STUDIES ON CHRONIC COR PULMONALE EXPERIMENTAL INVESTIGATIONS FROM VIEWPOINTS OF DYNAMIC MORPHOLOGY

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THERE have been many clinico-pathological studies on the chronic cor pulmonale and majority of them have been investigated comparing with the functional findings in the living body and the morphologic changes in the post mortem examination.

During past twelve years, such investigations have been also in progress in our laboratory¹⁻⁶ which should be a certain means for establishing a correlation among the electrocardiographic, phonocardiographic and/or pulmonary functional findings and the patho-anatomic findings of the heart. In clinico-pathological observations in human, however, the pathological materials obliged as to base upon only a fragmental, sectional and static sample, in which no more only a phase of the pathologic process is revealed. It is very difficult to systematize the pathologic process of a disease from such a fragmental finding.

With this standpoint for backing, the present study was designed to demonstrate the systematic process of the morphologic changes in the right ventricle in accordance with the advancement of the pulmonary affection.

MATERIALS AND METHODS

Five hundred twenty-five german rabbits, u-15, were used. Four hundred forty-eight rabbits were infected with mycobacterium bo-

vinus and the remained seventy-seven animals were not infected.

1. Making the pulmonary affection

In order to make a gradually advancing tuberculous affection, four hundred forty-eight rabbits were injected with 0.002 mg/kg of Mycobacterium bovinus intravenously at four weeks after B.C.G. vaccination. For the purpose of long term observation, three hundred ninety-eight rabbits out of the all infected animals were treated with some kinds of antituberculostatics initiating on the 90th day after the infection, and the treatment was continued for three months.

2. Sacrifice of the animals

After the infection, four to six rabbits were sacrificed every week with chloroform. Their hearts and lungs, including those of the cases which died of pulmonary tuberculoses, were investigated.

3. Estimation of tuberculous affection in the lungs (Fig. 1)

The trachea cut off at one to two cm from carina, and the lungs isolated as carefully as possible from the heart and other surrounding tissues, and weighed. The correlation of the weight of the lungs and the quantitative component of the tuberculous affection in the lungs was preliminary examined as follows. The lungs were all prepared using the method of formalin steam fixation which had been reported by Weibel.⁷ The large sections were made up and the ratio of quantitative component of the affected part to whole lung capacity was calculated using the "point counting method"⁸⁻¹⁰ under the submicroscopic level (6 times).

The weight of the normal lungs was remained less than seven grammes, and it was increased in

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Fig.1. Correlation of weight of lungs and quantitative component of affected part to whole lung capacity.

parallel with tuberculous affection. Therefore, the weight of the lungs at the time of dissection was used as the indicator which represented the degree of the pulmonary affection. In this paper, the findings of the heart were compared with the weight of the lungs.

4. Macroscopic observations of the heart

The heart was fixed for one to two weeks in four percent formalin solution. Then, both atria were removed by scissors along the anteroventricular groove. The right ventricular free wall was amputated from the intraventricular septum. To separate the left ventricular free wall from the septum was so difficult to delimit, that the both fractions were treated as left side of ventricle. After the tissues on the surface of the wall, such as fat, were discarded, the weight, surface area and the thickness of the free walls were measured.

i) Weighing of the ventricular wall: For unification of the observed method and condition, some preliminary examinations were performed. The moisture excessive on the surface of

the wall at a time taking out the formalin solution was softly wiped out with gauze, and then the ventricular weight was weighed. Repeating this procedure, the value of the weight became stable (see to underlines in Tab. I). This stabilized value W was estimated as the weight of the ventricular wall.

ii) Measurement of ventricular area: The inside surface of the wall was extended on the graphic paper, cutting into suitably when the wall could not be extended all over. The edge of it was drawn and the area was determined by planimeter at the level of 10 mm².

iii) Calculation of ventricular thickness: There are some difficult problems on the determination of the thickness of rabbit's ventricular wall. For example, the wall, especially right side, is too thin to measure with the usual method, and little is known about the defining location to be most significant. If the specific gravity of the ventricular wall is 1.0 (1.02 to 1.26 by our preliminary examination), then the average value of the thickness T of the whole wall is derived from the following formula,

$$T = \frac{V}{F} = \frac{W}{F} \dots\dots\dots 1)$$

where V is the volume of the ventricular wall, and F is the area of it. In this paper, the value T was used as the indicator of the thickness.

