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Research Article

To determine the myocardial response to prolonged pressure-loading and unloading, kittens weighing 0.8-1.2 kg underwent pulmonary artery banding, which initially elevated right ventricular (RV) systolic pressure by 10-15 mm Hg. 52 and 76 wk later; RV weight/body weight had increased by approximately 80%. Total RV hydroxyproline had increased significantly, whereas hydroxyproline concentration was unchanged from that of nonbanded animals of comparable age. In isometrically contracting RV papillary muscles, peak active force was significantly less at 76 wk (3.3 +/- 0.8 [SD] g/mm2 than at 52 wk (5.1 +/- 0.8 g/mm2) or in nonbanded animals (4.8 +/- 0.8 g/mm2). Velocity of muscle shortening at comparable loads was unchanged after 52 wk but was significantly less after 76 wk. In nonstimulated, slowly stretched muscles, passive stiffness constants, alpha and beta, derived from delta = alpha(e beta epsilon - 1), where delta is instantaneous stress and epsilon is Lagrangian strain, were unchanged by banding. The band was removed after 52 wk in additional animals that were studied 24 wk later. In those animals with normal RV pressures at death, hypertrophy had regressed and hydroxyproline concentration was comparable to that of nonbanded and banded animals; Active and passive mechanical function remained normal. In this model, changes in hydroxyproline parallel changes in muscle mass, and passive stiffness remains normal during development and regression of hypertrophy. […]

Myocardial Hydroxyproline and Mechanical Response to Prolonged Pressure Loading Followed by Unloading in the Cat

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ABSTRACT To determine the myocardial response to prolonged pressure-loading and unloading, kittens weighing 0.8–1.2 kg underwent pulmonary artery banding, which initially elevated right ventricular (RV) systolic pressure by 10–15 mmHg. 52 and 76 wk later; RV weight/body weight had increased by ~80%. Total RV hydroxyproline had increased significantly, whereas hydroxyproline concentration was unchanged from that of nonbanded animals of comparable age. In isometrically contracting RV papillary muscles, peak active force was significantly less at 76 wk (3.3±0.8 [SD] g/mm^2 than at 52 wk (5.1±0.8 g/mm^2) or in nonbanded animals (4.8±0.8 g/mm^2). Velocity of muscle shortening at comparable loads was unchanged after 52 wk but was significantly less after 76 wk. In nonstimulated, slowly stretched muscles, passive stiffness constants, α and β, derived from $\sigma = \alpha(e^{\beta\epsilon} - 1)$, where σ is instantaneous stress and ϵ is Lagrangian strain, were unchanged by banding. The band was removed after 52 wk in additional animals that were studied 24 wk later. In those animals with normal RV pressures at death, hypertrophy had regressed and hydroxyproline concentration was comparable to that of nonbanded and banded animals; Active and passive mechanical function remained normal. In this model, changes in hydroxyproline parallel changes in muscle mass, and passive stiffness remains normal during development and regression of hypertrophy. Removal of the pressure load after prolonged hypertrophy prevents or retards the late development of myocardial dysfunction.

INTRODUCTION

It is well accepted that myocardial hypertrophy may regress and depressed myocardial function improve in humans (1, 2) and experimental animals (3, 4) after removal of the hypertrophic stimulus. Unfortunately, this is not a universal occurrence and myocardial dysfunction may persist (1, 2, 5). The reasons for these therapeutic failures are unclear but myocardial fibrosis from prolonged overload often is incriminated as a major factor in the persistent dysfunction (6, 7). However, several studies have observed no increase in myocardial collagen or connective tissue concentration in patients dying with long-standing myocardial hypertrophy (8–10). An unanswered question and one that might contribute to these conflicting results regarding myocardial fibrosis is whether increased connective tissue that accompanies the hypertrophic process regresses when the stimulus for hypertrophy is removed. For example, connective tissue may increase proportionately to other myocardial constituents during hypertrophy but not regress or even continue to increase after removal of the stimulus, resulting in an increase in connective tissue concentration as muscle mass declines.

Experimental studies of the hydroxyproline response to the development and regression of hypertrophy have produced conflicting data that may be due in part to differences in methods for producing hypertrophy. Baratosova (11) et al. have demonstrated convincingly that the connective tissue response to a hypertrophic stimulus depends on the type of stimulus applied. However, other factors must contribute to these disparate results since proportionate (11–15) and disproportionate (16–19) increases in collagen have been reported in hearts in which hypertrophy was produced solely by pressure-loading. Furthermore, regression (20) as well as lack of regression (13, 21) of collagen has been observed in these hearts as hypertrophy regressed.

