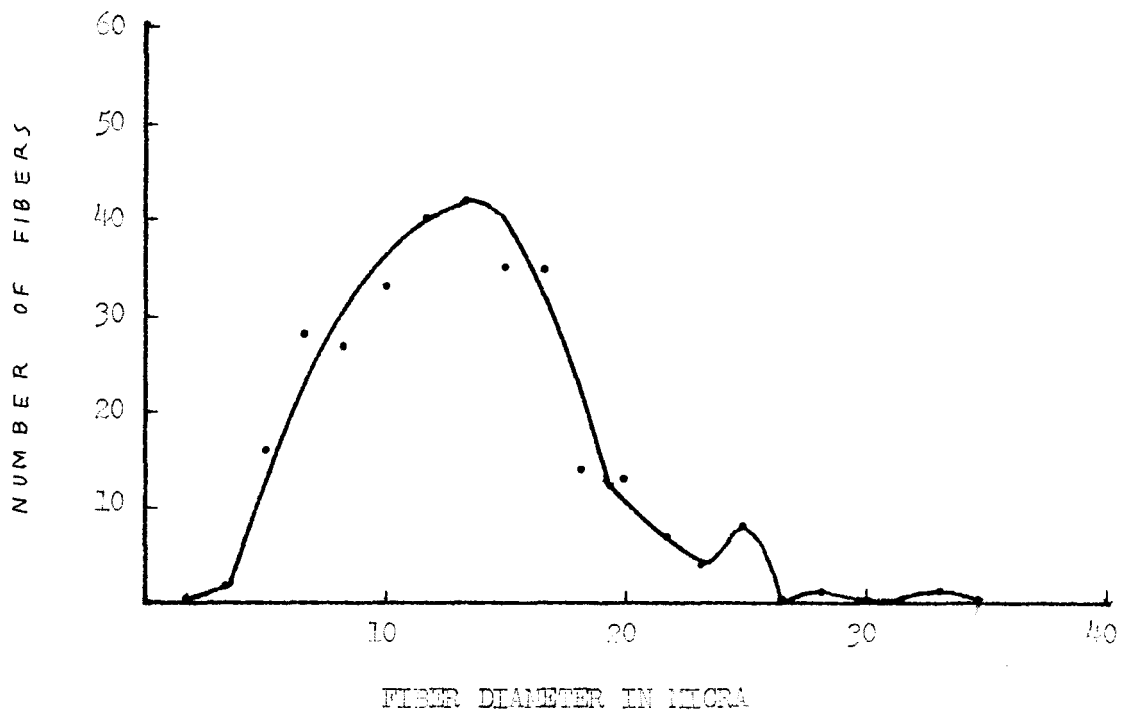


Fig. 1.-Frequency distribution of fiber diameters from the left ventricle of Rat 1

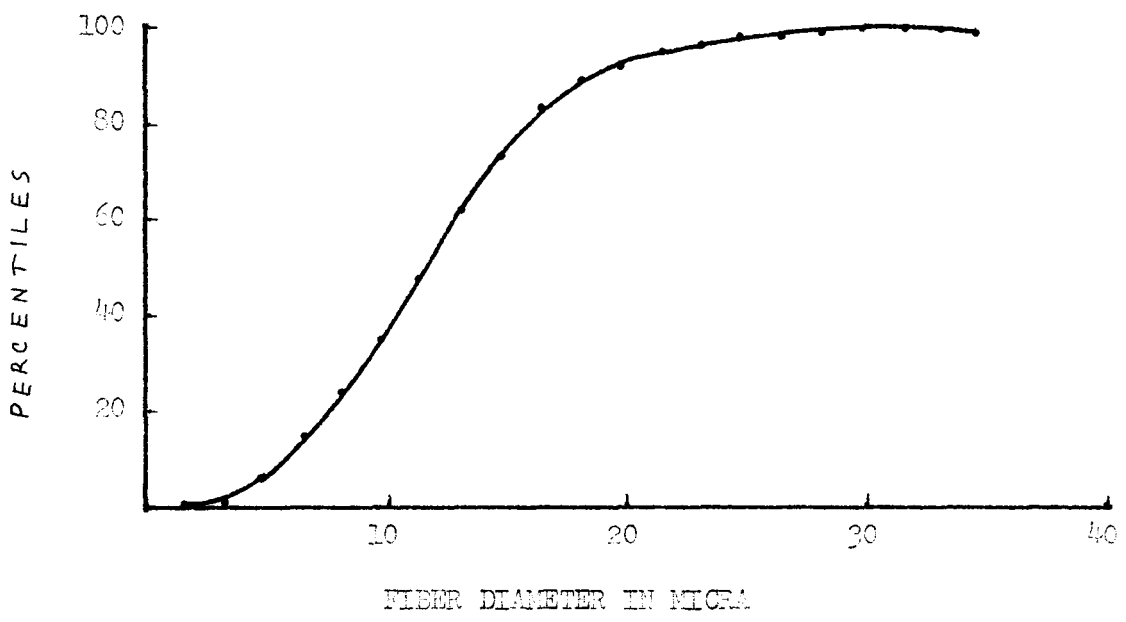


Fig. 2.-Cumulative frequency distribution of fiber diameters from the left ventricle of Rat 1

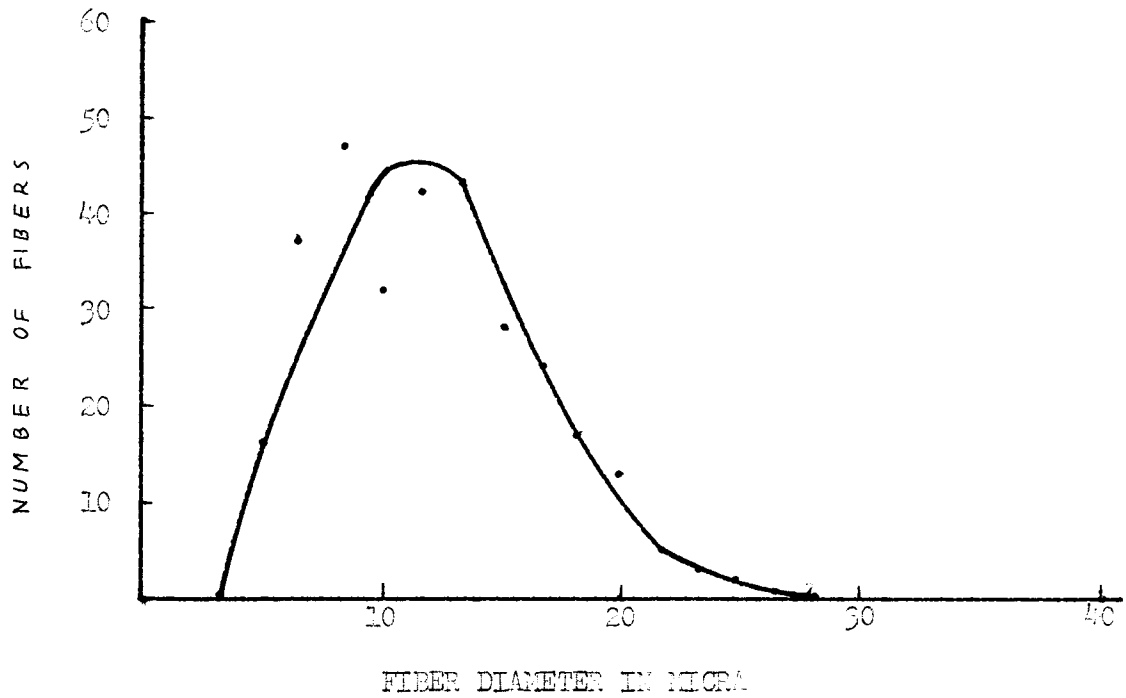


Fig. 3.-Frequency distribution of fiber diameters from the left ventricle of Rat 2

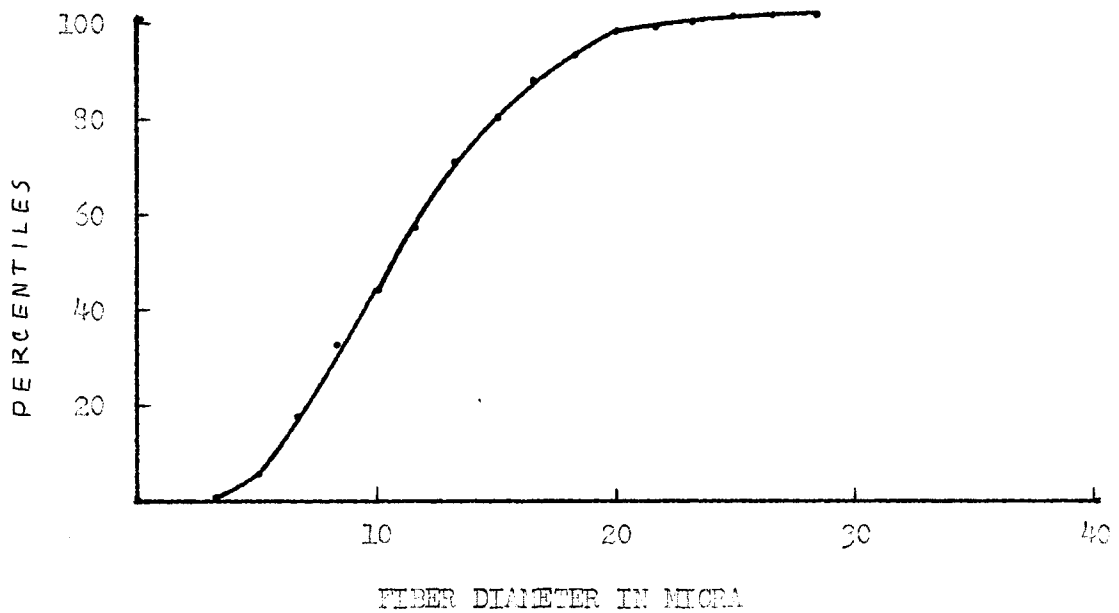


Fig. 4.-Cumulative frequency distribution of fiber diameters from the left ventricle of Rat 2

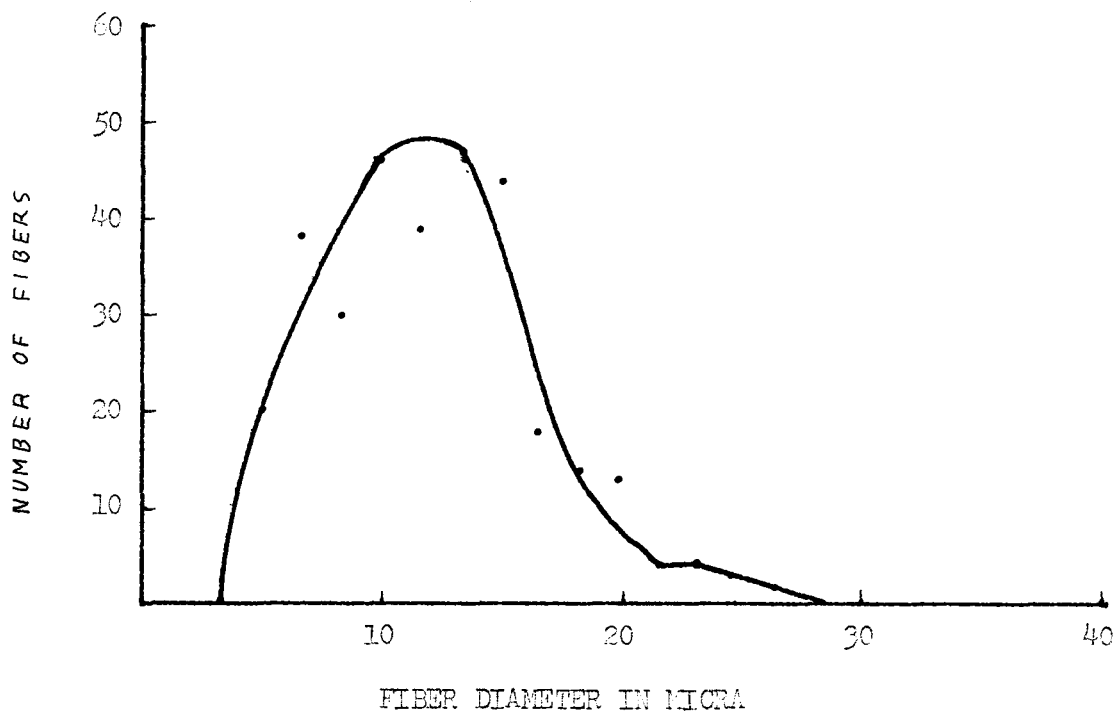


Fig. 7.- Frequency distribution of fiber diameters from the left ventricle of Rat 4

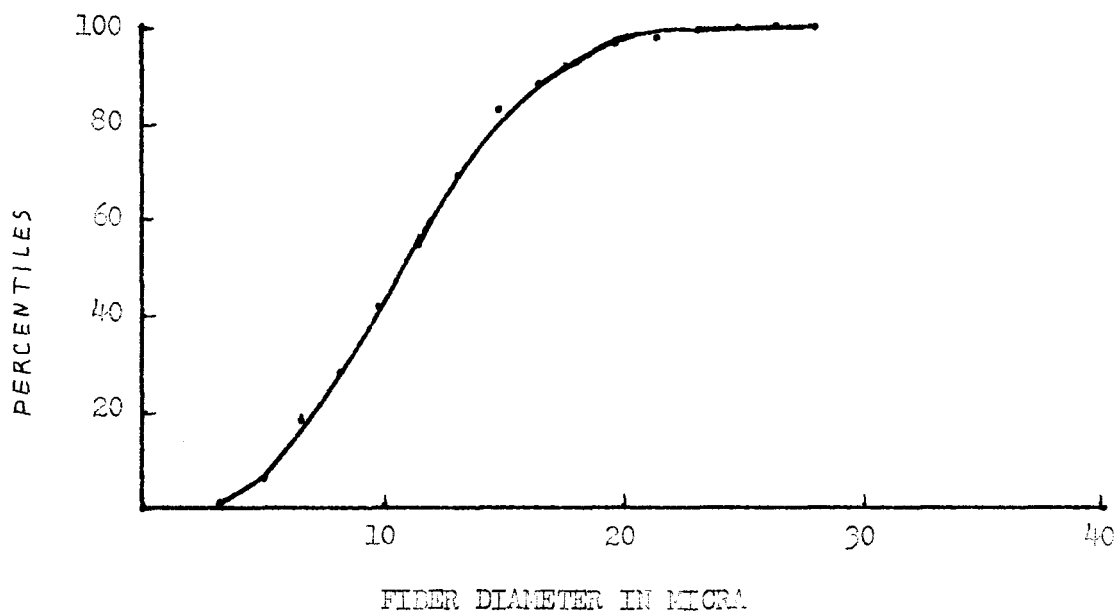


Fig. 8.-Cumulative frequency distribution of fiber diameters from the left ventricle of Rat 4

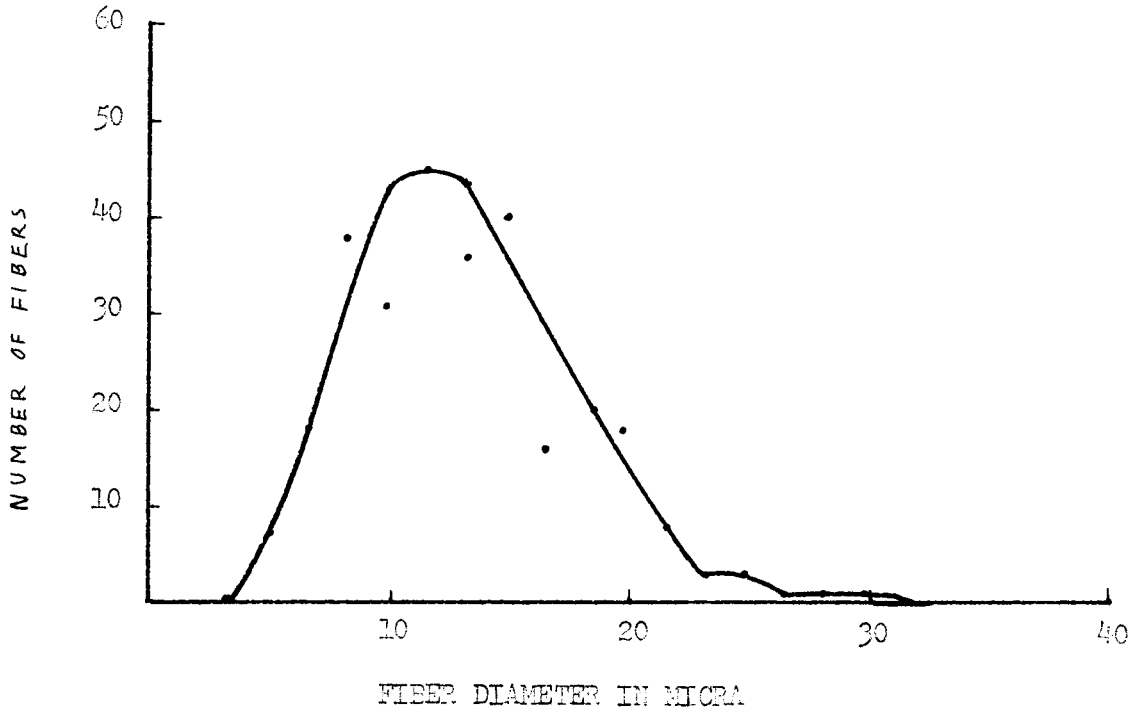


Fig. 9.- Frequency distribution of fiber diameters from the left ventricle of Rat 5