iv) Evaluation of obtained values: The values which were obtained by means of above methods were ranged considerable wide on the weight, the area and the thickness, even if in normal rabbits (Tab. II). Therefore, these ratios were used as the indicator estimating the macroscopic changes of the ventricular wall, such as the weight ratio (weight of right ventricle/weight of left ventricle), the area ratio (surface area of extended right ventricle /left ventricle) and the thickness ratio (thickness of right ventricle/left ventricle).

5. Microscopic observations of the heart

Three hundred seventy-seven rabbits out of all were selected at random for microscopic observations. After the macroscopic observations, 3 mm width of rectangular sections were taken from each ventricular wall at two-third height on the line from apex to basis. Five micron thickness of the microscopic sections were prepared.

The method for the counting the fiber, however, had not been so well established, that the morphometric problems on it were also examined in this paper.

RESULTS

TABLE I DETERMINATION OF VENTRICULAR WEIGHT

		Number of repeated time						
		1.	2.	3.	4.	5.	6.	7.
Right ventricle	1	1.09	1.07	1.04	<u>1.02</u>	1.02	1.02	
	2	1.01	0.99	0.97	0.96	<u>0.95</u>	0.95	0.95
	3	1.09	1.08	<u>1.07</u>	1.07	1.07		
	4	1.54	1.52	<u>1.50</u>	<u>1.49</u>	1.49	1.49	
	5	1.10	1.07	1.06	<u>1.05</u>	1.05	1.05	
	6	0.86	0.83	0.80	0.79	<u>0.78</u>	0.78	0.78
	7	0.66	0.64	<u>0.63</u>	0.63	0.63		
	8	0.88	0.86	0.84	<u>0.82</u>	0.82	0.82	
	9	0.78	0.74	0.71	<u>0.70</u>	0.70	0.70	
	10	1.02	0.99	0.98	<u>0.96</u>	0.96	0.96	
Left ventricle	11	3.48	3.44	3.40	3.36	<u>3.34</u>	3.34	3.34
	12	2.89	2.84	2.80	2.79	<u>2.78</u>	2.78	2.78
	13	3.45	3.38	3.36	<u>3.34</u>	3.34	3.34	
	14	3.80	3.74	3.70	3.66	<u>3.65</u>	3.65	3.65
	15	3.22	3.18	3.15	3.10	<u>3.09</u>	3.09	3.09
	16	2.77	2.70	2.68	2.64	<u>2.63</u>	2.63	2.63
	17	2.42	2.40	<u>2.39</u>	2.39	2.39		
	18	3.06	3.01	2.97	<u>2.96</u>	2.96	2.96	
	19	2.65	2.59	2.56	<u>2.52</u>	2.52	2.52	
	20	3.93	3.87	3.84	<u>3.82</u>	3.82	3.82	

Stabilized values were shown by underline.

TABLE II MEAN VALUES OF NORMAL VENTRICLE

	Right ventricle	Left ventricle	Ratio (Right/Left)
Weight (mg)	790 ± 160	3100 ± 650	0.248 ± 0.019
Area (mm ²)	638 ± 119	885 ± 145	0.72 ± 0.06
Thickness (mm)	1.22 ± 0.11	3.45 ± 0.36	0.352 ± 0.024

1. Macroscopic findings of the ventricular wall

i) Relation of weight ratio and weight of lungs (Fig. 2): The mean values of the weight were 790 mg in right ventricle and 3100 mg in left side in normal rabbits. The value of the weight ratio was calculated 0.25 ± 0.02 . This value increased to 0.05 in proportion with the increase with the weight of lungs.

