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METABOLIC STUDIES OF HYPERTROPHY AND FAILING MYOCARDIUM: EVALUATION OF MITOCHONDRIAL AND SARCOPLASMIC RETICULUM FUNCTION

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Chun-Fu Peng, Marvin L. Murphy, Karl D. Straub, and James R. Phillips, (1981) Metabolic studies of hypertrophy and failing myocardium: evaluation of mitochondrial and sarcoplasmic reticulum function. *Bull. Inst. Zool., Academia Sinica* **20**(2): 49-57. An experimental model of aortic stenosis was used in the rabbit to induce myocardial hypertrophy and failure which were carefully defined. An increase of phosphofructokinase activity and normal mitochondrial function including oxidative phosphorylation and calcium uptake was observed in the hypertrophied and failing myocardium. In contrast, studies of sarcoplasmic reticulum from hypertrophied and failing myocardium showed a significant decrease in the rate of calcium uptake compared to normal. This observation of decreased movement of ionized calcium across the sarcoplasmic reticulum membrane may be related to the altered contractile state commonly associated with the hypertrophied and failing myocardium.

Information continues to evolve about metabolic changes that occur with cardiac hypertrophy. Major foci of interest have been in abnormalities of energy production, protein synthesis, calcium metabolism, and in the relationship of these abnormalities to altered contractile properties of hypertrophied, or failing hearts[6,18,34,35,41].

Reported data are variable and may be related to rapidity of the development of hypertrophy, the magnitude of hypertrophy achieved, or the nature of the stimulus to produce hemodynamic stress, i.e., pressure or volume overload. In addition, the stage of the myocardium at the time of data collection, whether hypertrophied only or hypertrophic and failing, has not been systematically evaluated; definition and standardization of the stages of hypertrophy and heart failure are difficult to achieve. For these reasons, interpretation of published data has resulted in conflicting conclusions about metabolic alterations in hypertrophy and failure.

In the past decade, a number of reports have presented evidence showing involvement of membrane systems such as mitochondria, sarcoplasmic reticulum, and sarcolemma vesicles in cardiac function and metabolism[3,6,7,9,10,15]. One of the unique properties of these membranes is the regulation of the movements of Ca^{++} which has been shown to play an important role in the myocardial contraction and relaxation process[3,9,10,15]. For example, in addition to oxidative phosphorylation, mitochondria are able to accumulate and/or release Ca^{++} under certain circumstances[2,21,23]. Similarly, sarcoplasmic reticulum is able to bind, accumulate, and release Ca^{++}[8,11,17,27]. These observations suggest that alteration of subcellular regulation of calcium movements may paly a role in the altered mechanical activity

of hypertrophied or failing myocardium.

This study utilized an experimental rabbit model of surgically induced aortic stenosis to induce a subacute form of cardiac hypertrophy. In this model it was possible to differentiate hypertrophied from failing myocardium. Metabolic studies of the myocardium were performed and the results analyzed for normal, hypertrophied, and failing hearts.

METHODS

White rabbits weighing 2–3 kg were anesthetized with 1% Nembutal (30 mg/kg). The animals were intubated and maintained on a small animal respirator at 50 respirations per minute using a tidal volume of 18–20 cm^3. Adequate oxygenation was evaluated by periodic arterial sampling. A thoracotomy was performed and an ameroid band, small enough to induce significant stenosis, was placed around the aorta according to our previous experience[26]. At ten days, due to the hygroscopic properties of the ameroid band, maximal aortic constriction can be expected from this method and results in subacute induction of stenosis. Control animals also had thoracotomy including aortic dissection, without banding, and had the procedure performed the same day as the banded animals. Hence, paired animals, one banded and one control, were followed concurrently. Animals were sacrificed at four to six weeks post banding. Rabbits were sacrificed by a blow to the head to avoid potential anesthetic effect on metabolic measurements.

At the time of sacrifice body, lung, and heart weights were recorded. Specific right and left ventricular masses were measured after dissection to separate the two chambers. Ratios for total heart weight, right and left ventricular weight, and lung weight compared to body weight were determined. Histology of lung tissue was routinely examined.