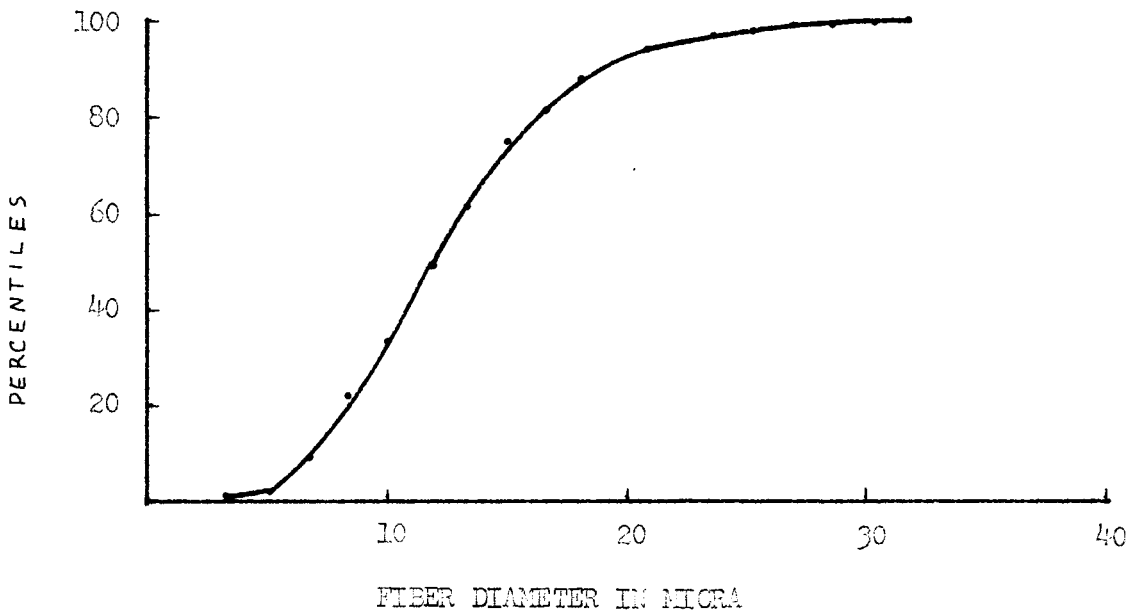


Fig. 10.-Cumulative frequency distribution of fiber diameters from the left ventricle of Rat 5

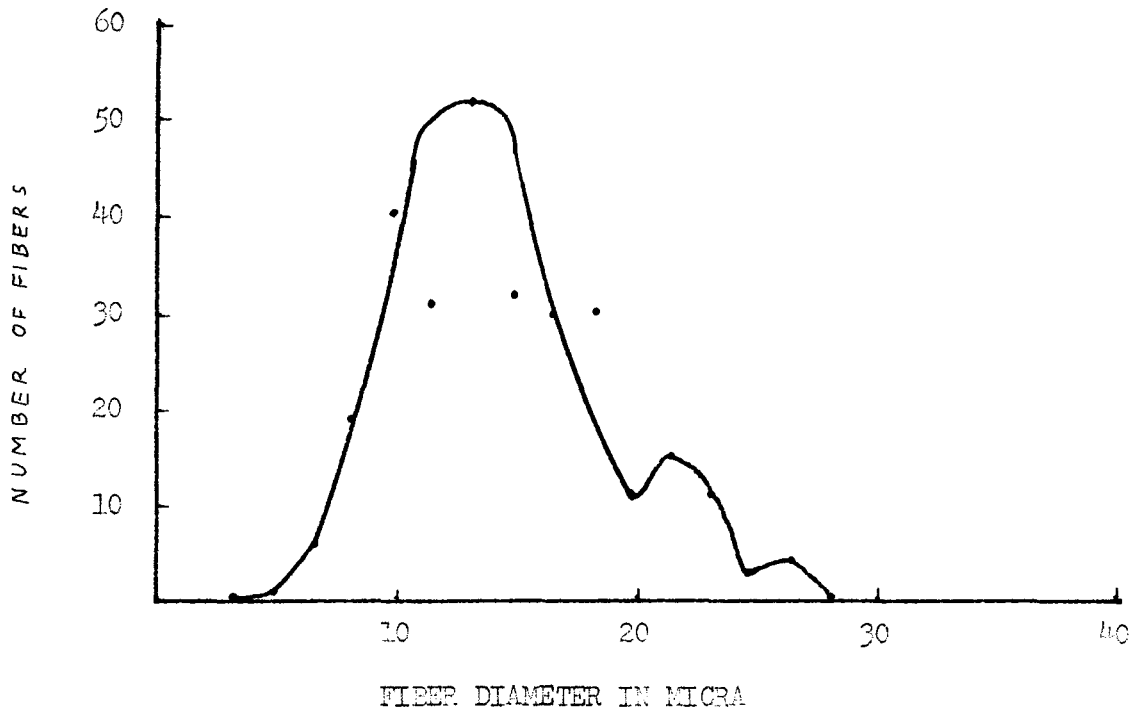


Fig. 11.-Frequency distribution of fiber diameters from the left ventricle of Rat 6

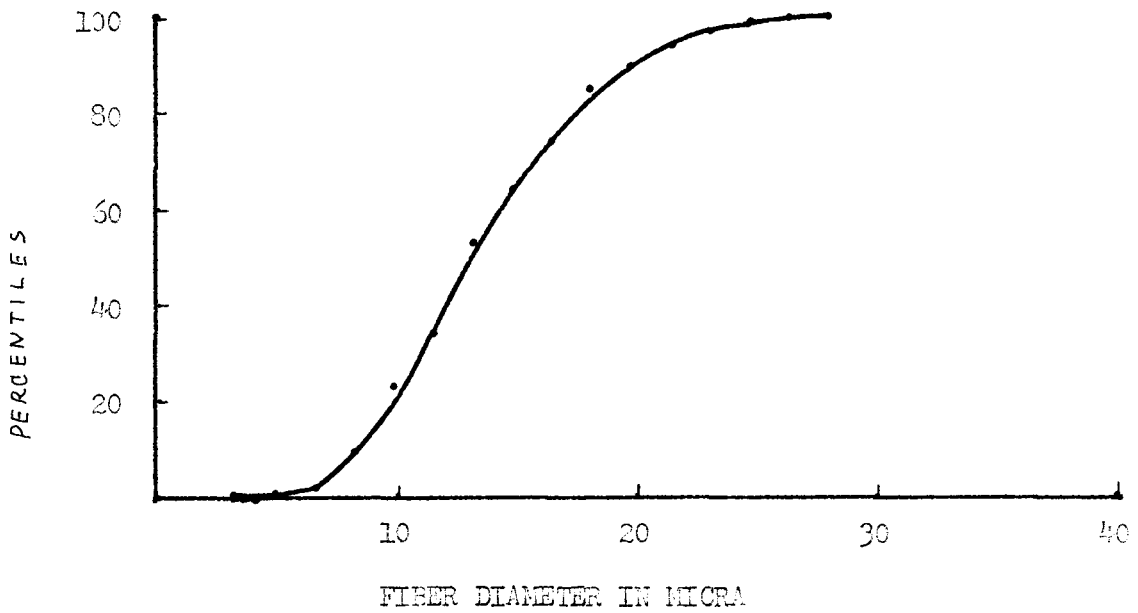


Fig. 12.-Cumulative frequency distribution of fiber diameters from the left ventricle of Rat 6

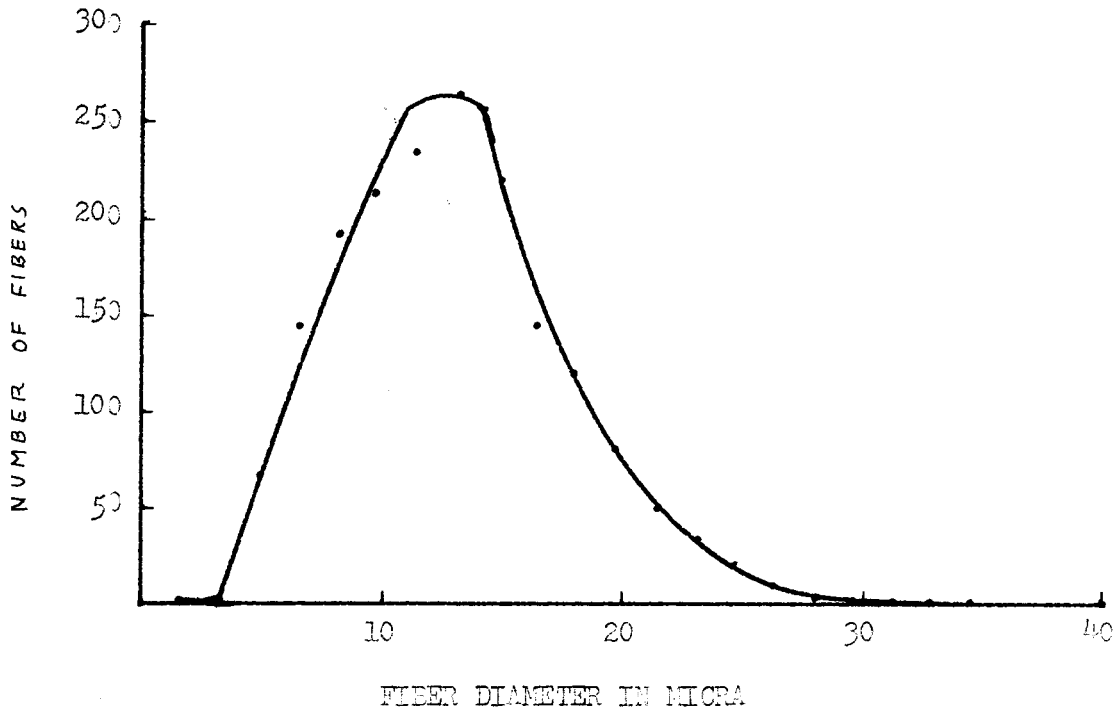


Fig. 13.-Frequency distribution of fiber diameters from the left ventricles of Rats 1 through 6

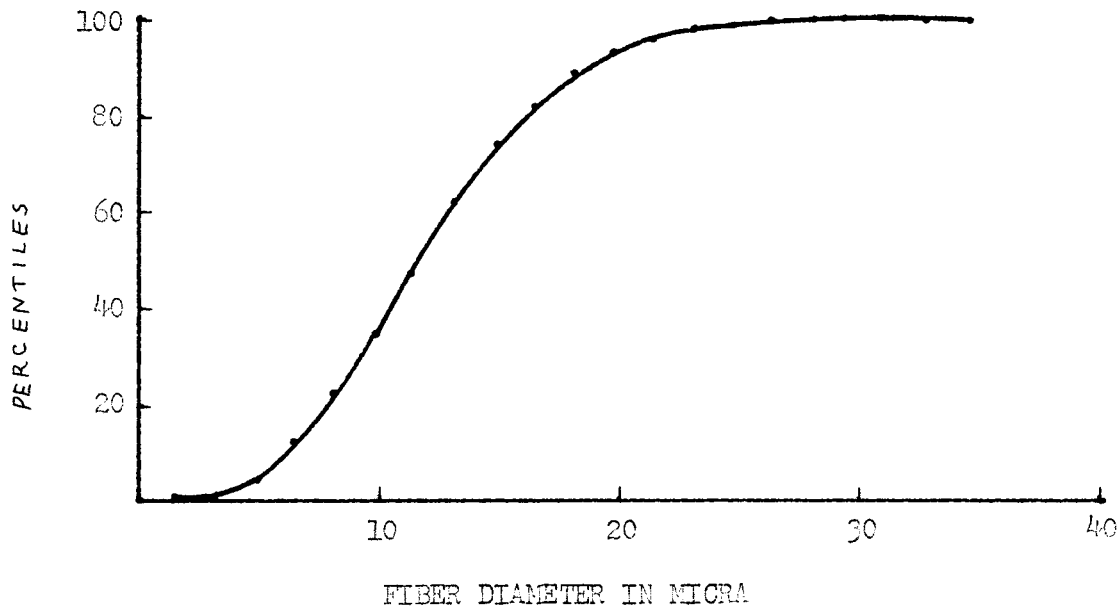


Fig. 14.-Cumulative frequency distribution of fiber diameters from the left ventricles of Rats 1 through 6

b. right ventricle

The frequencies of fiber sizes in the right ventricles of the six rats individually, and of the six right ventricles when grouped together were tabulated (Table 3). As in the left ventricles, the majority of fiber sizes in the right ventricle were between five and twenty-five micra.

TABLE 3.-Frequency distributions of fiber diameters in the right ventricles of six normal rats

Diameters in Micra	Number of Fibers						Total
	R1	R2	R3	R4	R5	R6	
1.65	0	0	0	0	0	0	0
3.30	1	0	0	0	1	1	2
4.95	12	15	13	15	2	2	59
6.60	35	32	26	31	23	9	156
8.25	23	45	30	51	38	19	200
9.90	39	54	32	42	56	31	254
11.55	45	56	39	44	49	45	278
13.20	44	38	43	39	52	48	265
14.85	36	32	41	32	36	54	231
16.50	18	23	29	19	30	27	156
18.15	17	13	22	25	8	19	104
19.80	15	19	12	12	6	16	80
21.45	7	8	7	6	1	6	35
23.10	6	2	5	2	1	8	24
24.75	5	2	5	0	1	1	14
26.40	3	0	2	1	1	1	8
28.05	0	0	0	0	0	1	1
29.70	0	0	0	0	0	0	0
31.35	0	0	0	0	0	0	0
33.00	1	0	0	0	0	0	1
34.65	0	0	0	0	0	0	0
Totals	307	339	306	319	306	292	1869

Figures 15, 17, 19, 21, 23, and 25 are plotted frequency distributions of fiber sizes in the individual right ventricles of the six rats. Figure 27 shows the frequency distribution of fiber sizes

in the six right ventricles collectively. Again the curves are unimodal and relatively symmetrical. They are remarkable similar to the curves shown for the left ventricle.

Figures 16, 18, 20, 22, 24, and 26 are graphs of the data for each of the six right ventricles; Figure 28 is a graph of the cumulative frequency distribution of the combined right ventricles.

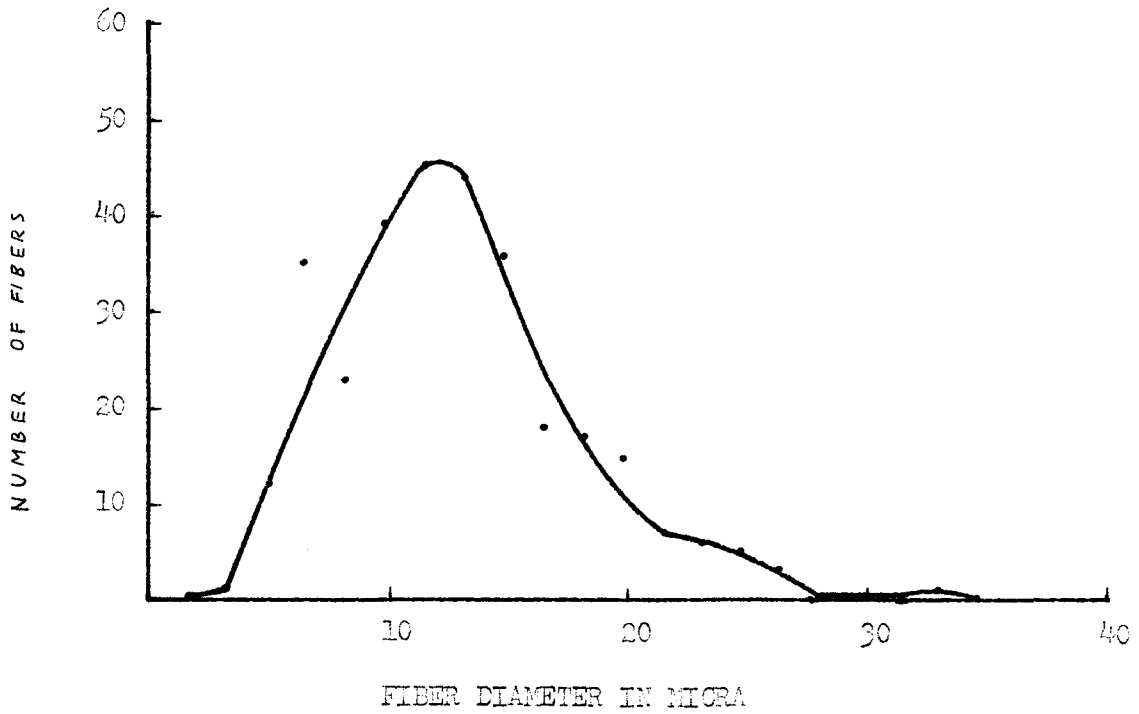


Fig. 15.-Frequency distribution of fiber diameters from the right ventricle of Rat 1