ii) Relation of area ratio and weight of lungs (Fig. 3): The mean values of the surface area in normal ventricles were 638mm² in right ventricle and 885 mm² in left side. The value of the area ratio was calculated 0.72 ± 0.06 . This ratio

was rapidly enhanced to 1.03 at the weight of the lungs increased in slight degree (10–30 g). In the moderately advanced stage of the pulmonary affection (30–75 g), the value remained in such high level without reference to increase of the weight of the lungs. In far advanced stage (lung-weight; more than 75g), the ratio was enhanced again.

iii) Relation of thickness ratio and weight of lungs (Fig. 4): The mean values of the thickness in normal ventricles were 1.2 mm in right ventricle and 3.5 mm in left side. The value of the thickness ratio was calculated 0.35 ± 0.02 . The value was firstly reduced to 0.25 with

Fig. 2. Relation of weight ratio and weight of lungs.

Fig. 4. Relation of thickness ratio and weight of lungs.

Fig. 3. Relation of area ratio and weight of lungs.

Fig. 5. Macroscopic changes of right ventricle.

increase in weight of the lungs, and then it changed contrariwise toward higher (to 0.45).

iv) Three dimensional changes of the right ventricle (Fig. 5): The left ventricle varied not or minimal (1.12 times on the weight, 1.09 times on

the surface area and 1.08 times on the thickness at the stage of far-advanced pulmonary affection). There were no statistical correlations between them. From the results, the changes of each obtained ratio may be founded on the changes of the right ventricle. To simplify the comparison, the average values of the each ratio in normal rabbits were converted into 1.0 on the

Fig.6. Morphometry of myocardial fiber from longitudinally cut fiber.

figure.

In the stage of the minimal advanced pulmonary affection (weight of lungs; from 9 to 25 g), the thickness of the right ventricular free wall was firstly decreased and the area of it was dominantly enlarged to 1.5 times than the normal rabbits, but there was no changes on the weight (= volume) of the ventricular wall. At the next stage (weight of lungs; 35 g), the area was

no more extended. In opposition, thickening of the wall was set in. On the followings (weight of lungs; from 45 to 75 g), the weight and the thickness of the right ventricular wall was increased to 1.7 times and 1.2 times than the each normal value. At the far-advanced stage (weight of the lungs; over 75g), the right ventricular wall was enlarged again and the average size of it was increased to the level which is about 1.9 times in volume, 1.5 times in surface area and 1.4 times in thickness of the normal ventricle.

2. Morphometric examination of myocardial fiber

Two different microscopic samples on the myocardial fiber, namely the transversely cut and the longitudinally cut fibers, are usually obtained. The thickness of the right ventricular wall in rabbit, however, is so thin to take enough number of the transversely cut fibers on a limited field of microscopic vision, that the method for counting the fiber on the longitudinal section must be examined preliminary. By means of

Fig.7. Sampling size of counting fiber.

TABLE III RELATION OF WIDTH OF MYOCARDIAL FIBER AND LENGTH OF NUCLEUS

Width of Fiber (u)	No. of counted	Length of nucleus (Frequency)							
		8.3	10.2	12.1	14.0	15.9	17.8	19.7	21.5 u
7-8	736 (100)	19 (3)	114 (15)	159 (22)	232 (31)	148 (20)	49 (6)	9 (2)	6 (1)
10-12	682 (100)	10 (2)	90 (13)	143 (21)	240 (35)	137 (20)	45 (6)	14 (2)	3
14-16	421 (100)	6 (2)	68 (16)	85 (20)	132 (31)	82 (20)	29 (7)	13 (3)	6 (1)

Fig.8. Comparison of values of diameter measured from longitudinally cut fiber and from transversely cut fiber.

counting the fiber, the nucleus of which cross a line drawn optionally on a microscopic field, the random sampling will be possible (Fig. 6). If the thicker fiber, in such a procedure, should have usually longer nucleus than in the thinner fiber, a possibility would exist that the former is selected more frequently for counting. Consequently, a sampling error for the population will arise. An experiment of this subject was performed.

i) Relation between width of fiber and length of nucleus (Tab. III): As to three kind of fibers, each width of which was different (7.5, 10.0 and 14.5 μ), the length of each nucleus was measured on the microscopic section. The length of the nucleus in each group ranged from 8 to 21 μ , but their sampling distribution were equal. The result indicated that the exceptional or indefinite sampling error owing to width of the fiber by taking aim at the nucleus, is hard to find.