The left ventricle was minced in the cold room and homogenized in a medium containing 250 mmol•litre^{-1} sucrose, 20 mmol•litre^{-1} Tris buffer, pH 7.5 with a Polytron PT10 homogenizer. Mitochondria were isolated by a method similar to that previously described[36], and suspended in a medium containing 0.2 mmol•litre^{-1} Hepes, (N-2, hydroxyethyl piperazine-N^1-2-ethanesulfonic acid) buffer, pH 7.4 and 250 mmol•litre^{-1} sucrose after the final washing. Mitochondrial protein was determined by the biuret method[20]. Oxygen uptake was determined pholarographically with the Clark oxygen electrode[4]. The ejection of H^+ during the Ca^{2+} uptake by mitochondria was followed with a recording pH electrode as previously described[12]. Appopriate corrections were made for the buffering capacity of the medium and suspended mitochondria by addition of standardized HCl or NaOH in each experiment. Ionic calcium uptake was also followed with $^{45}Ca^{2+}$ as described previously[33]. Aliquots of the incubation mixture were withdrawn at selected time intervals after the addition of $^{45}CaCl_2$ and rapidly filtered through a millipore filter[25]. The radioactivity of $^{45}Ca^{2+}$ of each sample was determined with a Beckman liquid scintillation spectrometer, model 1650.

Sarcoplasmic reticulum vesicles from normal, hypertrophied, and failing hearts were isolated from the left ventricle by the method of Harigaya and Schwartz[14]. Ca^{2+} uptake by sarcoplasmic reticulum was determined with the use of murexide dye and dual beam spectrophotometer[14,25]. Accumulation of Ca^{2+} by sarcoplasmic reticulum was also examined by the radioisotope $^{45}Ca^{++}$ millipore filtration technique. The results obtained by the millipore filter method were consistent with those obtained by the dual beam spectrophotometric method. The possibility that the observed Ca^{++} uptake was due to mitochondrial contamination was excluded by the following evidence: (1) the isolation of sarcoplasmic reticulum was done in the presence of 5 mmol•litre^{-1} sodium azide, (2) intact rabbit heart mitochondria were incubated with $^{45}Ca^{++}$ in the presence of 5 mmol•litre^{-1} sodium oxalate, 2 mmol•litre^{-1} ATP, and 3 mg mitochondrial protein. Under such conditions there was no significant uptake of $^{45}Ca^{++}$ by mitochondria. It is thus unlikely

that Ca^{++} uptake by sarcoplasmic reticulum in this study under the described conditions could result from the mitochondrial contamination. Sarcoplasmic reticulum protein concentration was determined by the biuret method.

Phosphofructokinase activity was assayed following homogenization of 5.0 gm left ventricular myocardium and diluted ten-fold with Tris 10 mmol·litre^{-1} ethylenediaminetetraacetic acid (EDTA), 2 mmol·litre^{-1} buffer pH 8.0. A half milliliter of the initial homogenate was saved for protein determination using the method of Lowry, *et al*[22]. Homogenate was centrifuged at 4°C for 30 minutes at 700×g. The supernatant was saved for phosphofructokinase assay spectrophotometrically according to methods adapted from Mansour[23].

RESULTS

Table I shows gross pathological data for all animals entered into the study. Body weights were comparable for the control and banded animals but total heart weight, left ventricular weight, left ventricular body weight ratio, right ventricular weight, right ventricular body weight ratio, lung weight and lung weight/body weight ratio were significantly different. Eighty-eight pairs of rabbits (control plus banded) were operated. Of these, 39 banded with the concurrent control (except in two instances when the control rabbit died) were used in this report. Of the remaining, 29 banded rabbits could not be used because of premature death. The causes of death were as follows: anesthetic death 4; ruptured aorta post-banding 8; congestive heart failure 10; hemorrhage in lungs at sacrifice 2; other causes 7. The number of animals (pairs) in which a specific biochemical assay was performed is indicated in each figure.

TABLE I.
Gross pathological data from normal and banded rabbits

	Control	Banded
Number of Animals	57	59
Body Wt (kg)	3.3±0.4	3.4±0.5
Total Heart Wt (gm)	7.8±1.6*	11.8±2.7
LV Wt (gm)	4.6±0.8*	7.0±1.4
LV Body Wt Ratio (10^{-3})	1.3±0.2*	2.2±0.4
RV Wt (gm)	1.4±0.4*	2.0±0.6
RV Body Wt Ratio (10^{-3})	0.4±0.1*	0.6±0.2
Lung Wt (gm)	12.1±3.3*	16.7±6.1
Lung Wt/Body Wt Ratio (10^{-3})	3.8±1.0*	5.2±2.0