To provide further information in this area, we measured right and left ventricular hydroxyproline after prolonged pulmonary artery banding in cats and observed the response of this measure of connective tissue

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to removal of the band and regression of hypertrophy. We also measured right ventricular papillary muscle mechanics in these hearts to determine if any observed alterations in hydroxyproline were associated with changes in mechanical function.

METHODS

Kittens weighing between 0.8 and 1.2 kg (12–20 wk of age) were anesthetized with intraperitoneal sodium pentobarbital (35 mg/kg), the chest was opened, and a band of sufficient size to elevate right ventricular systolic pressure by 10–15 mmHg was placed around the main pulmonary artery. This reduced the cross-sectional area of the vessel by ~60%. The band was composed of silastic tubing through which passed a copper wire of selected length and a silk suture. The suture was tied to maintain band size and was attached to surrounding fibrous tissue to prevent band migration. The animals were again anesthetized 52 or 76 wk later, the hearts removed, and the thinnest right ventricular papillary muscle isolated. The muscle was placed in a myograph containing modified Kreb's solution of the following concentration (in millimoles): Na^+, 144; K^+, 4.0; Ca^{++}, 2.5; Mg^{++}, 0.5; $H_2PO_4^-$, 1.0; HCO_3^-, 25; Cl^-, 128; and glucose, 5.6—all maintained at 30°C. The solution was bubbled vigorously with 95% O_2–5% CO_2, which produced a pH of 7.4 and $Po_2 > 500$ mmHg. The nontendinous end of the muscle was held by a plastic clip attached to a short metal rod that passed through the bottom of the bath and was connected to a Statham model Gl-4-250 force transducer (Statham Instruments, Inc., Oxnard, CA). The tendinous end was secured by a short silk suture to the long arm (10:1 ratio) of a lever, which in turn was attached to a displacement transducer (model R4BS; Schaevitz Engineering, Pennsauken, NJ). The compliance of the system without muscle but including the suture was 2 μm/g and the equivalent mass of the lever system was 150 mg. Micrometers located above the lever permitted us to obtain isometric contractions (while varying muscle length by known amounts) or isotonic contractions under various afterloads (while maintaining initial muscle length constant).

The muscle was stimulated at a frequency of 12/min and initially allowed to contract isotonically under a 0.5-g preload for 45–60 min at which time extent and rate of shortening were constant. Thereafter, with initial muscle length held constant, muscle shortening was recorded after increments in afterload until the muscle contracted isometrically. Maximal velocity of shortening was determined electronically. The muscle was then again allowed to contract isotonically at a 0.5-g preload until shortening had returned to previous values. Isometric contractions were then produced and length-tension relations were recorded from a length where active force development was just apparent to a length at which force first declined from its maximum.

To normalize shortening velocity for differences in muscle length, muscle length at the initial preload was measured by means of a calibrated reticle and shortening was expressed as muscle length per second. To normalize force for differences in muscle size, muscle length (Lmax)[1] was measured at peak active force development, and cross-sectional area was determined from this length and the wet weight of the muscle, while assuming the muscle to be a cylinder with a specific gravity of 1.0.

Passive stiffness of the muscle was assessed as described by Glantz and Kernoff (22). Stimulation of the muscle was discontinued and the muscle was stretched at a constant rate of 0.2 mm/min using a constant-speed motor attached to the lever-displacement transducer complex. The muscle was stretched from zero resting-tension to the resting tension that produced peak active force determined previously. The motor was stopped at this point and muscle length was measured. Force was measured after each 0.1-mm increment in muscle length and stress-strain relations were determined using instantaneous stress (σ [force/instantaneous cross-sectional area]) and Lagrangian strain, ϵ, derived from $(l - l_0)/l_0$, where l_0 is unstressed muscle length. We determined l_0 at the point at which resting tension first deviated from the zero force-line by using the length of the muscle measured as described above and the change in length from the zero force-line recorded by the displacement transducer. As we reported previously (15), repeated measurements of unstressed length revealed a reproducibility of ±8%. From the stress-strain data, the elastic constants α and β were calculated from $\sigma = \alpha\,(e^{\beta\epsilon} - 1)$. Elastic stiffness ($d\sigma/d\epsilon$) was calculated from $d\sigma/d\epsilon = \beta\sigma + \beta\alpha$. Elastic stiffness-stress relationships for each group were determined by averaging elastic stiffness values calculated at each of four comparable stress points plus that at Lmax in individual animals of each group. The theory pertaining to and derivation of these expressions has been described by Glantz (23) and the importance of elastic stiffness as it relates to stress has been emphasized by Mirsky and Parmley (24).