ii) Sampling size of counting fiber (Fig. 7): The problem on the sampling size of the counting fiber was also examined. It is thought that the ventricular wall consists of innumerable myocardial fibers. The real value of the mean diameter of myocardial fibers in a ventricle was calculated from the average value of 21800 fibers, error distribution of which obtained normal curve. The correlation with each confi-

Fig.9. Relation between net-weight of ventricular wall and radius of myocardial fiber.

dence limit ($p = 0.01$) of the average value according to number of counted fibers and the real value is derived as follows,

$$\bar{x} - \left(2.5 \frac{s}{n}\right) < \bar{x} < \bar{x} + \left(2.5 \frac{s}{n}\right) \dots \dots 2)$$

wherein s is the standard deviation of the mean and n is the number of the counted fibers. The coefficient 2.5 is defined as the normal deviation of the sample, its value when p is 0.01 and for given degree of freedom ($n = 1$) can be found in Student's T-table. These relations were drawn as shown in Fig. 8. It seems that the error limit when 500 fibers are counted on a ventricle is remained less than three percent for the result ($p = 0.01$).

iii) Comparison of the values of diameter measured from longitudinally cut fiber and from transversely cut fiber (Fig. 8): It may be necessary to compare the obtained values by means of this method with the result from the other method. The counting method using the geometric probability based on the theory of Buffon's needle problem were served for this test. Rabbit's ventricular wall is so thin to take a enough number of the roundly sliced fiber on a section, that sixty-five cases of human ventricle were supplied for the subject. The result obtained from the transversely cut fiber were somewhat larger than the longitudinal one. There was, however, no remarkable difference between

them.

iv) Determining the diameter of myocardial fiber: From the above results, the morphometry was performed on the longitudinally cut fiber under the light microscopic field of 320 magnifications. The average diameter was calculated from the values of more than 500 fibers.

v) Determining the length of myocardial fiber: A structural model has been considered that the ventricular wall consists of N number of cylindric myocardial fibers. The quantitative fraction of the fiber for a whole wall (net-V) is able to be determined using a point counting method on a transversely cut section. Setting the specific gravity of the myocardial fiber is 1.0,

$$\begin{aligned} \text{net-V} &= \text{net-W} \\ &= r^2 \cdot l \cdot N \end{aligned} \quad \dots\dots\dots 3)$$

where l is length of a fiber and r is radius of a fiber. However, to determine the length of a myocardial fiber on a microscopic section is impossible in today. Suppose that the total length L of the myocardial fibers contained in a ventricle is, $L = N \cdot l$, the L is able to calculate from the cross sectional area of the fibers, such as,

$$L = \text{net-W} / S \quad \dots\dots\dots 4)$$

vi) Microscopic architecture of normal ventricle: In both ventricles, the heavier walls were consisted of thicker fibers. If the total length of the fiber in a wall is always constant, 6.48 km in a right ventricle and 20.00 km in a left side, values of which were obtained from three young rabbit, four weeks old, the correlation between the net-weight and the radius of the fiber in each ventricle was shown as in figure 9. In the right side, all cases were plotted on this theoretical curve, but the heavier cases in the left side were dotted under the curve. It suggested that the cross sectional area of the fiber was increased with growing of the rabbit, but the length of the fiber was not so markedly changed. The phenomenon in the left side is thought that so remarkable large number of the fiber consist in it that the deviation from the curve is due to the summed up value of a few elongation of each fiber.

3. Microscopic findings of ventricular wall

i) Relation between cross sectional area of myocardial fiber and weight of lungs (Fig. 10): The cross sectional area of the myocardial fiber, even if in the normal group, was ranged from 70 to $100\mu^2$ in the right ventricle and 80 to $100\mu^2$ in the left. There are considerable variations in susceptibility of individual animals. Therefore,

Fig.10. Relation between cross sectional area of fiber and weight of lungs.

Fig.11. Relation between total length of fiber and weight of lungs.