* $p<0.001$
(Wt—weight; LV—left ventricular; RV—right ventricular)

Fig. 1 shows a distribution of animals in control and banded groups according to left ventricle body weight ratio (LV/BW). It is noted that 47 out of the 57 rabbits in the control group had a LV/BW ratio of 1.1 to 1.5 times 10^{-3}. On the other hand, the majority of banded animals have a greater LV/BW ratio in the 1.6 to 3.0 times 10^{-3} range. A bell-shaped distribution curve for both control and banded animals is demonstrated. Fig. 2 shows the distribution of animals in control and banded groups according to the ratio of lung weight/body weight. A greater number of animals in the banded group have higher lung weight/body weight ratios. The hypertrophied hearts are thus defined as an increased left ventricle body weight ratio without increasing the lung weight/body weight ratio. Animals

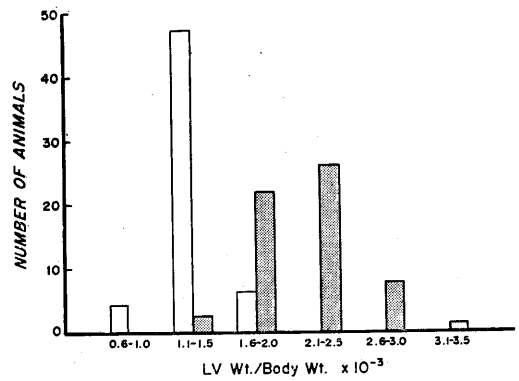

Fig. 1. The distribution of the left ventricle body weight ratio is shown. Control animals are shown in clear bars and banded animals in hatched bars. Note a bell-shaped distribution in both groups of animals.

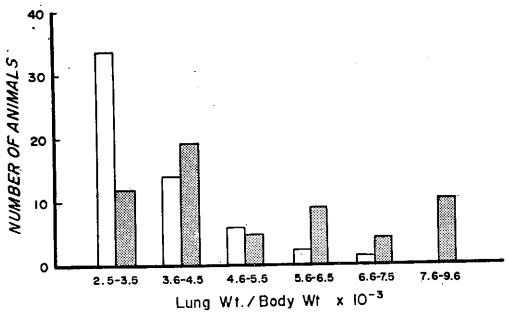

Fig. 2. The distribution of the lung weight/body weight ratio is shown. Control animals are shown in clear bars and banded animals in hatched bars. Banded animals tend to have a higher lung weight/body weight ratio.

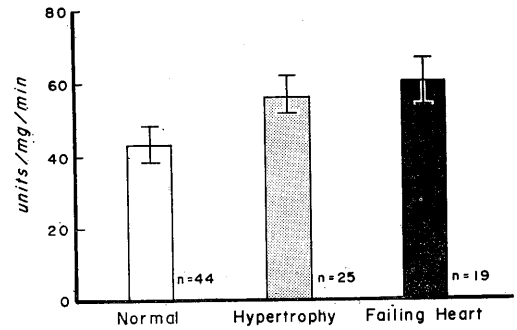

Fig. 3. Phosphofructokinase activity in units/mg protein/min is shown for the three animal groups, normal, hypertrophied, and heart failure. Activity is significantly higher in the latter two groups.

considered to have congestive heart failure were defined as those having a lung weight/body weight ratio above 4.8 times 10^{-3} which is one standard deviation above the control animal lung weight to body weight ratio of 3.8 ± 1.0 times 10^{-3} (Table I). Routine microscopic examination of the lungs for evidence of pulmonary edema was attempted. Special thin lung sections embedded in plastic were also used. Blind readings by a pathologist of lung sections prepared by both methods showed no consistent changes in either control or banded animals, and therefore microscopic evidence for pulmonary edema was not used in this study as an index of congestive heart failure.

Phosphofructokinase, the key regulatory enzyme in glycolysis and the Embden-Meyerhof pathway, was studied. Fig. 3 shows that phosphofructokinase activity in hypertrophied and failing hearts was significantly elevated compared to the normal preparations. However, no significant difference was observed in phosphofructokinase activity between hypertrophied and failing hearts. These results suggest the supply of carbon moieties for oxidative metabolism and anaerobic energy-generating reaction in hypertrophied and failing hearts was activated.