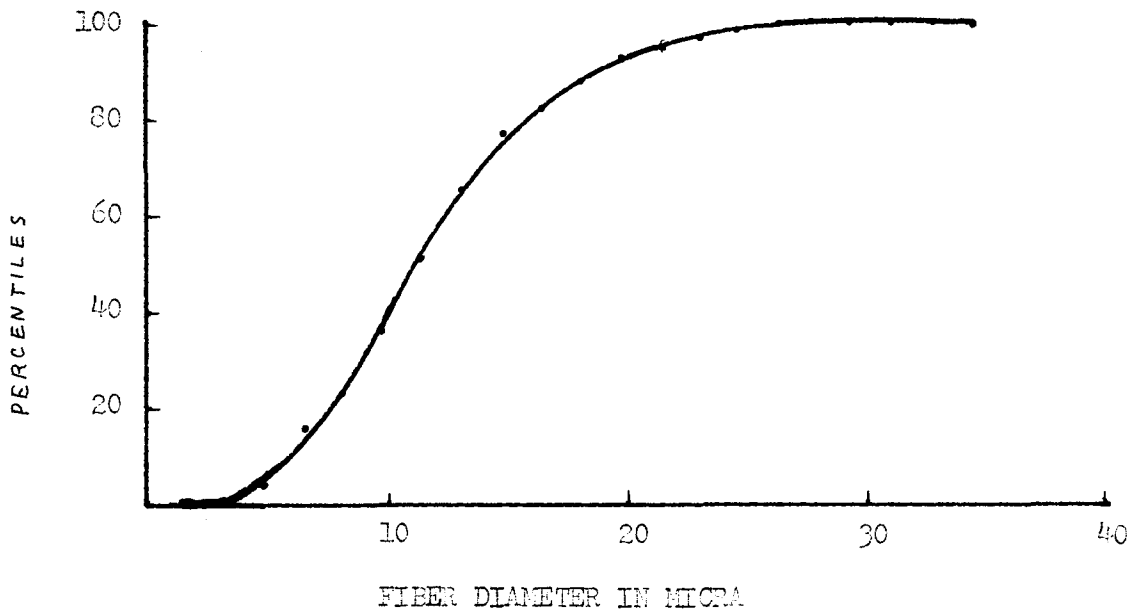


Fig. 16.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 1

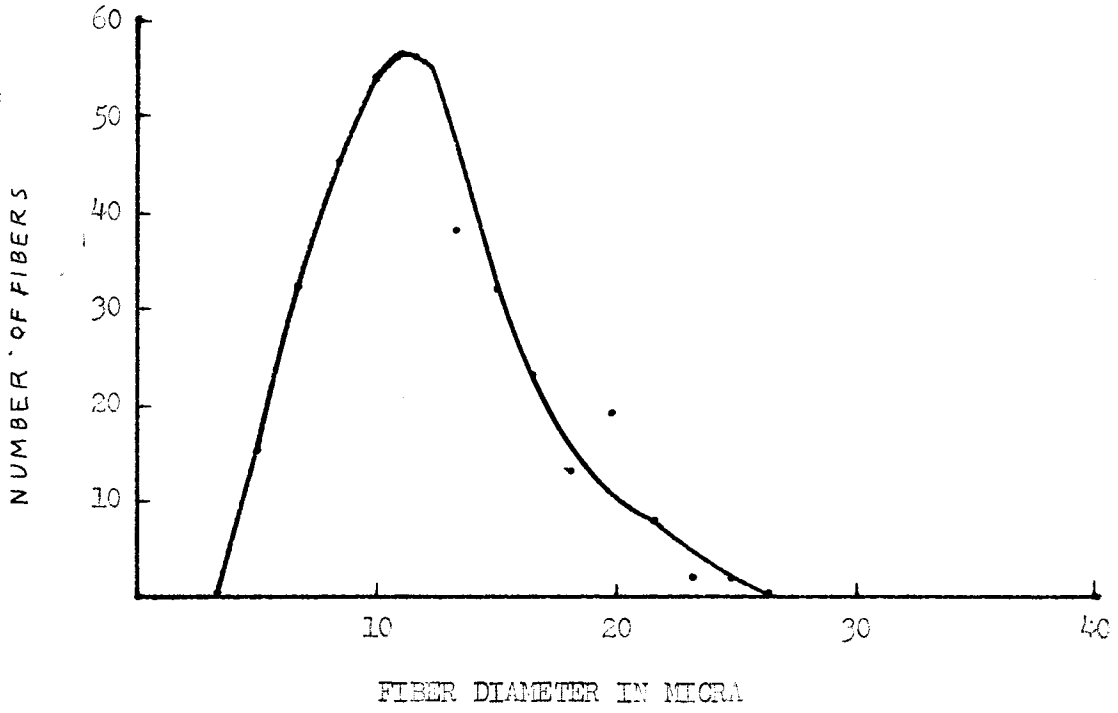


Fig. 17.-Frequency distribution of fiber diameters from the right ventricle of Rat 2

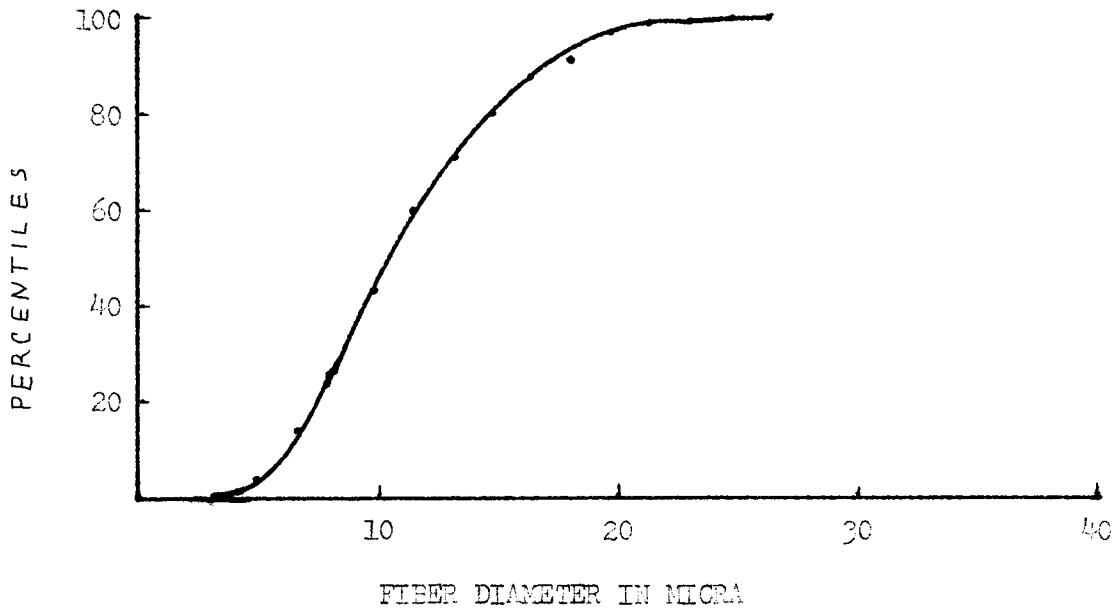


Fig. 18.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 2

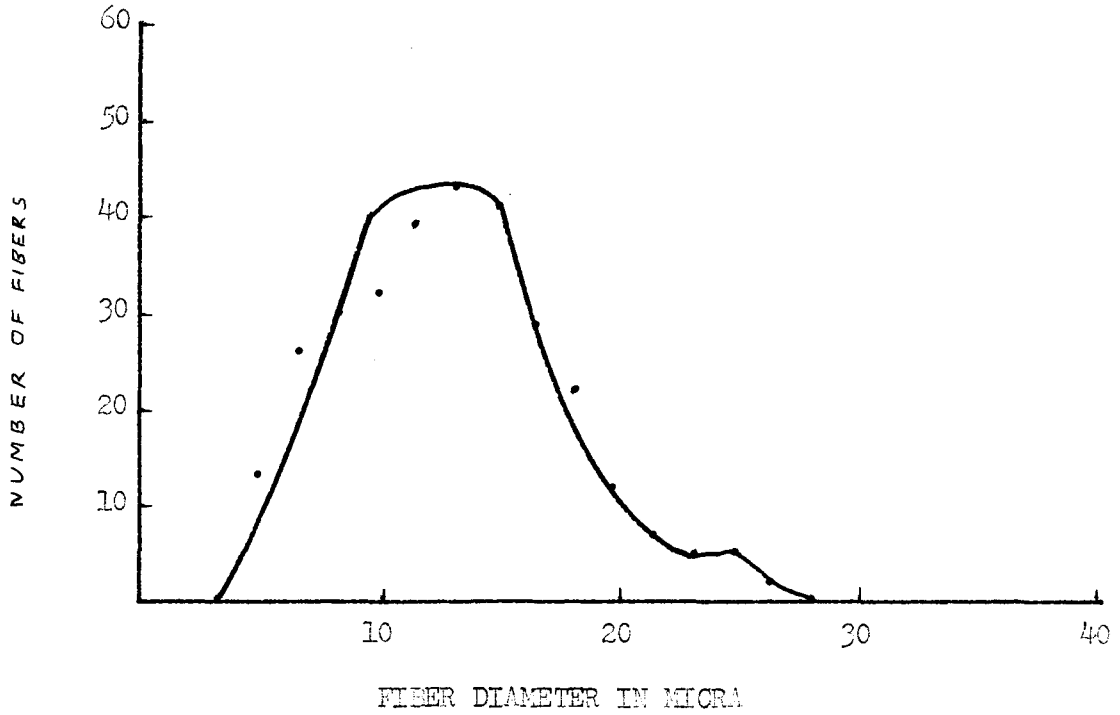


Fig. 19.-Frequency distribution of fiber diameters from the right ventricle of Rat 3

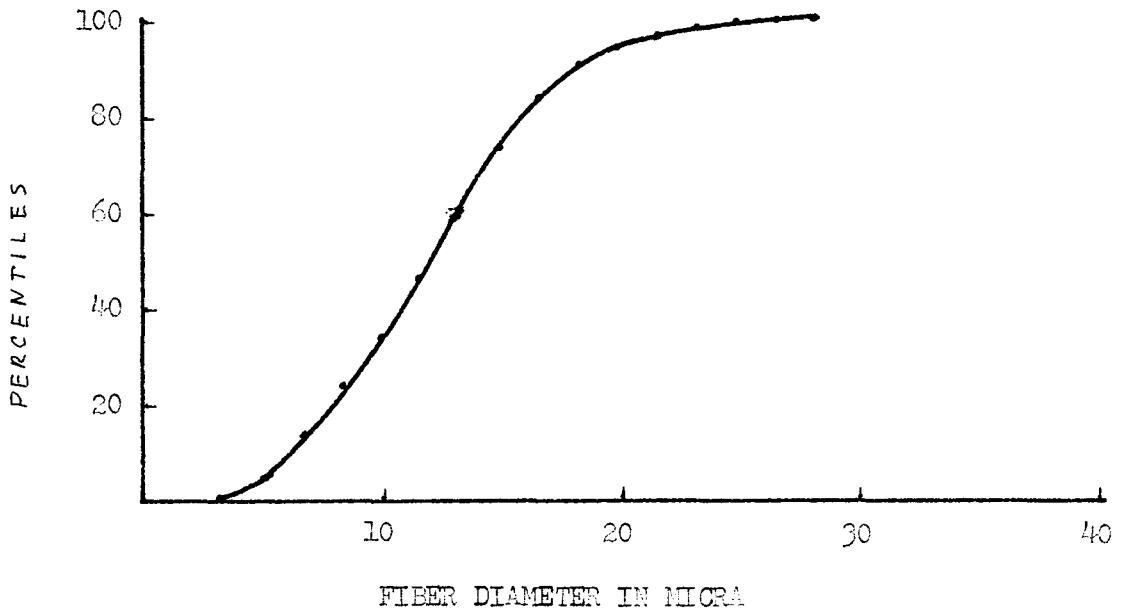


Fig. 20.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 3

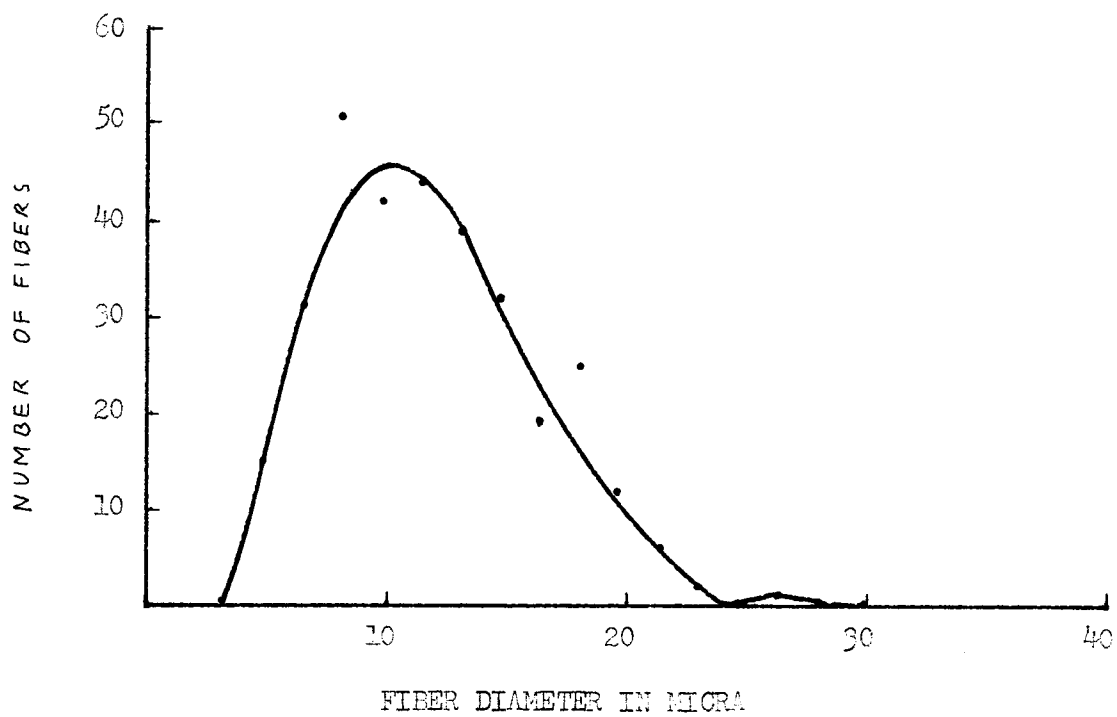


Fig. 21.-Frequency distribution of fiber diameters from the right ventricle of Rat 4

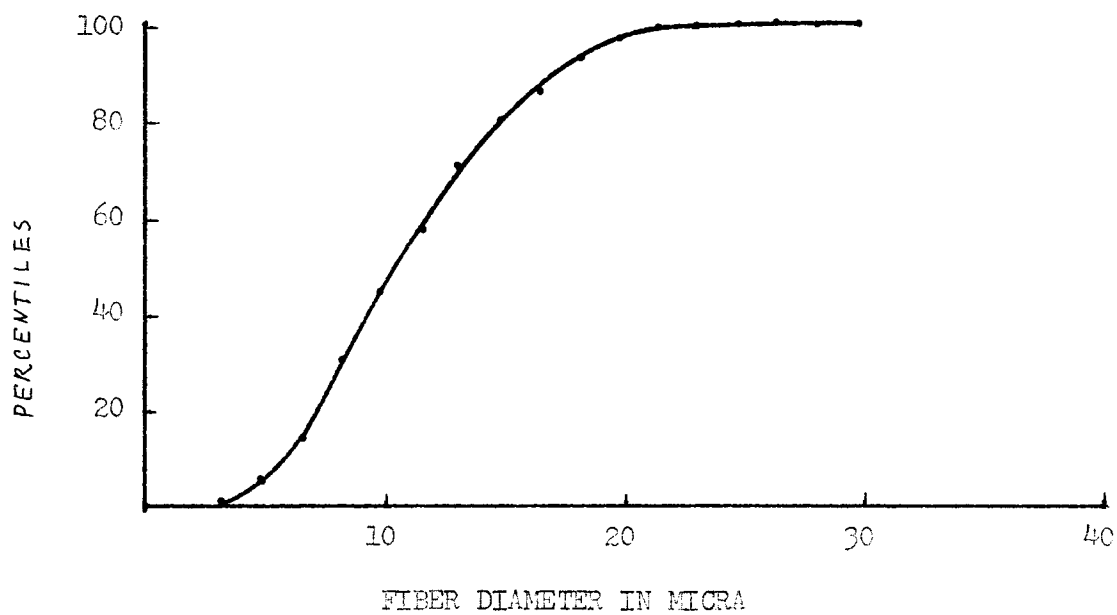


Fig. 22.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 4

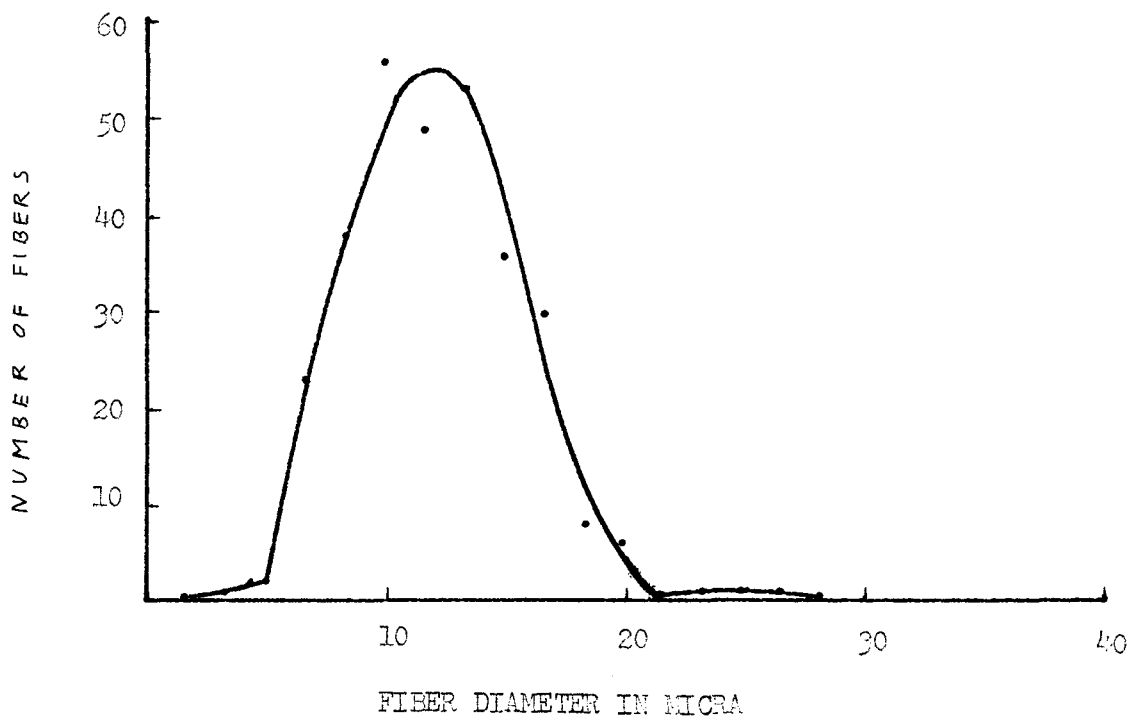


Fig. 23.-Frequency distribution of fiber diameters from the right ventricle of Rat 5