Fig.12. Morphologic changes of myocardial fiber.

the morphologic changes of the myocardial fiber were difficult to be estimated by each individual value. The ratio of the cross sectional area of the right ventricle to the left (right side / left side) was not ranged widely (0.09 ± 0.04). So that the value of the ratio was used as the indicator representing the morphologic changes of the myocardial fiber. In proportion to the increase in weight of the lungs, the value of the ratio was firstly reduced to 0.05, then it became increased contrariwise to more than 1.30 in far advanced cases.

ii) Relation between total length of fiber and weight of lungs (Fig. 11): The value of the total length of the fiber in normal ventricle ranged from 5.0 to 7.6 km in the right ventricle and 15.0 to 22.0 km in the left. The ranges for obtained values were too wide to estimate the morphologic changes of the fiber. Therefore, the ratio of the total length of the fiber in the right ventricle to the left was compared. The mean value of the normal ratio was 0.28 ± 0.02 . In the stage of minimal increased in weight of the lungs, the value was rapidly enhanced, and became unchangeable in the next stage. In far advanced stage, the value increased again.

iii) Three dimensional changes of myocardial fiber (Fig. 12) To simplify the comparison, each average value of the ratio were converted into 1.0. In the minimal advanced pulmonary affection, the cross sectional area of the myocardial

fiber in the right ventricle was drastically reduced and the length of it was rapidly elongated, but there was minimal or no change on its volume. At the moderately advanced stage (weight of lung: 35 to 75 g), the length of the myocardial fiber remained in the value, but the cross sectional area of the fiber became increased contrariwise in proportion to the advancement of the pulmonary affection. In far advanced stage (weight of lung; more than 75 g), the length of the myocardial fiber was elongated again. The size of the myocardial fiber at this stage was increased to the level which was about 1.4 times in the cross sectional area, 1.3 times in the length and 1.9 times in the volume of the normal fibers.

DISCUSSION

Making the pulmonary affection: By means of the infection with the various quantity of *Mycobacterium bovis*, the various kinds of clinical course of the experimental rabbits tuberculosis are able to prepare. Some conditions on this subject have been reported by Freerksen¹² and the others.^{13,14} In this paper, referring to their results, the rabbits were vaccinated with B.C.G. four weeks before infection. The mean survival period was enough in preparing a gradually advancement of pulmonary affection. For the purpose of more long term observation, the majority of infected rabbits were treated with some dosage of Streptomycin and INH. Under this condition, the present observation was continued for ninety-two weeks, which may be long enough for the viewpoint of "chronic".

Estimation of the pulmonary affection: A relationship was found to exist between the weight of lung and the quantitative component of the affected part for a whole lung capacity. There are, however, some essential unsolved problems on this subject. For example, how the shrinking of the volume induced by formalin-aldehyde after the fixation should be corrected, and how the cavity on the counting-procedure should be dealt? During past two years, our group has investigated on these subjects, but remains obscure in detail. Further studies into this problem are necessary.

Methodologic problems on macroscopic observations: Not only Müller's¹⁵ and Fulton's¹⁶ works, but also many studies¹⁷⁻²⁵ have been reported on the significance of the heart weight. But the conditions of the determining the heart weight in their works were discrepant, and the technique of it was scarcely to find in detail.

Soaping the formalin solution for a certain period, the weight of ventricle has reduced²⁶. After the fixation in four percent of formalin solution for a week, in our preliminary examination, the weight of rabbit ventricle was reduced to from five to fourteen percent. And the decrease of the weight, moreover, was rapid in the first or second day, and was gradual afterward. Referring to the results and the influence of rigor mortis in this paper, the ventricular weight was determined at a week after the fixation. Therefore, the obtained value may be satisfied with the reproducibility for the result.