Mitochondrial function from normal, hypertrophied, and failing hearts is shown in Table II. With glutamate substrate as an example, mitochondria isolated from hypertrophied and failing hearts have a slightly increased respiratory rate compared to the control group. However, the difference was not significant. Likewise, neither ADP/0 ratios nor respiratory control ratios show a significant difference between normal, hypertrophied, and failing hearts. A similar result was obtained with malate plus pyruvate or succinate plus rotenone (data not included in Table II). When ethylenediaminetetraacetic acid, 1 mmol·litre^{-1}, was included in the asaay medium, the rate of ADP-induced state 3 respiration was unchanged in mitochondria from normal and hypertrophied hearts but decreased in mitochondria from failing hearts. This was comparable to the data obtained by others[37]. Furthermore, the inclusion of ethylenediaminetetraacetic acid results in improvement of ADP/O ratios and respiratory control ratios in all three preparations of mitochondria. This suggests that divalent cations in the absence of chelating agents may interfere with mitochondrial oxidative phosphorylation, and this interference is more notable in mitochondria from failing hearts than in mitochondria from normal and hypertrophied hearts.

Ionized calcium uptake by mitochondria was studied in the presence of succinate plus rotenone. Fig. 4 shows that the rate of Ca^{++} uptake by mitochondria from hypertrophied and failing hearts decreases slightly as judged by

TABLE II.

Mitochondrial function from normal, hypertrophied, and failing rabbit hearts: reaction mixture contained 10 mmol. litre^{-1} hepes, pH 7.4, 100 mmol. litre^{-1} KCl, 100 mmol. litre^{-1} sucrose, inorganic phosphate (NaH_2PO_4, 2 mmol. litre^{-1}), sodium glutamate (5 mmol. litre^{-1}), ADP (300 n moles), mitochondrial protein (1.0-1.25 mg/ml) in an final volume 1.7 ml at 30°C.

Substrate	Number of animals	State 3 respiration (N Atoms O/mg/min)	ADP/O	RCR
Glutamate (No EDTA)				
Normal	28	176±26	2.6±0.3	4.4±1.2
Hypertrophy	14	195±29	2.5±0.3	4.4±1.4
Failure	14	182±27	2.5±0.3	4.1±1.2
Glutamate (EDTA 1 mmol. litre^{-1})				
Normal	10	164±36	2.9±0.2	8.0±2.6
Hypertrophy	5	171±26	2.8±0.2	7.9±3.0
Failure	5	125±37*	2.9±0.3	6.2±3.2

* $p<0.05$

(ADP—adensione diphosphate; O—oxygen; RCR—respiratory control ratio)

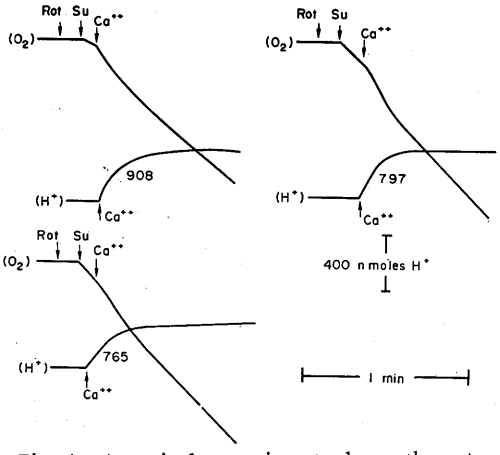

Fig. 4. A typical experiment shows the rate of Ca^{++} uptake by mitochondria from normal, hypertrophied, and failing hearts. Experimental systems contained 100 mmole•litre^{-1} sucrose, 100 mmol. litre^{-1} KCl, 1 mmol. litre^{-1} inorganic phosphate, 1 mmol. litre^{-1} Hepes buffer,and pH 7.4. Rotenone (1 μg/mg mitochondrial protein), succinate (5 mmol. litre^{-1}), mitochondria (1.0-1.25 mg/ml) and $^{45}Ca^{++}$ (400 n moles) were added as indicated in a total volume of 2 ml. Numbers adjacent to H^+ tracing are expressedas n mole H^+ ejection per minute per milligram protein. There is no signi ficant difference in the rate of Ca^{++} uptake by mitochondria among the three groups.

the rate of H^+ ejection during Ca^{++} uptake by mitochondria. A similar result was observed with the use of NADH-linked substrates, e. g., glutamate or malate plus pyruvate (data not shown). The steady state Ca^{++} accumulation was also determined with the use of $^{45}Ca^{++}$. Spontaneous Ca^{++} release was not observed in any of these mitochondria under the conditions used in this study. This was judged by the H^+ movement (H^+ uptake would have occurred if Ca^{++} release had occurred) as well as $^{45}Ca^{++}$ radioisotope measurement. Fig. 5 shows that there was not significant difference in normal, hypertrophied, and failing hearts for the steady state amount of total Ca^{++} accumulation. These results demonstrate that although state 3 respiration shows a slight decrease in failing hearts, the rest of the mitochondrial function, e. g., ADP/0 ratio, respiratory control ratio, and calcium transport are not significantly different.