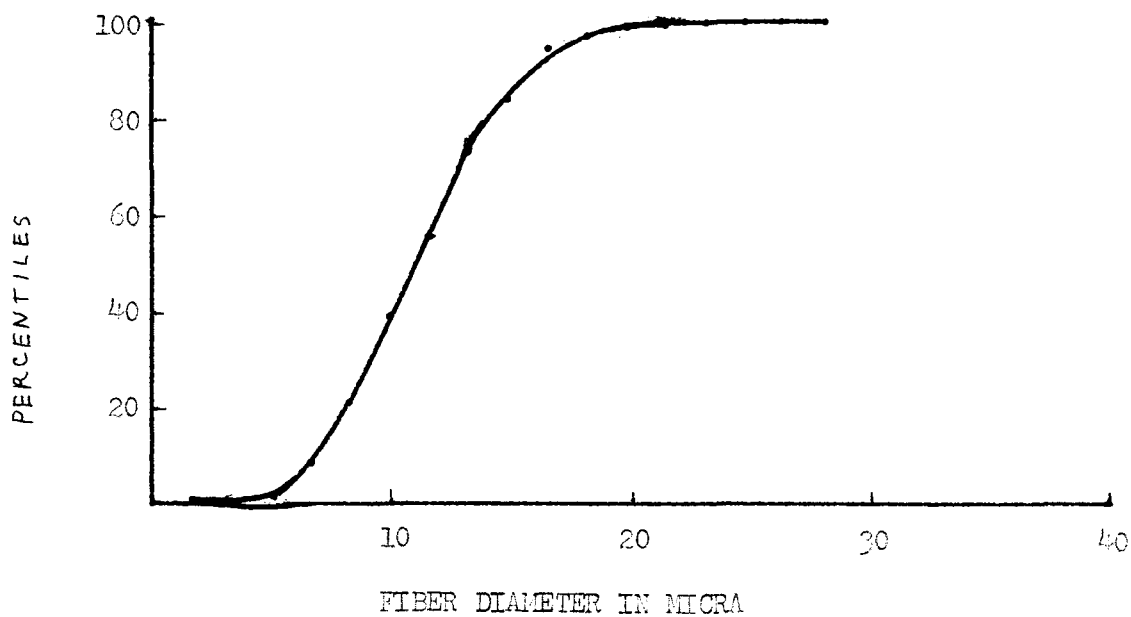


Fig. 24.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 5

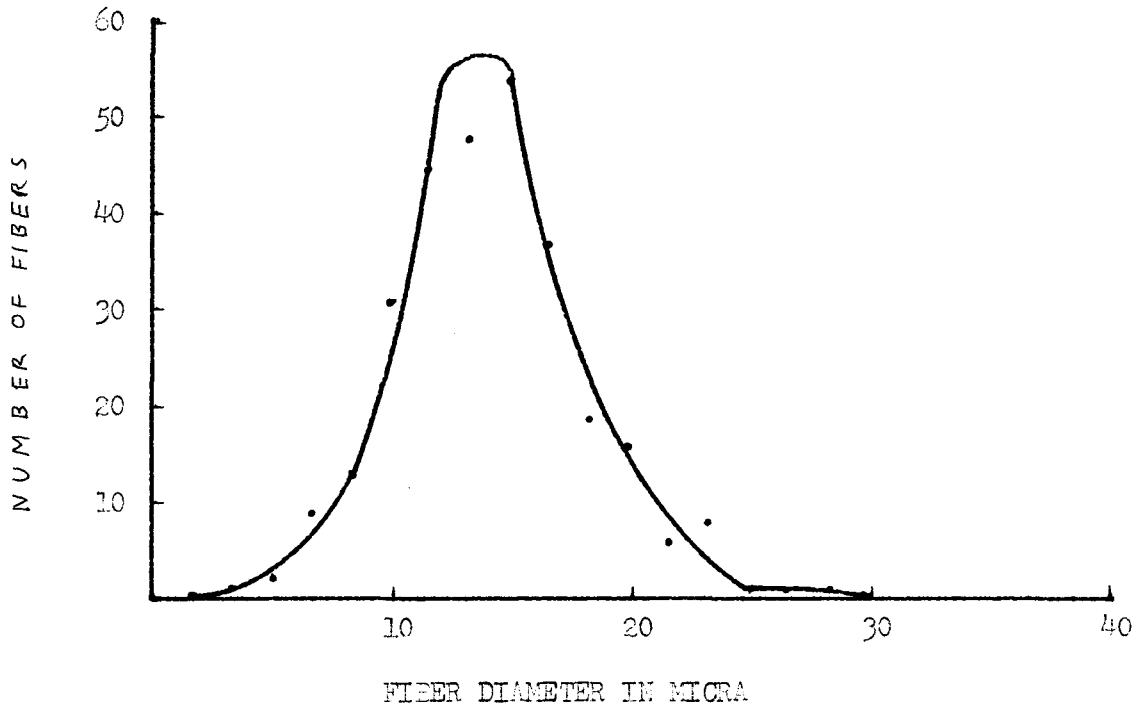


Fig. 25.-Frequency distribution of fiber diameters from the right ventricle of Rat 6

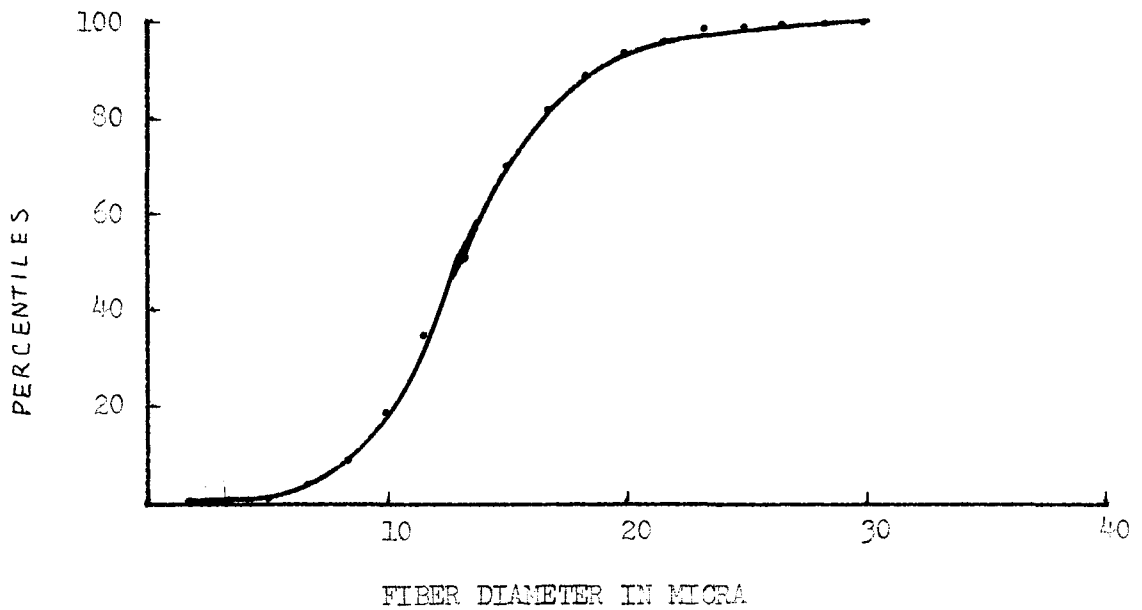


Fig. 26.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 6

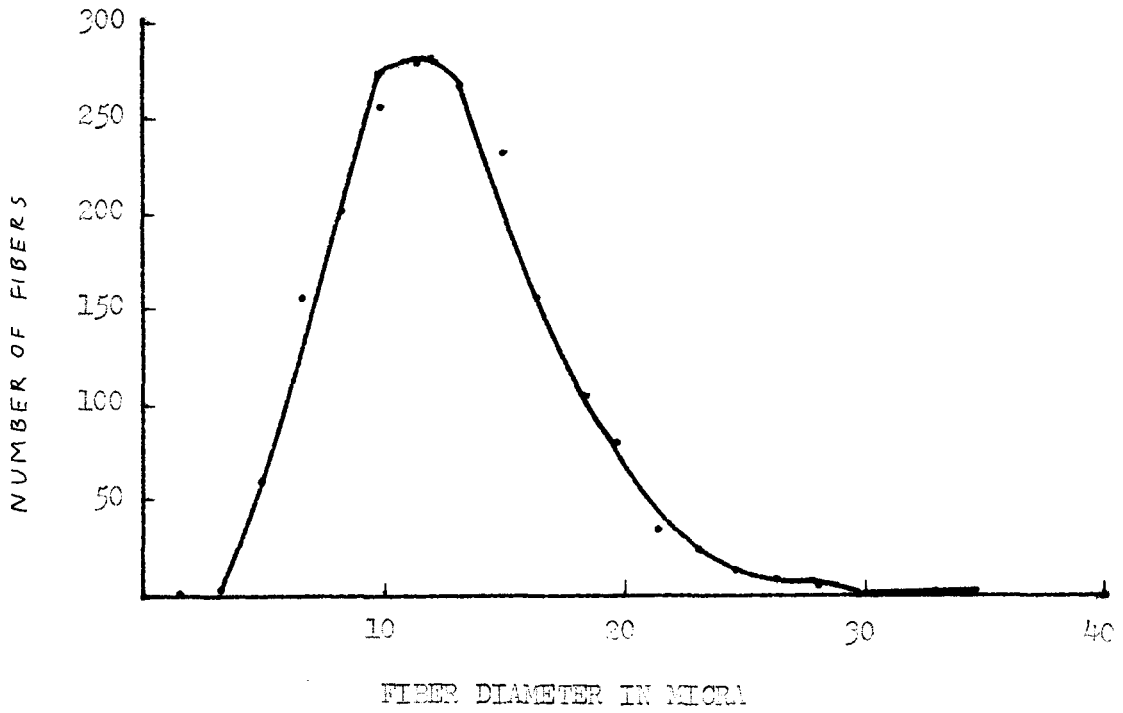


Fig. 27.-Frequency distribution of fiber diameters from the right ventricles of Rats 1 through 6

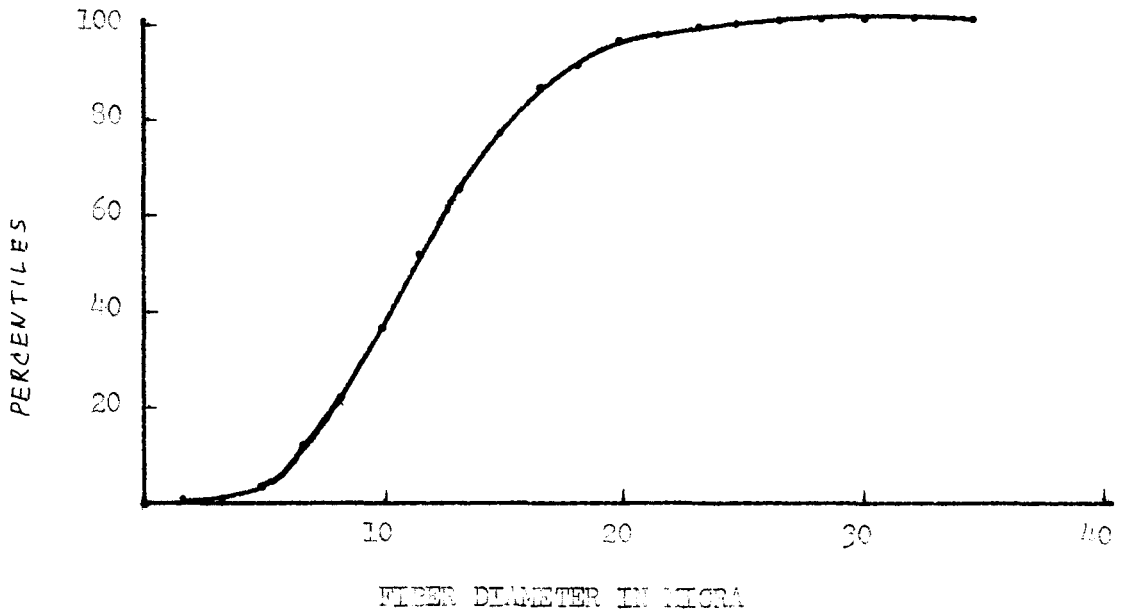


Fig. 28.-Cumulative frequency distribution of fiber diameters from the right ventricles of Rats 1 through 6

c. central tendency

A summary of the statistical data pertinent to fiber size in the six normal rats is shown in Table 4. The age is given in months; the weight refers to body weight in grams.

TABLE 4.-Statistical summary of central tendency data for six normal rats

Number of Fibers	Age	Wgt	Mean±S.D.		Median		Mode		C.V. %	
			LV	RV	LV	RV	LV	RV	LV	RV
306	3.0	562	12.9±5.0	12.8±5.0	11.8	11.5	13.3	12.0	38.8	39.1
310	3.0	527	12.0±4.5	12.0±4.3	10.8	10.6	11.3	11.5	37.5	35.8
297	6.0	543	13.7±4.8	13.0±4.6	13.5	12.0	13.3	13.3	35.0	35.4
319	6.0	495	12.0±4.5	12.0±4.3	10.9	10.7	12.0	10.0	37.5	35.8
286	6.5	499	13.1±4.6	12.0±3.6	11.8	11.0	11.5	12.0	35.1	30.0
284	7.0	522	14.5±4.5	14.1±4.0	13.2	13.1	13.3	14.4	31.0	28.4
Average	6.3	526	13.0±4.7	12.7±4.3	11.9	11.5	12.4	12.3	36.3	33.9

*LV - Left ventricular data; RV - Right ventricular data; C.V. - Coefficient of variation

The means and standard deviations were computed from the tabulated frequency distributions and are given in terms of microns. The modes, as read from the frequency distribution curves, and the means, as read from the cumulative frequency curves, are also given in microns. The coefficient of variation refers to variation in size of the individual fibers from the mean, and this is given in percentage.

2. Fiber counts

Individual fields had a fiber count range from eight to nineteen. Since the field size was constant (3136 square micra), the number of fibers in a field was dependent on the diameter of the fibers. Most fields contained a mixture of fiber sizes and had counts of ten to fifteen. High counts, sixteen to nineteen, were due to the presence of many small fibers, ten micra or less; low counts, eight or nine, indicated a majority of fibers were over sixteen micra. As the number of fields counted in each ventricle was increased, the variation of the total number of fibers per ventricle was decreased. When the total number of cells counted in each ventricle was greater than two hundred, the variation between ventricles became very small and the reproducibility increased.

A comparison of fiber counts from the left and right ventricles of each rat was made (Table 5). There was no significant difference between ventricles in any of the rats. In addition, high and low counts of each ventricle were compared, and there was no significant difference between ventricles of different rats (Table 5).

3. Capillary-fiber ratios

The capillary count per field ranged from six to twenty, and there was a direct relationship between the capillary count and the fiber count in each field. By comparing the number of capillaries with the number of fibers per field, the capillary-fiber ratio was determined. The mean ratio for twenty-five fields was then calculated for each ventricle. Again there was close reproducibility of results between ventricles and between rats. The means, standard deviations,

and coefficients of variation are shown in Table 6.