The right ventricular free wall was dissected from the left ventricle plus septum and the weight of each specimen was obtained. Hydroxyproline was measured by using a modification of method "A" of Bergman and Loxley (25) as used previously in our laboratory (15). Duplicate measurements were made on each specimen with an average difference between determinations of 5%.

In additional animals banded for 52 wk, the band was removed and 24 wk later the animals were reanesthetized and right ventricular pressure was measured by direct needle puncture of the ventricle. In those animals with systolic pressures ≤ 35 mmHg (the highest right ventricular systolic pressure measured in nonbanded cats) the above studies were undertaken.

Similar studies were performed in nonbanded cats entered into the study at 12–20 wk of age and killed 52–76 wk later.

Statistical analyses were performed using analysis of variance (26).

RESULTS

Individual values for control animals, 52-wk banded, 76-wk banded, and those unbanded for 24 wk are given in Tables I–IV, respectively. Mean values for selected variables are presented in Table V. Force-velocity relationships for these groups are illustrated in Fig. 1. Right ventricular mass assessed from right ventricular/body weight ratio increased by ~80% in animals banded for 52 wk; but the active mechanical properties of this group were quite similar to their respective values in nonbanded animals. By 76 wk right ventricular mass averaged 87% above controls, a value insignificantly different from that of 52 wk-banded animals.

[1] *Abbreviation used in this paper:* Lmax, maximum muscle length.

TABLE I
Values in Control Animals

				Hydroxyproline					
				Content		Concentration			
n	RV/BW	X sec	PF	RV	LV	RV	LV	β	α
	g/kg	*mm²*	*g/mm²*	*mg*		*mg/100 g*		*g/mm²*	
1	0.51	1.3	3.7	1.5	4.2	0.8	0.5	20.8	0.06
2	0.70	1.7	4.1	1.5	4.3	0.7	0.5	10.6	0.09
3	0.65	1.3	4.8	2.3	7.9	0.9	0.7	13.4	0.08
4	0.52	1.0	6.2	2.5	3.0	1.2	0.3	13.0	0.06
5	0.51	0.9	5.4	2.9	7.6	1.3	0.7	14.1	0.15
6	0.71	1.4	3.8	3.1	6.1	1.4	0.6	13.6	0.07
7	0.61	1.1	4.9	4.7	10.7	1.7	1.0	14.6	0.05
8	0.70	1.2	5.3	3.4	10.0	1.4	0.9	18.9	0.10
9	0.54	1.4	5.0	2.6	8.9	1.4	0.9	17.4	0.03
10	0.96	1.7	4.1	5.6	8.0	1.7	0.7	12.6	0.05
11	0.82	1.8	4.4	4.7	7.4	1.7	0.6	25.9	0.02
12	0.70	1.6	4.7	4.1	9.0	1.5	0.7	18.2	0.09
Mean ± 1 SD	0.68	1.4	4.8	3.2	7.3	1.3	0.7	16.1	0.07
	0.14	0.3	0.8	1.3	2.4	0.4	0.2	4.3	±0.03

RV, right ventricle; LV, left ventricle; BW, body weight; X sec, cross-sectional area; PF, peak developed force; β and α, passive elastic constants.

However, peak force and velocity of shortening at any given load were significantly less than their respective values in either nonbanded or 52-wk banded animals. Left ventricular/body weight ratio was unchanged in both banded groups (Table V).

In six of eight unbanded animals, right ventricular systolic pressure was <35 mmHg at the time of death, whereas in the remaining two unbanded animals, systolic pressure was 40 and 45 mmHg. Data from the latter two animals was not included because resolution

TABLE II
Values in Animals Banded for 52 Wk

				Hydroxyproline					
				Content		Concentration			
n	RV/BW	X sec	PF	RV	LV	RV	LV	β	α
	g/kg	*mm²*	*g/mm²*	*mg*		*mg/100 g*		*g/mm²*	
13	1.41	1.0	5.7	5.4	5.2	1.1	0.6	15.6	0.15
14	1.45	1.0	5.5	2.8	4.4	1.0	0.5	9.2	0.13
15	0.94	1.5	—	3.4	4.2	0.9	0.4	10.1	0.10
16	1.73	—	—	7.4	5.3	1.2	0.6	—	—
17	1.38	0.9	4.9	3.5	6.6	1.0	0.6	16.2	0.03
18	0.70	1.4	4.9	4.3	4.9	1.4	0.9	11.7	0.08
19	1.17	1.8	3.7	5.6	4.2	1.6	1.1	19.1	0.02
20	0.61	1.5	5.6	3.7	5.6	1.6	0.9	13.9	0.16
Mean ± 1 SD	1.2*	1.3	5.1	4.5	5.1	1.2	0.7	13.7	0.10
	0.4	0.3	0.8	1.5	0.8	0.3	0.2	3.6	0.06