The dilatation of the ventricular cavity has been signified the elongation of the myocardial fiber, and it has been expressed as the enlargement of the ventricular cavity. There have been three methods for objective estimation of the dilatation, namely the volumetry of ventricular cavity by *Linzbach*²⁷ the determining the diameter of the cavity by *Tanizaki*²⁸ and the measuring the surface area of the wall by *Cullen*²⁹. Their technique, however, are not always easy on the standpoint of the reproducibility, in human ventricle. But Cullen's method is easy to use on the rabbit ventricle, because the wall is thinner enough to extend, even after the fixation. The volume of the ventricular cavity was reduced to about fourteen percent on the chest film³⁰ and thirty three percent on the anatomic finding³¹ by rigor mortis. In this study, the volume of the ventricular cavity was reduced to from five to eleven percent in right ventricle and twenty-five to thirty-eight percent in the left, due to influence of the fixation and rigor mortis. Taking these phenomena into consideration, the ventricular area was determined at seven days after the dissection, when the influence of both became almost standardized.

Morphometric problems on myocardial fiber: In recent years the views of an integral geometry or a geometric probability have been introduced into the quantitative morphology by *Weibel*³²⁻³⁵ *Chalkley*³⁶ and *Suwa*³⁷. But little works^{5,38-41} have been done on the morphometry of the myocardial fiber. Two methods for counting the width of the myocardial fiber have been used. *Ishida*^{38,39} *Linzbach*⁴⁰ and *Gregg*⁴¹ calculated the average diameter of the myocardial fibers from the proportion of the muscular component to stroma on the roundly sliced fibers. *Suwa*³⁷ determined it applying the theory of Buffon's needle problem^{42,43} from the

viewpoint of the geometric probability. These methods, however, are only able to use for determining on the section, fibers in which were transversely cut. But the rabbit's ventricle is too thin to take a enough number of the transversely cut fiber on a microscopic field. Therefore, the counting method for the longitudinally cut fibers were examined in this paper. The objective sampling will be possible by means of counting fiber nucleus of which cross the line drawn at random on the microscopic field of vision. The random line can be drawn by movement of a imagined point on the micrometer. The length of the nucleus which is a target for sampling distributed equal in spite of the width of the myocardial fiber. If the enough number of counting fiber which is able to represent a ventricle were determined, the faultless sampling would be obtained. The enough number of the sampling size was derived as formula 2. From such examinations, the mean value of the diameter were calculated from more than 500 fibers, after due consideration of the variety of the sample. It is logical to believe that the observed error basing on sampling size is less than three percent for the result.

*Hort*⁴⁴ counted the length of sarcomeres (compacta) in a myocardial fiber and analysed some problems on the elongation of it. To measure the compacta of a fiber is a strenuous work. In this paper, the total length of the all fibers in a ventricle was calculated. This method may be satisfied with a theoretical view, but it is nothing but the indirect measurement for the length of the myocardial fiber.

Relation between the changes of the ventricular wall and the degree of the pulmonary affection: The findings of the ventricle, both macroscopic and microscopic, will be discussed in accordance with the advancement of the pulmonary affection. The dilatated changes of the right ventricle was appeared firstly in most initial advancement of pulmonary affection. The ventricular wall enlarged and thinned, and the myocardial fiber elongated and became slender. But the volume of them remained equal. These morphologic changes were advocated theoretically by *Moritz*⁴⁵ as a most initial change against the load. *Eyster*⁴⁶ had observed that the enlargement of the left ventricular cavity arised at third or sixth day after the operation in his experimental aortic stenosis. From the aboves the dilatation of the right ventricle observed at the initiate stage of the pulmonary affection may

be most sensitive changes of the ventricle against the pulmonic load. After that, the thickness of the ventricular wall and the width of the fiber were increased slightly. And then, the dilatable changes stopped. *Khorat*⁴⁷ *Aldinger*⁴⁸ and *Katagiri*⁴⁹ pointed out macroscopically in their works of experimentally pulmonic stenosis that the dilatation is the previous stage of the hypertrophy. It seems, this stage is the shifting phase from the dilatating changes to the hypertrophic one.

In the next stage, the right ventricular wall and the myocardial fiber were thickened with increase in its volume. But in the early stage of this period the wall and the fiber were thinner than the normal. On the course of this change, the thickness of the wall and the cross sectional area of the myocardial fiber became increased to the level which was larger than the normal. *Büchner*⁵⁰ and *Nieth*⁵¹ defined that the ventricular hypertrophy is the morbid overgrowth of the ventricular wall due to increase in size of the myocardial fiber. From their viewpoints this course is able to divide into two stage on the size of the myocardial fiber, one is the prehypertrophic stage and the other is the hypertrophic.