TABLE 5.-Comparison of fiber counts between ventricles within each normal rat and between high and low counts for each ventricle

Rat	Left Ventricle	Right Ventricle	\bar{d}^*	SE \bar{d}	c	P
1	306	307	1	24.78	0.081	>0.05
2	310	339	29	25.48	1.138	>0.05
3	297	306	9	24.56	0.366	>0.05
4	319	319	0	25.26	0.000	>0.05
5	286	306	20	24.34	0.822	>0.05
6	284	292	8	24.00	0.393	>0.05
Average	300	310	10	24.74	0.445	>0.05
Totals	1802	1869	67	60.60	1.122	>0.05
LV	High Count Rat 4	Low Count Rat 6				
	319	284	35	24.56	1.420	>0.05
RV	High Count Rat 2	Low Count Rat 6				
	339	292	47	25.12	1.870	>0.05

* \bar{d} - Difference between counts; SE \bar{d} - Standard error of the difference; c - The ratio of the difference to the standard error of the difference; P - The probability that the two compared values are not significantly different

TABLE 6.—Capillary-fiber ratios for right and left ventricles in six normal rats

Left Ventricle	Capillary Count	Fiber Count	Ratio Mean±S.D.	Coefficient of Variation in Per Cent
Rat				
1	274	306	0.895±0.283	31.6
2	260	310	0.856±0.222	25.9
3	284	297	0.990±0.260	28.3
4	278	319	0.877±0.215	24.5
5	253	286	0.883±0.174	19.7
6	272	284	1.000±0.203	20.3
Average	270	300	0.917±0.230	25.1
Right Ventricle				
1	273	307	0.836±0.273	30.8
2	282	339	0.832±0.303	36.4
3	291	306	0.951±0.275	29.4
4	274	319	0.859±0.331	38.5
5	281	306	0.935±0.310	33.5
6	270	292	0.925±0.344	37.2
Average	279	311	0.898±0.331	36.9

B. Young Hearts

Four male Sprague Dawley rats, six weeks old, weighing approximately one hundred grams, were studied. The myocardial capillary counts, fiber counts, and capillary-fiber ratios were determined and

tabulated (Table 7). This table shows that the capillary counts were reasonably consistent in each ventricle of different rats. However, the fiber counts differed greatly among rats and this caused wide variations in the capillary-fiber ratios.

TABLE 7. -Capillary counts, fiber counts, and capillary-fiber ratios in four six-week-old rats

Rat	Left Ventricle			Right Ventricle		
	Capillary Count	Fiber Count	Capillary-Fiber Ratio	Capillary Count	Fiber Count	Capillary-Fiber Ratio
7	279	293	0.952	314	366	0.857
8	250	274	0.912	323	365	0.885
9	257	464	0.567	281	448	0.649
10	309	305	1.013	264	676	0.395
Average	274	324	0.861	290	514	0.567

Table 8 presents a comparison of fiber counts in the right and left ventricles of these young rats. There was a significant difference in each case. The counts of the right ventricle were always significantly higher than those of the left. This showed that the fibers of the right ventricle were smaller than those of the left ventricle.

Despite the fact that these rats were of the same breed, age, weight, and sex, these variations made it impossible to establish a constant size pattern for the six-week-old rat myocardium.

TABLE 8.-Comparison of fiber counts between ventricles in the four six-week-old rats

Rat	Right Ventricular Fiber Count	Left Ventricular Fiber Count	\bar{x}	$SE\bar{x}$	P
7	366	292	73	25.7	<0.01
8	365	274	91	25.3	<0.01
9	648	464	184	33.4	<0.01
10	676	305	371	31.3	<0.01
Average	514	334	180	28.9	<0.01

C. Hypertrophied Hearts

Four rats, numbered 11 through 14, that had been treated with DOCA and saline, were studied. Special attention was paid to the ability of the histological and statistical procedures to demonstrate changes in fiber size and capillary-fiber ratio.

Rats 5 and 6 were control animals that were kept at the same time and under the same conditions as the experimental rats. The only difference was that the experimental rats (11 through 14) were injected daily with DOCA and given only saline to drink while the control rats (5 and 6) were not injected and were given water to drink. Rats 5, 11, and 12 were killed after two weeks of treatment, when they were six and a half months old. Rats 6 and 13 were killed after four weeks of treatment, at seven months; and Rat 14 after six weeks of treatment at seven and a half months.

1. Right ventricle

Fiber sizes in the right ventricles of the experimental rats

were similar to fiber sizes in the right ventricles of the control rats. Table 9 shows the frequencies of fiber sizes in the right ventricles of the two control rats, and the four experimental rats. As in the normal myocardia, the majority of fiber sizes fell between five and twenty-five micra.

TABLE 9.-Frequency distributions of fiber diameters in the right ventricles of two normal, and four experimental rats

Diameters in Micra	Number of Fibers					
	Controls		Experimentals			
	R5	R6	R11	R12	R13	R14
1.65	0	0	0	0	0	0
3.30	1	1	1	0	0	0
4.95	2	2	13	1	7	9
6.60	23	9	25	6	23	16
8.25	38	13	27	15	29	26
9.90	56	31	34	28	37	38
11.55	49	45	43	46	40	51
13.20	53	48	48	42	47	44
14.85	31	54	34	52	31	40
16.50	30	37	31	44	26	27
18.15	8	19	22	24	14	21
19.80	6	16	15	9	10	0
21.45	1	0	0	7	12	11
23.10	1	8	5	6	10	3
24.75	1	1	3	3	4	3
26.40	1	1	0	1	2	1
28.05	0	1	1	1	2	0
29.70	0	0	0	0	1	0
31.35	0	0	0	0	0	0
Totals	306	292	310	285	295	298

Table 10 shows a comparison of right ventricular fiber counts of each experimental rat with the control rat closest in age. There were no significant differences between fiber counts in the right

ventricles of experimental and control rats.

TABLE 10.-Comparison of right ventricular fiber counts of control and experimental rats

Rats	Control	Experimental	\bar{x}	SE \bar{x}	P
5 & 11	306	310	4	24.82	>0.05
5 & 12	306	285	21	24.32	>0.05
5 & 11,12	306	298	3	24.58	>0.05
6 & 13	292	295	3	24.23	>0.05
6 & 14	292	298	6	24.29	>0.05

Table 11 shows the capillary counts, the fiber counts, and the capillary-fiber ratios of the two control right ventricles and the four experimental right ventricles. None of these measurements showed significant differences between experimental and control right ventricles.

Figures 29, 31, 33, and 35 are the plotted frequency distributions of the fiber size in the four experimental right ventricles. Once again, the curves were unimodal, relatively symmetrical, and compact, as were the frequency curves for the normal rats.

Figures 30, 32, 34, and 36 are the cumulative frequency distributions for the four experimental right ventricles.

TABLE 11.-Capillary counts, fiber counts, and capillary-fiber ratios in the right ventricles of two control rats and four experimental rats

Rat	Capillary Count	Fiber Count	Capillary-Fiber Ratio
5	281	306	0.935
6	270	292	0.925
11	285	310	0.921
12	256	285	0.898
13	267	295	0.904
14	287	290	0.962
Average	275	298	0.924

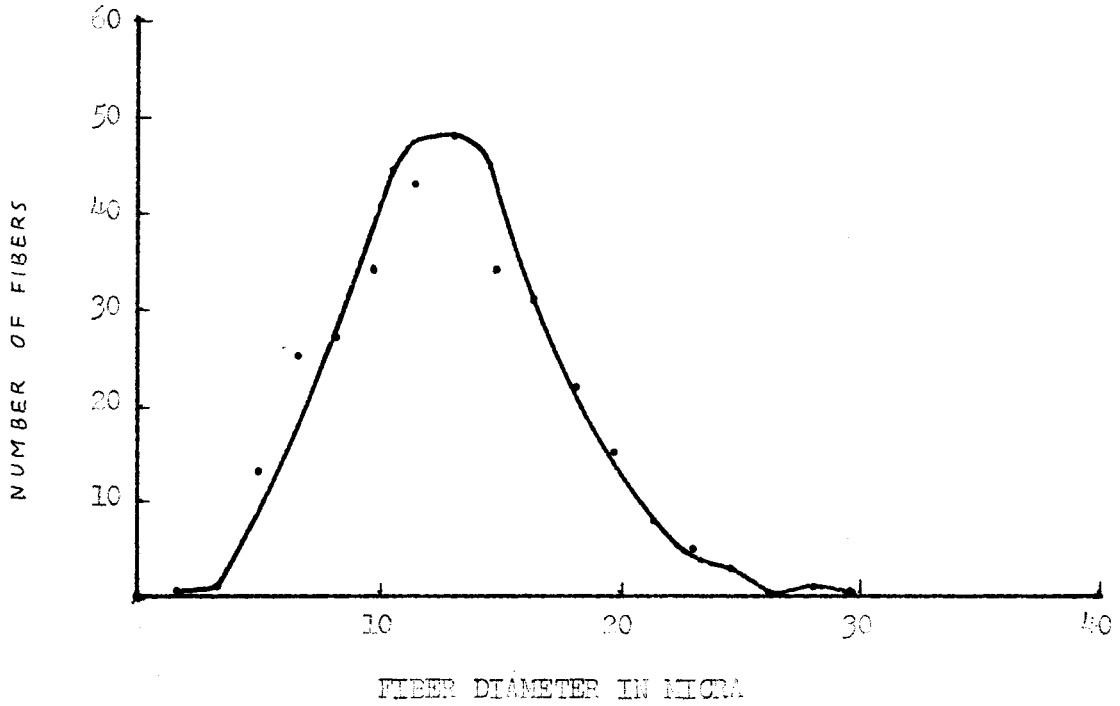


Fig. 29.-Frequency distribution of fiber diameters from the right ventricle of Rat 11

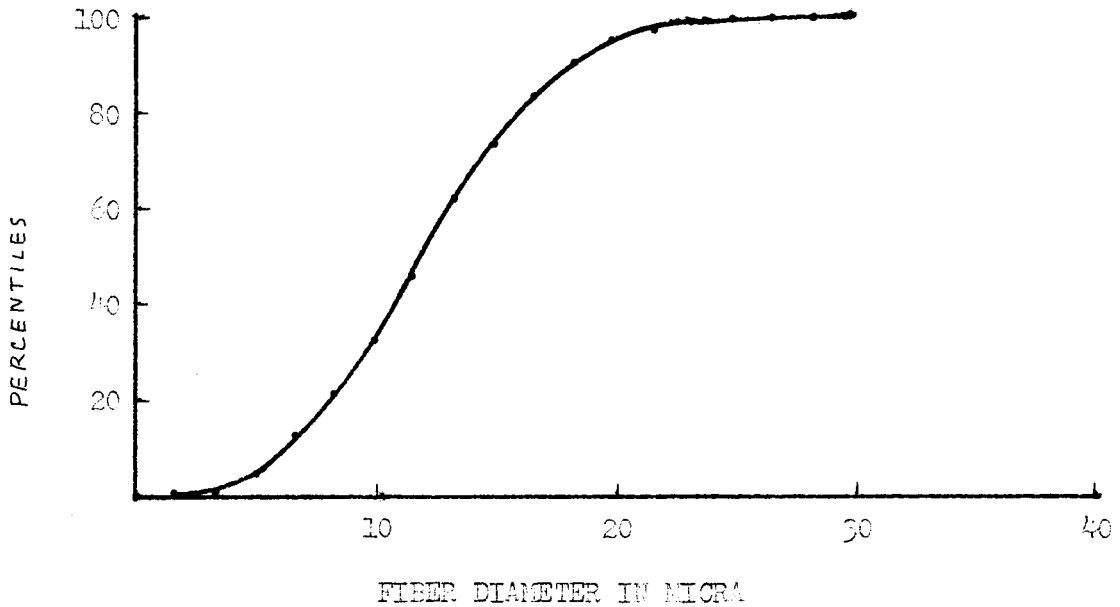


Fig. 30.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 11

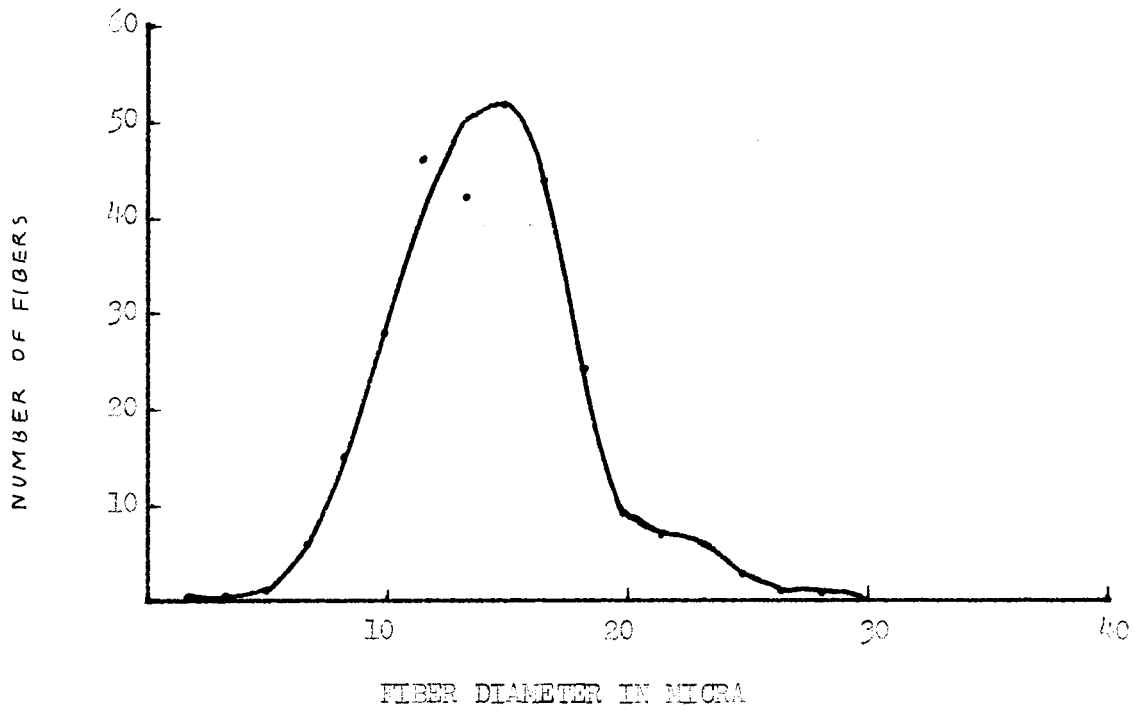


Fig. 31.-Frequency distribution of fiber diameters from the right ventricle of Rat 12

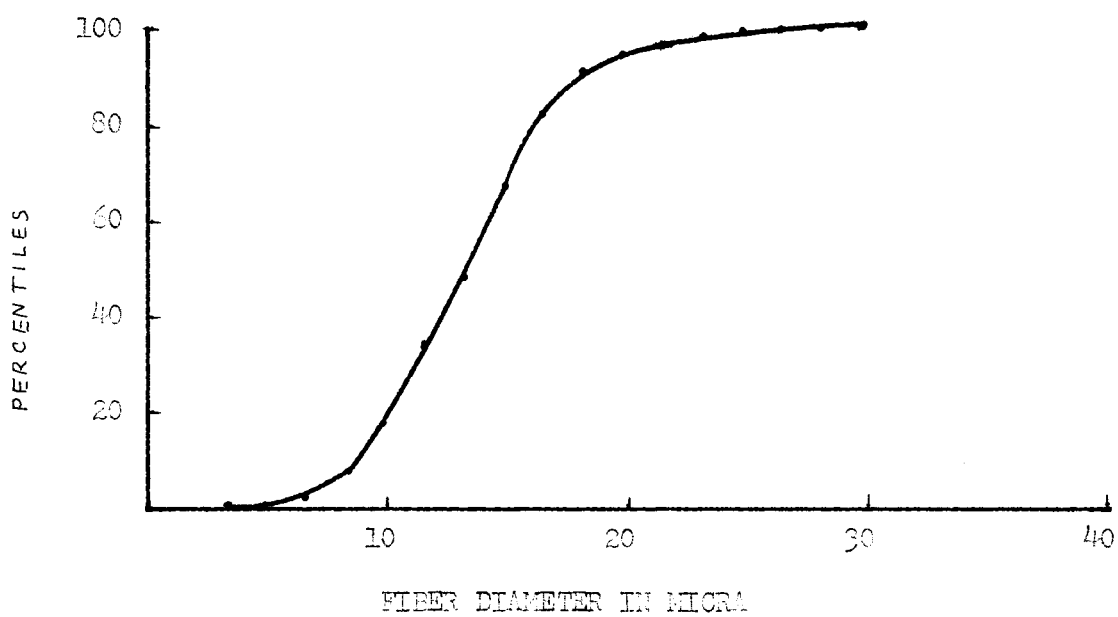


Fig. 32.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 12

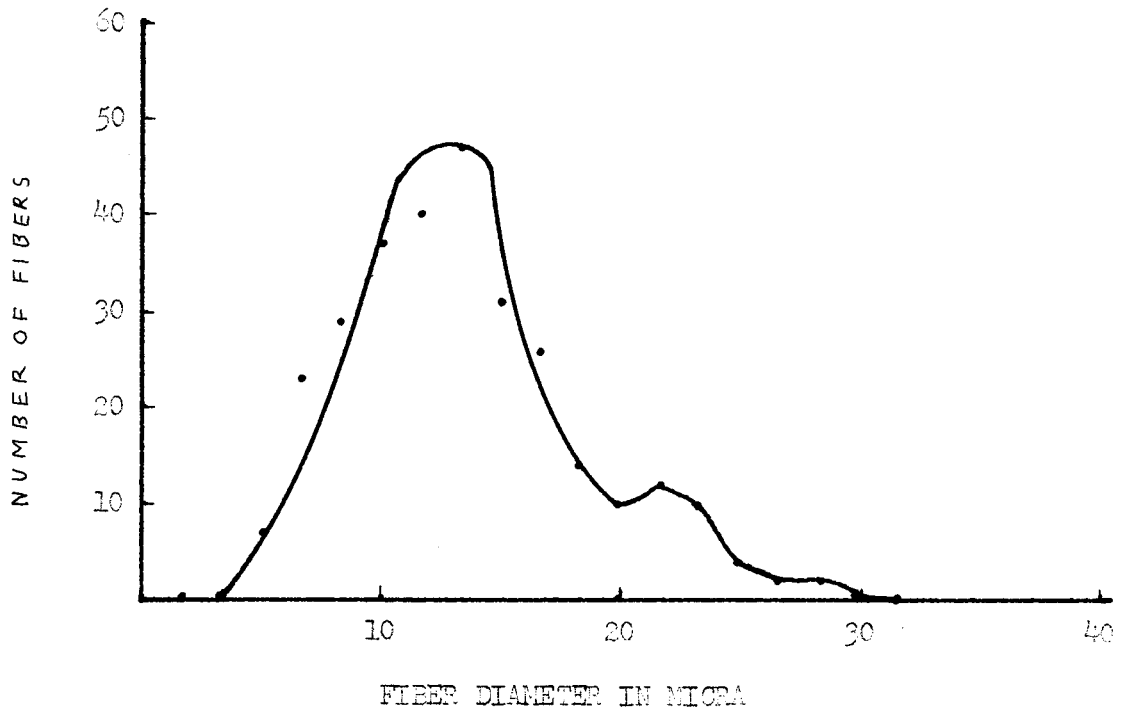


Fig. 33.-Frequency distribution of fiber diameters from the right ventricle of Rat 13