Fig. 6 demonstrates a typical tracing of Ca^{++} uptake by sarcoplasmic reticulum with the use of murexide. Sarcoplasmic reticulum isolated from failing hearts shows a significant decrease in the rate of Ca^{++} accumulation compared to control. Fig. 7 summarizes the

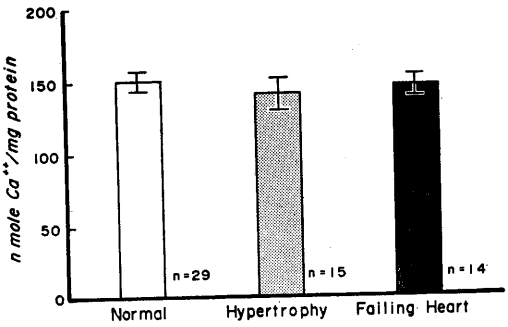

Fig. 5. The steady state amount of calcium uptake by mitochondria from normal, hypertrophied, and failing hearts is shown. The experimental conditions were the same as in Fig. 4. The reaction was termined 2 minutes after $^{45}Ca^{++}$ was added which allowed Ca^{++} accumulation to reach a steady state. The amount of Ca^{++} uptake by mitochondria among three groups is not significantly different.

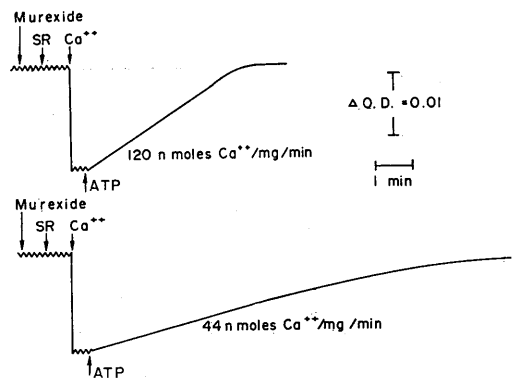

Fig. 6. An example experiment of the rate of calcium uptake in sarcoplasmic reticulum (SR) prepared from normal and failing myocardium is shown. The experimental systems contained 20 mmol. litre^{-1} Tris-meleate buffer, pH 6.9, 100 mmol. litre^{-1} KCl, 5 mmol. litre^{-1} $MgCl_2$, 3 mmol. litre^{-1} sodium oxalate, 0.3 mmol. litre^{-1} murexide, 0.9-1.0 mg protein, Ca^{++} (400 n moles) in a total volume of 3 ml. Incubation was at room temperature (23°C) and ATP (2 mmol. litre^{-1}) was added to initiate the reaction as indicated. The rate of uptake is significantly less in the failing myocardium compared to normal.

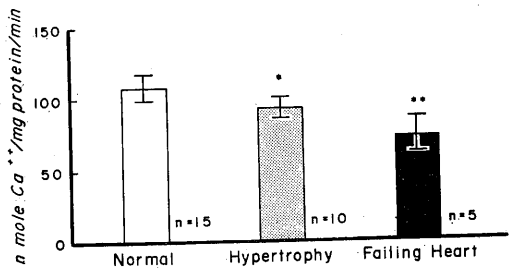

Fig. 7. This figure shows the mean of the rate of calcium uptake in sarcoplasmic reticulum prepared from 15 normal, 10 hypertrophied, and 5 failing hearts. The experimental conditions were the same as in Fig. 6. * $p<0.025$, ** $p<0.0125$ when compared to normal group.

rate of Ca^{++} accumulation by sarcoplasmic reticulum from normal, hypertrophied, and failing hearts for all experiments. There is a decreasing trend of the rate of Ca^{++} uptake by sarcoplasmic reticulum from normal to hypertrophied to failing hearts. The difference was significant between normal and hypertrophied ($p<0.025$) and between normal and failing hearts ($p<0.0125$) of the rate of Ca^{++} accumulation by sarcoplasmic reticulum.