There are some resarches on making the ventricular hypertrophy. They reported that the hypertrophy of right ventricle appeared at one hundred days in an experimental aortic insufficiency⁴⁶ and from two to eight days⁵² or eight weeks⁵³ in their pulmonic stenosis. On the microscopic level, *Meerson*⁵⁴ indicated that the cross sectional area of the myocardial fiber increased in about ninety-five percent within six months, and *Frobose*⁵⁵ in ten to fourty percent within three weeks in aortic stenosis. These observed the hypertrophic change in experimental heart disease. There is a few experimental work on the right ventricular hypertrophy due to pulmonary disease. *Kountz*⁵⁶ reported that the hypertrophy of right ventricle could not be observed in his experimental study on pulmonary emphysema. In present paper, the size of the myocardial fiber increased to 1.3 times in its cross sectional area and 1.9 times in volume. These results appearently indicated that the hypertrophic changes of the right ventricle resulting from experimental pulmonary disease is enough to observe. After the hypertrophic stage, the myocardial fiber lengthened again and the ventricular wall enlarged more. If the ratio of a myocardial fiber to capillary is constant, as *Linzbach*,^{31,57} *Roberts*⁵⁸ and *Shipley*⁵⁹ pointed

out, the oxygen supply in such a large fiber should be dominantly decreased. It is hardly thought that the fiber could not perform a normal metabolism and keep a normal function. In this stage the hypoxic changes of the myocardial fiber, such as fat or vacuole degeneration, were observed in seventy-seven cases in this stage. It is presumed that the elongation of the myocardial fiber in the final stage is based on not only the increase in haemodynamic overload, but also the relative anoxia⁶⁰⁻⁶³ due to increase in volume of the myocardial fiber.

Summarizing these findings, the morphologic changes in the right ventricle in accordance with the advancement of the pulmonary affection may be divided into following four phases; dilatation, prehypertrophy, hypertrophy and hypertrophic insufficiency. Through out the process, dilatation might be a rapid reaction against the haemodynamic load and the thickening of the fiber is slow-active reaction for it.

The patho-anatomic criteria of the chronic cor pulmonale should be determined already at the prehypertrophic stage, when the correlation of the rapid and slow reaction of the morphologic changes in right ventricle are considered. This view is based on not the static classification, as it were hypertrophy, but the dynamic division which is classified on the process of morphologic changes in the right ventricle. Such a view may be necessary for the elucidating the pathogeny of the chronic cor pulmonale, because the status of the disease is more functional than morphologic.

CONCLUSION

The morphologic changes in the right ventricle resulting from the disease which affects the structure of the lung, — so to speak, the morphology of chronic cor pulmonale — were investigated. Changes of the ventricular wall and the myocardial fiber were observed comparing with the degree of the pulmonary affection in experimental rabbits tuberculosis. The following conclusions appear to be justified.

1. The weight of the lung represented well the degree of the quantitative component of the tuberculous affection.
2. Morphometry of the myocardial fiber on the longitudinally sliced fibers were examined. The observed error for the result was remained less than three percent ($p = 0.01$) when five hundred fibers were counted from a ventricle.
3. The width of the myocardial fiber was correlated significantly with the weight of the

ventricle in normal rabbits.

4. In the stage of minimal advanced pulmonary affection, the right ventricular wall was thinned and enlarged, and the cross sectional area of the fiber in the right ventricle was reduced and its length was increased. Then, the thickness of the right ventricle and the cross sectional area of the fiber were increased. In the far-advanced stage, the volume of the fiber was increased to the level which was about 1.9 times the normal, the then the fibers were lengthened again.

Summarizing these findings, the morphologic changes in the right ventricle in accordance with the advancement of the pulmonary affection may be divided into following four phases; "dilatation, prehypertrophy, hypertrophy and hypertrophic insufficiency".

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