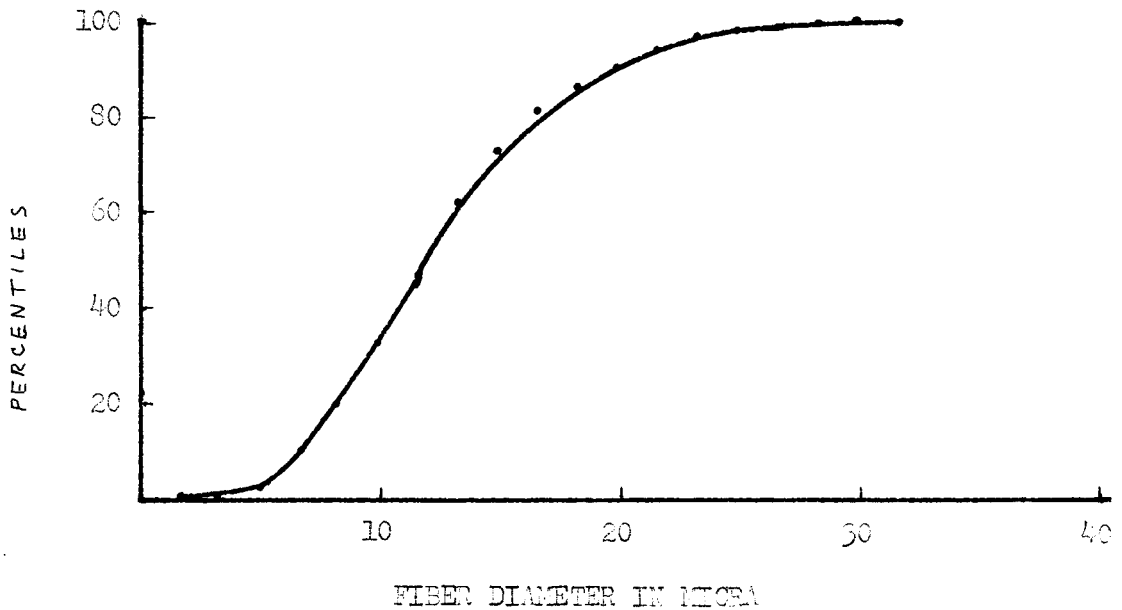


Fig. 34.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 13

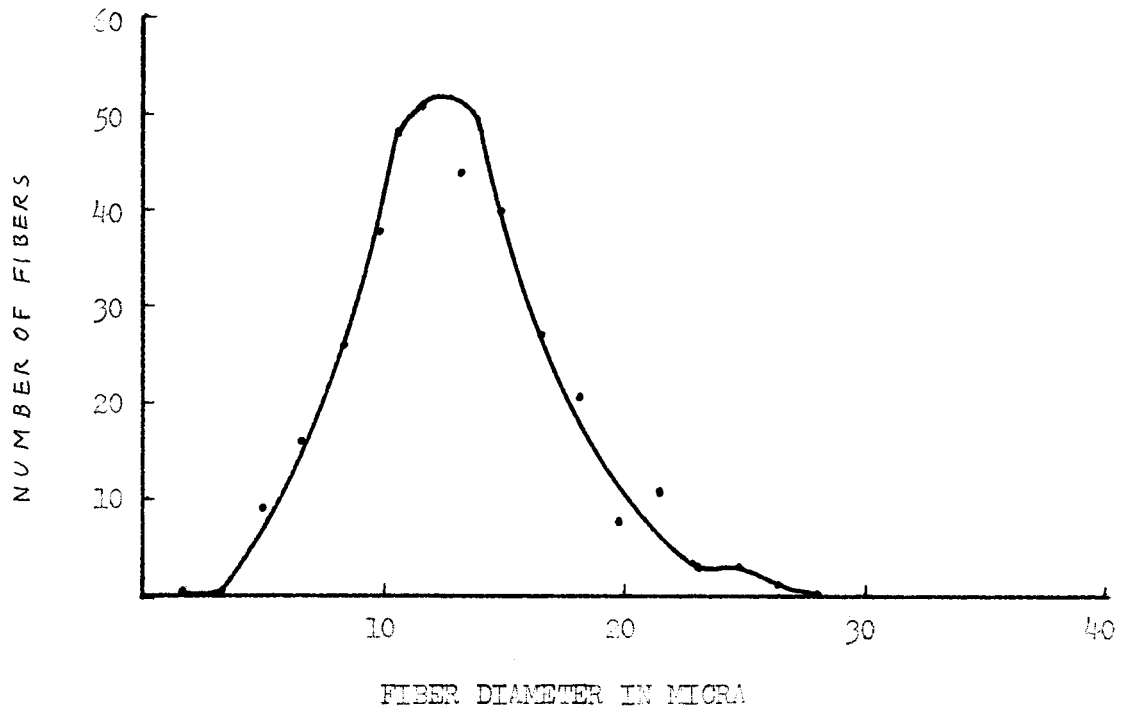


Fig. 35.-Frequency distribution of fiber diameters from the right ventricle of Rat 14

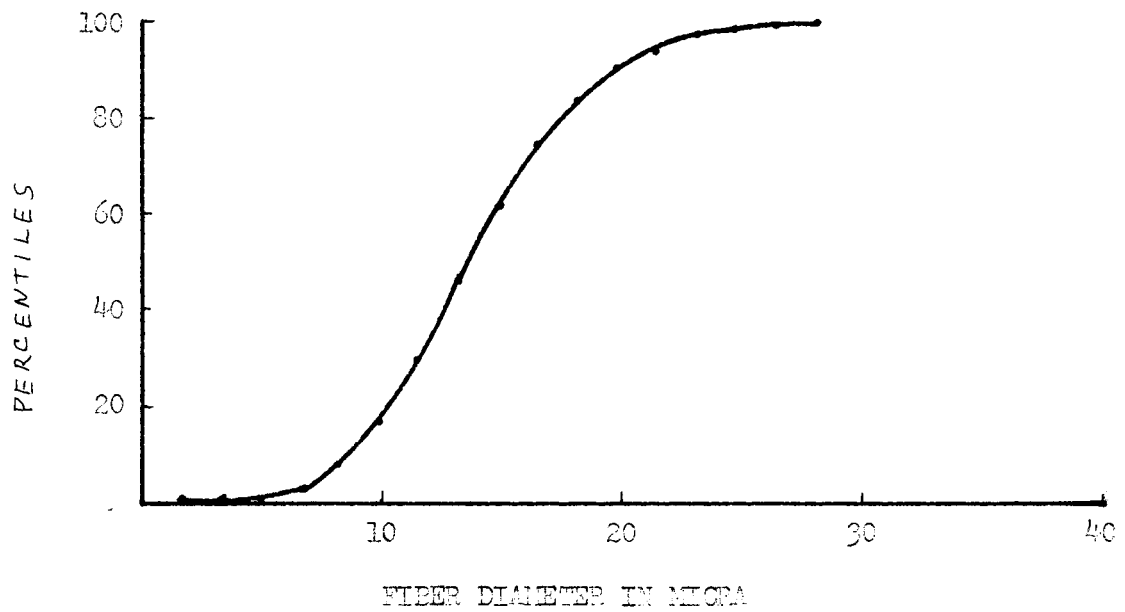


Fig. 36.-Cumulative frequency distribution of fiber diameters from the right ventricle of Rat 14

2. Left ventricle

The left ventricles of the four experimental rats showed a definitely altered pattern. Table 12 lists the frequencies of fiber sizes in the left ventricles of the two control and the four experimental rats. The fiber sizes no longer fell in the five to twenty-five micra range. There was a definite enlargement of fiber diameter with the majority of fibers between eleven and thirty-three micra (a twenty-two micra range). Therefore, the fibers enlarged but still remained in a fairly compact range.

Figures 37, 39, 41, and 43 show the plotted frequency distributions for the four experimental left ventricles. There was a definite shift toward the larger sizes. The curves are not unimodal nor are they symmetrical.

Figures 38, 40, 42, and 44 are graphs of the cumulative frequency distributions of the four left ventricles of the experimental rats. These curves are more spread out than those of the normal ventricles.

TABLE 12.-Frequency distributions of the fiber diameters in the left ventricles of two normal, and four experimental rats

Diameters in Micro	Number of Fibers					
	Controls		Experimentals			
	R5	R6	R11	R12	R13	R14
1.65	0	0	0	0	0	0
3.30	0	0	0	0	0	0
4.95	7	1	0	0	0	0
6.60	13	6	0	0	1	0
8.25	38	19	0	0	0	0
9.90	31	40	4	0	0	1
11.55	45	31	7	5	0	1
13.20	36	52	8	10	15	1
14.85	40	32	5	3	13	2
16.50	16	30	11	13	13	6
18.15	20	30	13	13	13	9
19.80	18	11	17	13	13	20
21.45	0	14	17	15	13	17
23.10	3	11	14	19	16	20
24.75	3	3	19	22	22	13
26.40	1	4	11	5	5	13
28.05	1	0	7	11	0	9
29.70	1	0	4	8	4	9
31.35	0	0	2	3	0	5
33.00	0	0	7	10	3	10
34.65	0	0	0	0	3	10
36.30	0	0	1	0	1	1
37.95	0	0	0	1	0	0
39.60	0	0	0	0	0	0
41.25	0	0	1	0	0	0
42.90	0	0	0	0	0	0
Totals	286	284	148	140	166	146

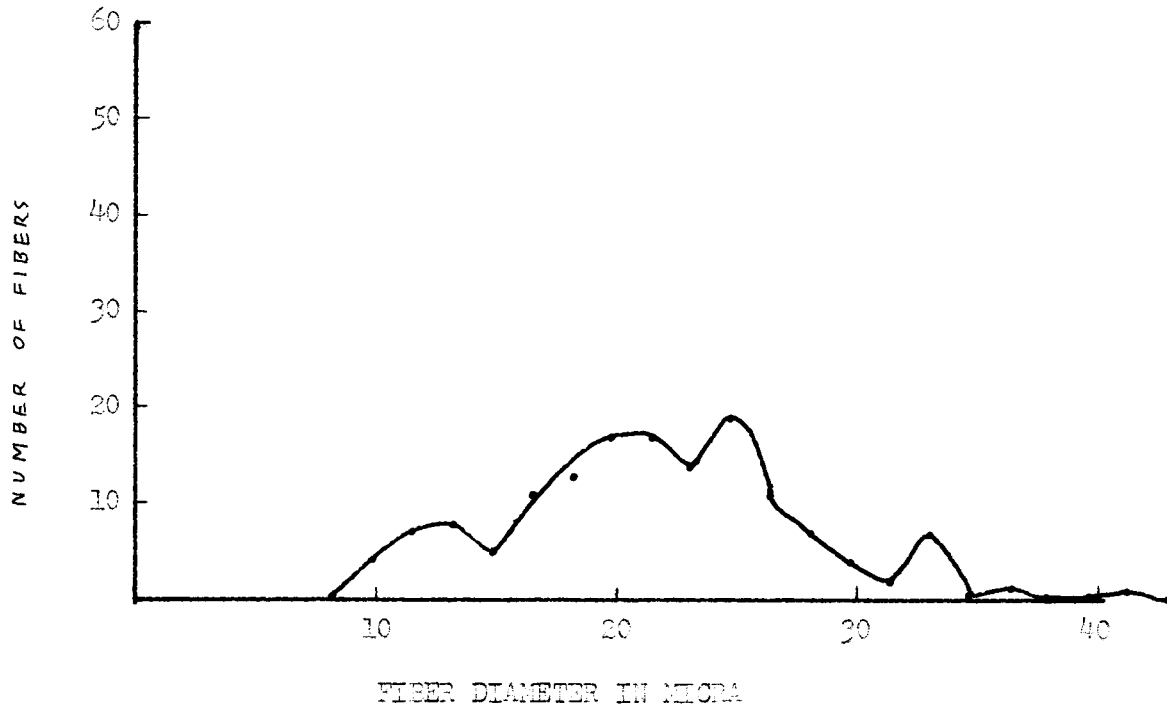


Fig. 37.-Frequency distribution of fiber diameters from the left ventricle of Rat 11

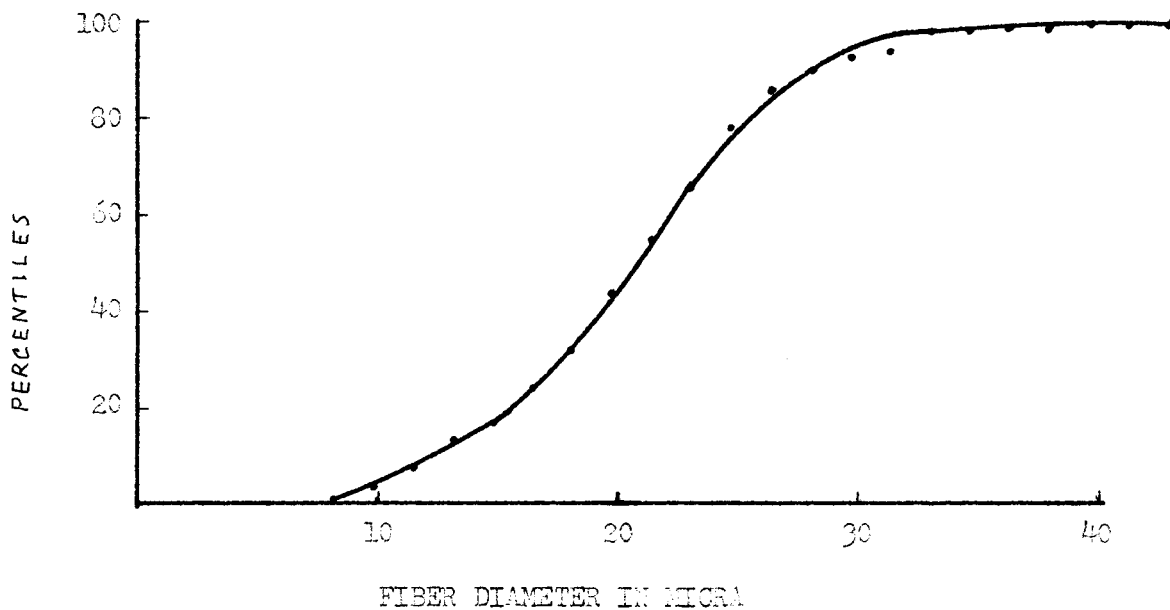


Fig. 38.-Cumulative frequency distribution of fiber diameters from the left ventricle of Rat 11