DISCUSSION

Our results show that sarcoplasmic reticulum function as measured by calcium uptake in the presence of oxalate is significantly decreased in hypertrophied and failing myocardium. Earlier studies have shown altered calcium binding and uptake in sarcoplasmic reticulum in failing hearts[13,14,16,38]. Hence, our results confirm this abnormality in Ca^{++} uptake in hypertrophied and failing heart tissue, but in our experiments we carefully distinguished between hypertrophied and failing heart preparations. This abnormality of the sarcoplasmic reticulum Ca^{++} pump may be responsible for altered movement of Ca^{++} ion to contractile proteins during excitation-contraction coupling. Theoretically, adverse effects of systolic contractile force and diastolic relaxation may result[6] and may be a factor in heart failure.

This study also shows that phosphofructokinase activity increases in hypertrophied and failing hearts. Although many factors regulate the glycolytic flux, the increased phosphofructokinase activity may imply an increase in the glycolytic pathway in the hypertrophied and failing hearts. In addition, oxidative energy supply and Ca^{++} accumulation by mitochondria was normal in all tissue examined.

This finding of relatively normal mitochondrial function and heightened phosphofructokinase activity in hypertrophied and failing hearts suggests that alterations of energy production cannot account for the heart failure in our model. Similar observations have been reported in the failing heart from some animal models and man[5,30,31,36]. However, other reports continue to show altered oxidative phosphorylation in congestive failure[32]. Conflicting data may be in part dependent upon the stage of failure when the tissue is obtained. Terminal heart failure with severe generalized derangement of tissue may account for these observations. In addition, different experimental isolation procedures of the tissue and various compositions of medium may alter experimental data[35]. For example, as demonstrated in this study, state 3 respiration, respiratory control rate, and ADP/0 ratios are changed when 1 $mmol \cdot litre^{-1}$ ethylenediaminetetraacetic adid is present in the reaction system. It has been known for some time that ethylenediaminetetraacetic acid may improve mitochondrial oxidative phosphorylation by removing calcium or other heavy metals during preparation. However, ethylenediaminetetraacetic acid may also remove Mg^{++} from mitochondria which is required for optimal mitochondrial function. This study demonstrates, in the presence of ethylenediaminetetraacetic acid, a decrease in the rate of mitochondrial energy production in failing heart myocardium. It is likely that mitochondrial function observed in the absence of chelating agents as in this study better simulates *in vivo* conditions.

Could a compensatory increase in sarcoplasmic reticulum that might accompany hypertrophy explain our findings? Previous studies suggest a compensatory increase in sarcoplasmic reticulum with hypertrophy[19], but others have suggested that sarcoplasmic reticulum is the least affected subcellar fraction[24,28,39,40]. It is unlikely that altered Ca^{++} uptake as we observed in hypertrophy and heart failure is due to a lesser quantity of sarcoplasmic reticulum, since the capacity (maximal accumulation) of Ca^{++} uptake by sarcoplasmic reticulum is unchanged in our study as well as reported by others[37].

The problem of defining the stage of heart failure in the experimental animal objectively remains, contributing to difficulty in comparing data of published reports. Previous investigators have employed commonly used hemodynamic measurements[36], while some have measured peak isovolumic presure as a parameter of heart failure[37]. Yet others have relied on the presence of overt evidence of peripheral edema or ascites using models of right heart failure[1]. In this experiment, we have carefully defined heart failure as an increased ratio between lung weight and body weight to distinguish from hypertrophy alone. The methodology of defining the stage of heart failure in various animal models remains a potentially significant problem in data comparison and is crucial if the observations made, altered subcellular organelle function, can be related to overt global myocardial failure; i.e., is it causal or simply an accompanying defect?

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心臟肥大和功能失效心臟其新陳代謝之研究

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本報告利用一特殊圈形環套圍大白兎之心臟升主動脈，促使動物引發心臟肥大，進而惡化使心臟功能失效，再觀察心臟細胞新陳代謝之變化。結果發現磷酸果糖激酶之活性稍增加，粒腺體之一般功能包括氧化磷酸化反應以及鈣離子的傳遞作用亦稍提高。相反的，於肥大及功能失效的心臟細胞，肌質網對鈣離子的吸收速率顯着降低。肌質網膜傳遞鈣離子的速率減緩，可能與心臟肥大及心臟功能失效時，常伴隨的心肌收縮狀態改變有關聯。