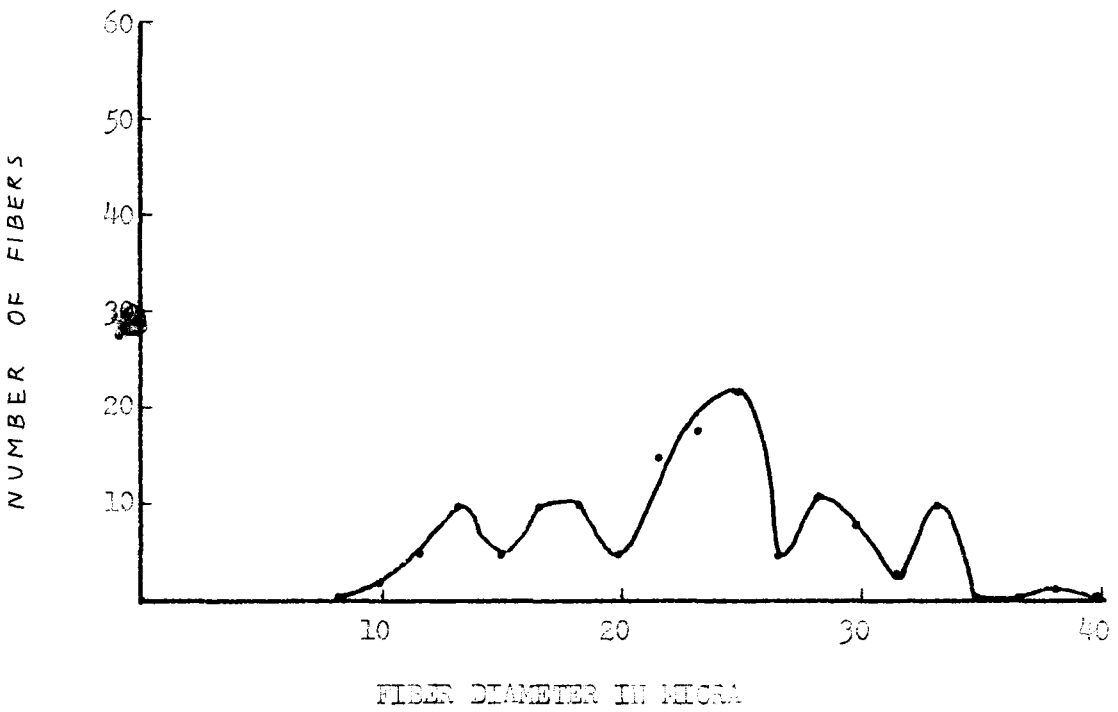


Fig. 39.-Frequency distribution of fiber diameters from the left ventricle of Rat 12

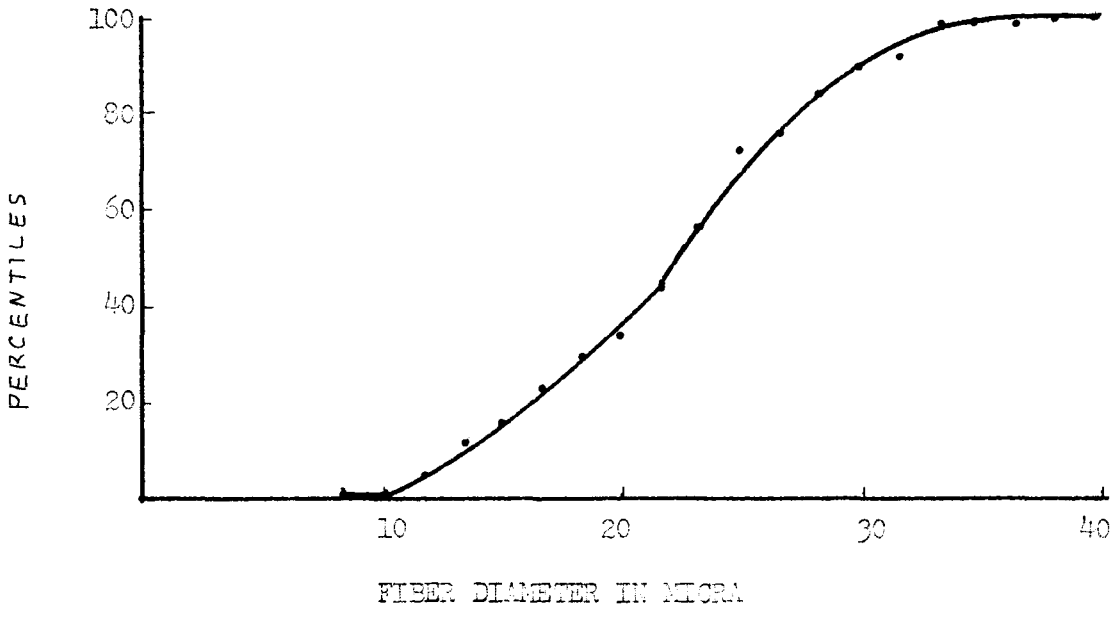


Fig. 40.-Cumulative frequency distribution of fiber diameters from the left ventricle of Rat 12

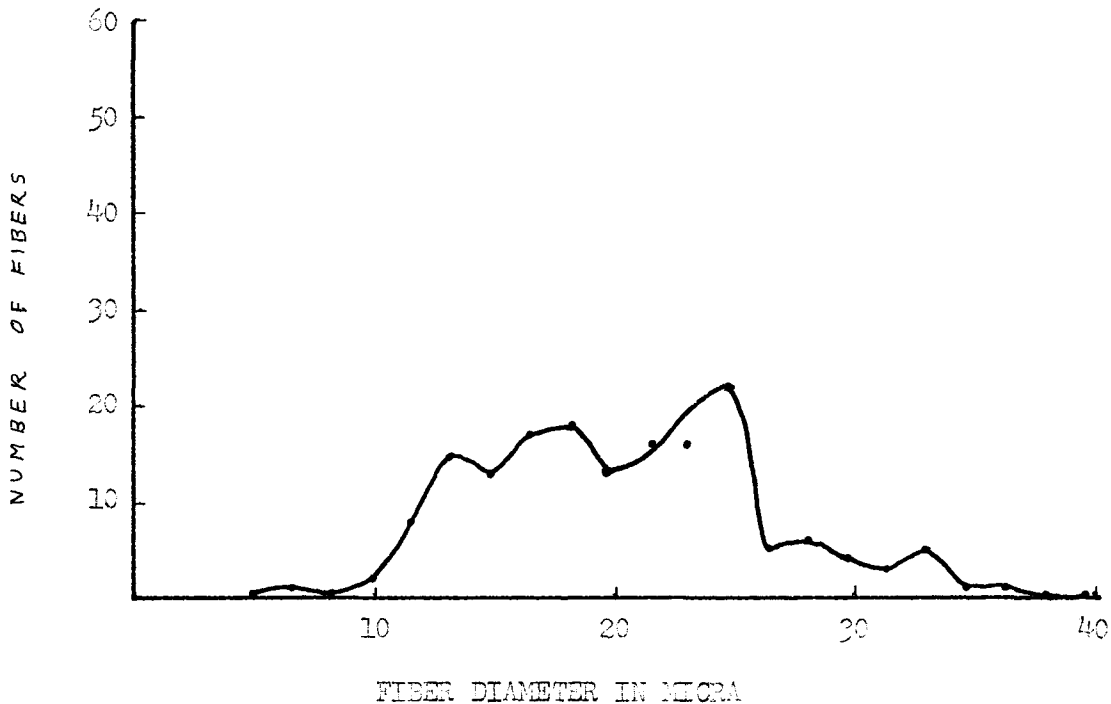


Fig. 41.-Frequency distribution of fiber diameters from the left ventricle of Rat 13

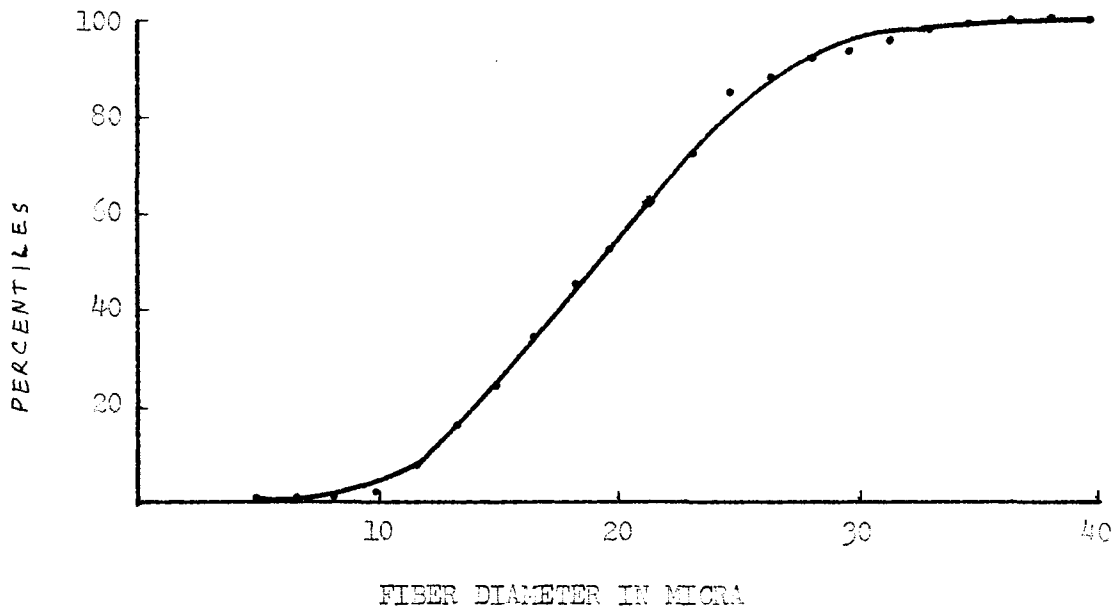


Fig. 42.-Cumulative frequency distribution of fiber diameters from the left ventricle of Rat 13

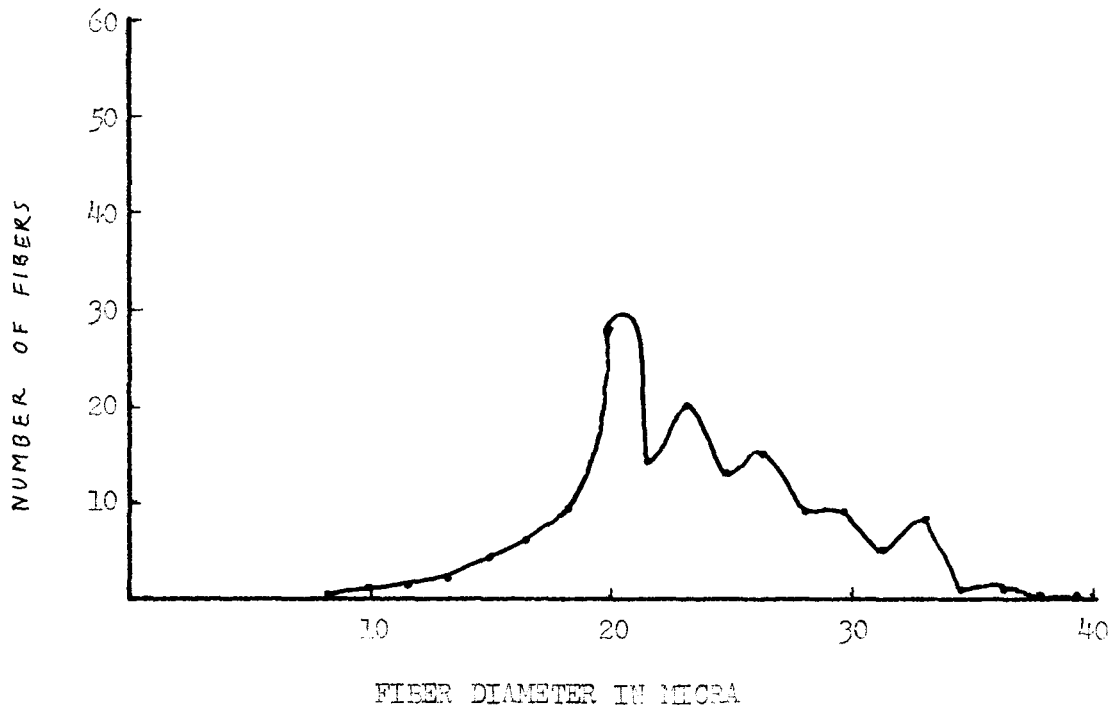


Fig. 43.-Frequency distribution of fiber diameters from the left ventricle of Rat 14

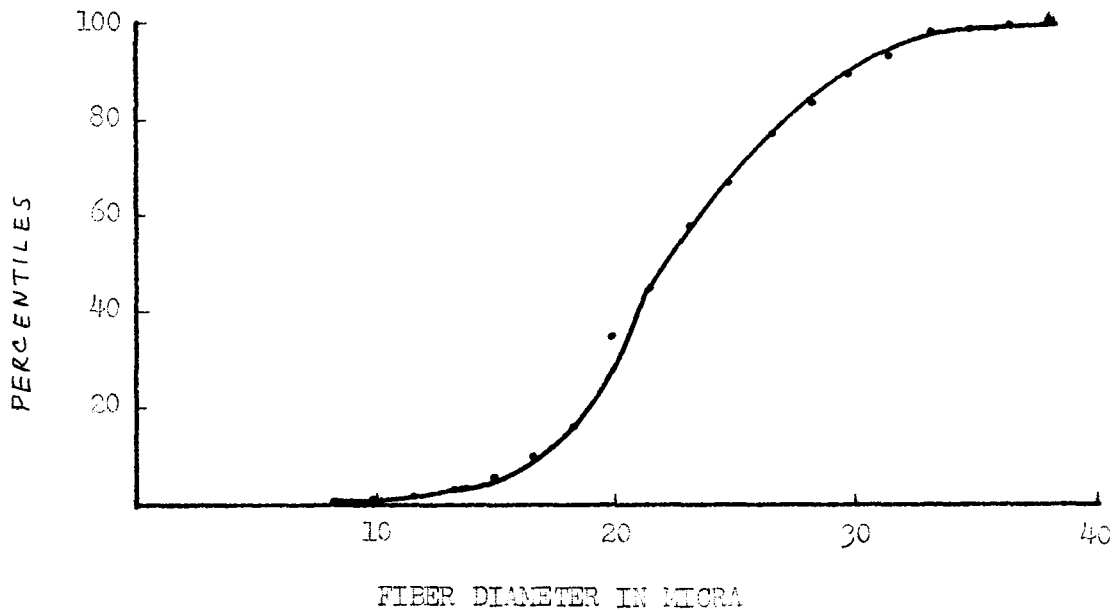


Fig. 44.-Cumulative frequency distribution of fiber diameters from the left ventricle of Rat 14

Table 13 presents a summary of statistical data pertinent to fiber size in the four experimental rats. Control rats 5 and 6 are included for comparative purposes. The age is given in months; the weight, in grams. Rx refers to the length of time of treatment with DOCA and saline, and this is given in weeks. The means, standard deviations, medians, and modes are given in micra. The modes are given for the right ventricle only, since the left ventricular distributions are multimodal.

TABLE 13.—Statistical summary of central tendency data for four experimental rats

Rat	Age	Wgt	Rx	Mean±S.D.		Median		Mode		C.V. %	
				LV	RV	LV	RV	RV	LV	RV	
Controls											
5	6.5	499	0	13.1±4.6	12.0±3.6	11.8	11.0	12.0	35.1	30.0	
6	7.0	522	0	14.5±4.5	14.1±4.0	13.2	13.1	14.4	31.0	28.4	
Average	6.8	511	0	13.8±4.6	13.5±3.8	12.5	12.5	13.2	33.5	29.2	
Experimentals											
11	6.5	603	2	21.5±5.9	13.0±4.6	20.7	11.9	12.7	27.4	35.4	
12	6.5	564	2	22.4±6.1	14.3±4.0	22.2	13.3	14.8	27.2	28.0	
13	7.0	505	4	20.3±5.8	13.4±5.1	19.3	12.0	13.2	28.6	28.1	
14	7.5	568	6	23.4±5.1	13.1±4.3	22.1	11.8	12.8	21.8	32.8	
Average	6.9	560	3.5	21.9±5.7	13.5±4.5	21.1	12.3	13.4	26.3	33.6	

The mean diameter of fibers from each left ventricle was noticeably larger than the mean diameter of those from the corresponding right ventricle. The standard deviations had increased only slightly

and the coefficients of variation decreased in the left ventricles, indicating that the fiber sizes were still compactly arranged.

Table 14 shows a comparison of fiber sizes between left ventricles of control and experimental rats and between right and left ventricles of experimental rats. There was a statistically significant increase in fiber size in the left ventricles of the experimental rats.

TABLE 14.—Comparison of fiber sizes between the left ventricles of control and experimental rats and between the right and left ventricles in each experimental rat

Rats	Left Ventricle		\bar{d}	SE \bar{d}	F
	Control	Experimental			
5 & 11	13.1 \pm 4.6	21.5 \pm 5.9	9.4	0.559	< 0.01
5 & 12	13.1 \pm 4.6	22.4 \pm 6.1	9.1	0.574	< 0.01
5 & 11, 12	13.1 \pm 4.6	22.0 \pm 6.0	9.9	0.567	< 0.01
6 & 13	14.5 \pm 4.5	20.3 \pm 5.8	5.8	0.519	< 0.01
6 & 14	14.5 \pm 4.5	23.4 \pm 5.1	8.9	0.501	< 0.01
Average	13.8 \pm 4.6	21.9 \pm 5.7	8.1	0.538	< 0.01
Experimentals					
	Right Ventricle	Left Ventricle			
11	13.0 \pm 4.6	21.5 \pm 5.9	9.5	0.556	< 0.01
12	14.3 \pm 4.0	22.4 \pm 6.1	8.1	0.558	< 0.01
11 & 12	13.7 \pm 4.3	22.0 \pm 6.0	9.3	0.557	< 0.01
13	13.4 \pm 5.1	20.3 \pm 5.8	6.9	0.539	< 0.01
14	13.1 \pm 4.3	23.4 \pm 5.1	10.3	0.495	< 0.01
Average	13.2 \pm 4.5	21.9 \pm 5.7	8.7	0.537	< 0.01

It should be noted that in the experimental rats, the left ventricular fibers enlarged very early, within two weeks, and no further enlargement took place with continued treatment.

Table 15 shows a comparison of fiber counts between experimental left and right ventricles, and there are significant differences.

TABLE 15.-Comparison of left and right ventricular fiber counts in four experimental rats

Pat	Right Ventricle	Left Ventricle	\bar{x}	SE \bar{x}	P
11	210	148	160	21.42	<0.01
12	285	140	145	20.60	<0.01
11 & 12	290	144	154	21.00	<0.01
13	295	166	189	21.40	<0.01
14	290	145	150	21.00	<0.01

A comparison of left ventricular fiber counts between each experimental rat and the control rat is shown in Table 16, and also between the various experimental rats is presented in Table 16. This shows that while there are significant differences between experimental and control rats, there are no significant differences between any of the experimental rats.

A comparison of capillary counts between left ventricles of control and experimental rats is shown in Table 17. In all cases the differences were statistically significant. A comparison of capillary counts from experimental rats is also included and there are no significant differences.

TABLE 16.—Comparison of left ventricular fiber counts between experimental and control rats and between experimental rats

Rats	Control Fiber Count	Experimental Fiber Count	\bar{x}	SE \bar{x}	P
5 & 11	206	140	170	20.64	<0.01
5 & 12	286	170	227	20.64	<0.01
5 & 11, 12	286	144	214	20.74	<0.01
6 & 12	204	166	188	21.22	<0.01
6 & 14	204	146	159	20.74	<0.01
11, 12 & 13			22	17.61	>0.05
11, 12 & 14			2	17.03	>0.05
13 & 14			20	17.67	>0.05

TABLE 17.—Comparison of left ventricular capillary counts between experimental and control rats and between experimental rats

Rats	Control Capillary Count	Experimental Capillary Count	\bar{x}	SE \bar{x}	P
5 & 11	253	175	214	20.69	<0.01
5 & 12	253	126	187	19.47	<0.01
5 & 11, 12	253	151	202	20.10	<0.01
6 & 12	204	152	192	20.20	<0.01
6 & 14	204	130	146	20.55	<0.01
11, 12 & 13			12	17.38	>0.05
11, 12 & 14			4	16.00	>0.05
13 & 14			14	17.03	>0.05

Table 18 shows the capillary-fiber ratios for the left ventricles of the experimental rats. The total number of capillaries per two hundred fields decreased. This was proportional to the decrease in fiber count and, therefore, the capillary-fiber ratio remained the same as in the normal left ventricles.

TABLE 18.--Capillary-fiber ratios for the left ventricles of four experimental rats

Rat	Capillary Count	Fiber Count	Ratio Mean±S.D.	Coefficient of Variation in Per Cent
11	175	148	0.962±0.190	19.9
12	126	140	0.913±0.338	37.0
11 & 12	151	144	0.939±0.264	28.4
13	152	166	0.919±0.244	26.5
14	138	141	0.974±0.263	27.3

Figures 45 through 48 are photomicrographs of the ventricles of Rat 6, a control rat, and Rat 74, an experimental rat.

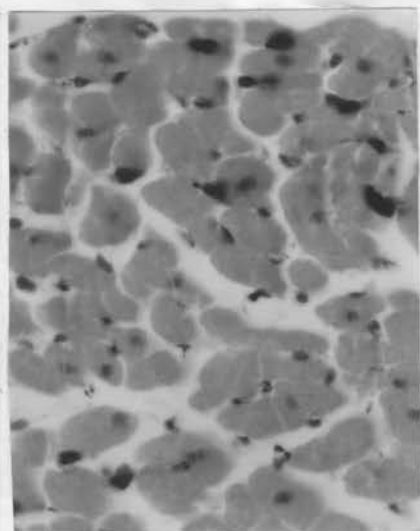


Fig. 45.-Right ventricle
of Rat 6 X470

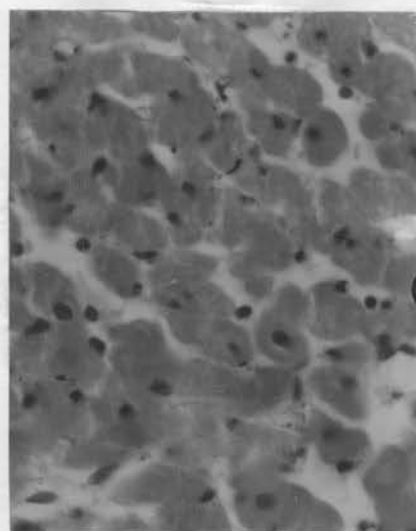


Fig. 46.-Left ventricle
of Rat 6 X470

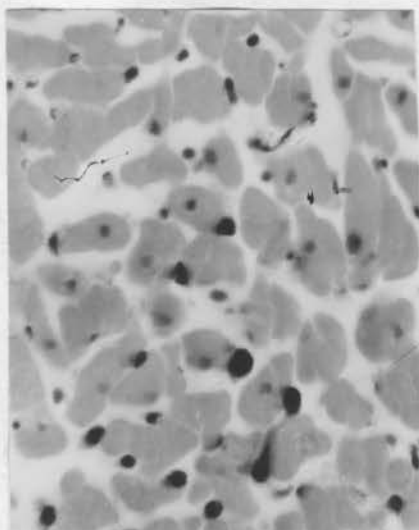


Fig. 47.-Right ventricle
of Rat 14 X470

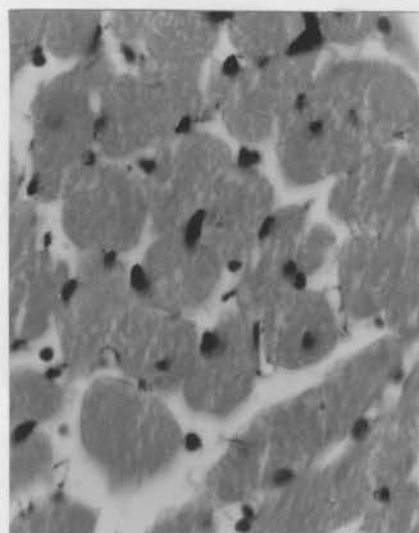


Fig. 48.-Left ventricle
of Rat 14 X470

IV. DISCUSSION

The purpose of this study was to determine whether the rat myocardium presents a consistent morphological pattern in relation to fiber size and capillary-fiber ratio.

It was found that, in the six-week-old rats studied here, there were great variations between the two ventricles of each rat as well as between ventricles of different rats. Theoretically, it should be possible to establish growth curves in which rats of a given age would all have a predictable fiber size and capillary-fiber ratio. This may be possible when littermates are used. However, complete studies of myocardial alterations often necessitate the use of such large numbers of animals as to exclude the possibility of using only littermates. Rats are usually obtained on the basis of age and weight. Despite the fact that the young rats of this study were of the same breed, age, weight, and sex, there were significant differences between ventricles and between rats in relation to fiber size and capillary-fiber ratio.

When one studies the data presented in this thesis, it becomes obvious that, in rats six months old or older, there is a high degree of consistency in regard to fiber size, capillary supply, and the quantitative relation between the two. These rats make good experimental animals since the controls have such a definite pattern.

For the purposes of this study, a rat of six months is considered to be adult. Although the rat continues to grow throughout its entire life, the growth after six months is minor and does not

introduce significant changes.

In some of the frequency distribution graphs, the curves were clearly unimodal. In the other graphs the points fell in such a way that a smooth curve could be drawn to give a single peak or, if the dots were connected on a strict point to point basis, there appeared to be several peaks. There are several possible explanations as to the multimodal appearance. It is known that in higher mammals, there are four different muscular bundles present in the myocardium (Mall, 1911). This may also occur in the rat and these bundles could contain fibers of different sizes. Also, cardiac muscle is highly branched. These branches, being smaller than the main muscle fibers, could account for any peaks in the lower range, below six micra. In addition, the micrometer used in this study measured with an accuracy of only 0.82 micra. Therefore, it was possible for fiber measurements to be misplaced one group higher or lower than the correct category. This could be corrected by an oil immersion technique or by the use of a filar micrometer. Also, the irregular shape of the fibers prevented accurate determination of diameters beyond certain limits and again fibers could be misplaced one group higher or lower than the correct category. Or it could be a fixation effect caused by unequal diffusion of the fixing fluid.

To get a clearer picture of the distribution, the six left ventricles were grouped and plotted collectively. The six right ventricles were then treated similarly. Had there been a true multimodal distribution, it should have been emphasized here. On the contrary

it disappeared with resultant smooth single-peaked curves. In addition, the means, medians, and modes for the ventricles individually and collectively were so similar that indications were again in favor of a unimodal distribution. A unimodal distribution does not imply that all the fibers were the same size. In the case of the six normal left ventricles, individual fibers ranged in diameter from three to thirty-three micra. However, the mode was at thirteen micra and fibers with smaller or larger diameters occurred to a lesser extent.

Within the limitations of this study, there appears to be a single population of fiber sizes in each ventricle. Moreover, this population is not significantly different between left and right ventricles in any one rat, nor between ventricles of various rats. Neither the weight of the rat, nor the difference in age, all being adult, affected the measurements. Therefore, in relation to fiber size, there is a constant morphological pattern characterizing the rat myocardium. It is possible to use adult rats in experimental research on fiber size since consistent control values may be obtained.

This study is not intended to establish absolute fiber sizes as they exist in the living rat. The paraffin method used here results in 15 to 20 per cent shrinkage (Lowe and Bate, 1948). However, as long as the method is consistent, the shrinkage is constant and all measurements are comparable. Where absolute values are desired, frozen sections may be considered. These do not survive multiple stain techniques very well, and therefore were not used in the present study. Another possibility of determining absolute size is to apply a shrinkage correction factor (Hereoux, 1957).

There is an inverse relationship between fiber diameters and fiber counts in a given area. Therefore, simple counts per unit area can be used as a general indication of fiber size. This is especially useful for a quick evaluation of pathological conditions, although measurements should be made for conclusive information, since excessive connective tissue or conditions such as edema could decrease the count without increasing the fiber sizes.

It should be noticed that since the right ventricle of the experimental rats has fiber sizes and capillary-fiber ratios essentially similar to the ventricles of the normal rats, the right ventricle of an experimental animal may serve as control when left ventricular changes are to be studied.

Inspection of the photographs shows the similarity between left and right ventricles of normal rats and right ventricles of experimental rats. Also, since the photographs were taken with a magnification of 470, the left ventricular hypertrophy of the experimental animals can be visually spotted. However, visual inspection is not a substitute for measurements and statistical analysis. It is necessary to consider over two hundred measurements in order to determine whether the distributions are unimodal or multimodal and to determine valid central tendency data that is capable of being duplicated.

V. SUMMARY

1. The purpose of this thesis was to determine whether the rat myocardium had a consistent fiber and capillary pattern that would make it suitable for experimental work.

2. From a literature review, the histological methods of previous investigators are described and their results are tabulated.

3. The histological and statistical procedures of the present study, chosen for their accuracy and simplicity, are presented in detail.

4. Fiber diameters in six-month-old rats were analyzed according to frequency and cumulative frequency distributions. It was determined that there was a single population of fibers in each ventricle and that there were no significant differences between right and left ventricles of each rat, or between ventricles of various rats.

5. Fiber counts and capillary counts were made and found to be in direct proportion to one another. A ratio of approximately 1:1 was found.

6. Fiber counts per unit area were found to vary inversely with fiber diameter; and, therefore, provide a quick method of evaluating fiber size.

7. Six-week-old rats were studied but, due to the variations of normal growth, no definite control values were obtained. Therefore, young rats, six-weeks-old, approximately one hundred grams in weight, are considered unsuitable for experimental work involving alterations

in myocardial fiber size and capillary supply.

8. The myocardia of four rats treated with DOCA and saline for two, four, and six weeks were studied. The right ventricles showed no significant deviations from normal. The left ventricular fibers were enlarged. The fiber counts and the capillary counts per unit area decreased in direct proportion to one another, thereby retaining the 1:1 capillary-fiber ratio of the normal myocardial tissue.

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BOSTON UNIVERSITY
GRADUATE SCHOOL

Thesis
EVALUATION
OF
MYOCARDIAL FIBER SIZE AND CAPILLARY SUPPLY
IN
THE RAT
by
PAMELA R. BERNARDINI
(B.S., Boston College, 1960)

Submitted in partial fulfillment of the
requirements for the degree of
Master of Arts
1962

ABSTRACT

The purpose of this study was to determine whether the rat myocardium had a consistent fiber and capillary pattern that would make it suitable for experimental work involving hypertrophy of the cardiac muscle fibers. Histological and statistical procedures were chosen on the bases of accuracy and simplicity.

Fiber diameters in six -month-old rats were analyzed according to frequency and cumulative frequency distributions. It was determined that there was a single population of fibers in each ventricle and that there was no significant difference of fiber size between right and left ventricles of each rat or between ventricles of various rats. The central tendency data were: mean \pm S.D., 12.9 ± 4.5 micra; median, 11.7 micra; mode, 12.3 micra; coefficient of variation, 35.1 per cent. The average body weight of these rats was 526 grams. The fibers were compactly grouped within a twenty micra range, from five to twenty-five micra. The frequency distribution curves were unimodal and symmetrical.

Fiber counts per unit area were found to vary inversely with fiber diameters and therefore, provide a quick method of evaluating fiber size.

Fiber and capillary counts were made and found to be in direct proportion to one another. The average capillary-fiber ratio was 0.908 ± 0.281 with a 31.0 per cent coefficient of variation. There were no significant differences between ventricles of each rat or

between ventricles of different rats in relation to fiber counts, capillary counts, and capillary-fiber ratios.

Therefore, it is possible to use adult rats, six months old or older, in experimental research on fiber size since consistent control values may be obtained in relation to the fiber size, capillary supply, and the quantitative relationship between the two.

Young rats, six weeks old weighing approximately one hundred grams were studied. Capillary counts were reasonably consistent for right and left ventricles. However, the fiber counts differed greatly among rats and this caused wide variations in capillary-fiber ratios. The averaged data for the left ventricles were: capillary count, 274; fiber count, 334; capillary-fiber ratio, 0.861. The average data for the right ventricles were: capillary count, 298; fiber count, 514; capillary-fiber ratio, 0.647. Despite the fact that these rats were of the same breed, age, weight, and sex, these variations made it impossible to establish a consistent fiber and capillary pattern in the six-week-old rat myocardium. Since clear control values could not be obtained, six-week-old rats were considered unsuitable for experimental work involving alterations in myocardial fiber size and capillary supply.

Experimental rats, six to seven and a half months old, with an average body weight of 500 grams, were studied. These animals were treated two to six weeks with 1 mg/day/rat of a long-acting preparation of corticosterone acetate (DOCA) and given saline instead of drinking water. This treatment is known to produce hypertension and left ventricular hypertrophy. It was expected that the cardiac muscle fibers

of the left ventricle would show evidence of hypertrophy, as a result of the hypertension. Comparison with the normal adult hearts was made to determine whether this had occurred. The purpose of this comparison was to determine whether the histological and statistical procedures were sufficiently sensitive to demonstrate myocardial alterations.

As it would be expected, the experimental right ventricles were not significantly different from the ventricles of the control rats. The average data for capillary and fiber counts and for capillary-fiber ratios in the experimental right ventricles were: capillary count, 274; fiber count, 297; capillary-fiber ratio, 0.921. The average data for fiber size were: mean \pm S.D., 13.5 ± 4.5 micra; median, 12.3 micra; mode, 13.4 micra; coefficient of variation, 33.6 per cent.

The left ventricles of the experimental rats showed a definitely altered pattern. Presumably as a result of induced hypertension, the fiber sizes no longer fell in the five to twenty-five micra range. There was a definite enlargement of fiber diameters with the majority of fibers between eleven and thirty-three micra (a twenty-two micra range). The fibers enlarged but still remained in a fairly compact range.

The frequency distribution curves showed a definite shift toward the larger sizes. The curves were not unimodal nor were they symmetrical.

The central tendency data for fiber size in the left ventricles of experimental rats were: mean \pm S.D., 21.9 ± 5.7 micra; median, 21.1 micra; coefficient of variation, 26.3 per cent. The modes are

not given because the distributions were multimodal and varied for different rats.

Fiber counts and capillary counts per unit area were significantly different from those of the control rats. However, capillary counts and fiber counts per unit area decreased in direct proportion to one another so that the capillary-fiber ratio gave a mean \pm S.D., of 0.945 ± 0.284 with a 27.7 per cent coefficient of variation. This is similar to the ratio of the normal control rats.

Since the right ventricle of the experimental rats has fiber sizes and capillary-fiber ratios statistically similar to the ventricles of the control rats, the right ventricle of an experimental animal may serve as control when left ventricular changes are to be studied.