For abbreviations see Table I.
* Values significantly different from control ($P < 0.05$).

TABLE III
Values in Animals Banded for 76 wk

				Hydroxyproline					
				Content		Concentration			
n	RV/BW	X sec	PF	RV	LV	RV	LV	β	α
	g/kg	mm^2	g/mm^2	*mg*		*mg/100 g*		g/mm^2	
21	1.13	1.8	4.2	7.6	4.3	1.6	0.9	22.8	0.04
22	1.10	1.9	2.8	5.0	5.4	1.3	0.9	19.7	0.08
23	1.24	—	—	4.7	6.7	1.4	1.1	—	—
24	1.21	1.0	3.4	4.8	2.4	1.7	0.8	15.1	0.03
25	1.14	1.0	2.0	2.1	2.1	1.3	1.0	13.9	0.05
26	1.90	—	—	14.6	10.5	1.9	1.0	—	—
27	0.93	1.6	3.7	10.9	15.9	2.0	1.1	17.4	0.08
28	1.37	1.4	3.4	5.5	12.4	0.9	1.1	25.9	0.02
29	0.82	—	—	5.6	4.0	1.5	0.8	—	—
Mean ± 1 SD	1.2*	1.4	3.3*	6.8	7.1	1.5	1.0	19.1	0.05
	0.3	0.4	0.8	3.8	4.8	0.3	0.1	4.6	0.02

For abbreviations see Table I.
* Values significantly different from control ($P < 0.05$).

of the elevated pressure was incomplete and complete regression of hypertrophy had not occurred. In the remaining six animals right ventricular systolic pressure fell immediately after unbanding from an average of 50±5 to 38±4 mmHg, with a further decline to an average of 28±4 mmHg at the time of death. Right ventricular hypertrophy regressed with the right ventricular/body weight ratio insignificantly different from that of nonbanded animals.

Banding resulted in an increase in total right ventricular hydroxyproline, but because of the small number of animals in each group and the wide range of hydroxyproline values, none of the differences among groups are significant statistically. However, combining data in the banded cats resulted in a highly significant difference in total hydroxyproline from that of nonbanded animals ($P < 0.01$). Left ventricular hydroxyproline values also varied widely but insignificantly

TABLE IV
Values in Animals 24 wk after Unbanding

				Hydroxyproline					
				Content		Concentration			
n	RV/BW	X sec	PF	RV	LV	RV	LV	β	α
	g/kg	mm^2	g/mm^2	*mg*		*mg/100 g*		g/mm^2	
30	0.80	0.9	5.0	4.8	6.2	1.3	0.5	10.2	0.15
31	0.84	1.9	4.0	9.8	22.7	1.5	1.1	13.6	0.04
32	0.90	1.3	4.6	3.4	7.1	1.4	0.8	12.5	0.05
33	0.90	1.5	3.7	3.7	7.0	1.2	0.8	6.1	0.07
34	0.80	1.0	4.6	5.3	13.5	1.4	1.0	23.0	0.02
35	0.51	0.7	5.1	2.9	8.9	1.3	0.9	20.3	0.11
Mean ± 1 SD	0.79	1.2	4.6	5.0	10.9	1.4	0.9	14.3	0.08
	0.15	0.4	0.6	2.5	6.4	0.1	0.2	6.3	0.05

For abbreviations see Table I.

TABLE V

Mean±1 SD Values for Body Weight (BW) Left Ventricular Weight (LV) and Right Ventricular Systolic Pressure (RV PR) in Various Groups

	n	BW	LV/BW	RV PR
		kg	*g/kg*	*mmHg*
Controls	12	2.8 ±0.3	2.1 ±0.4	25 ±4
52-wk banded	8	2.6 ±0.4	2.5 ±0.5	48* ±7
76-wk banded	9	3.0 ±0.6	2.4 ±0.4	53* ±9
24-wk unbanded	6	3.0 ±1.0	2.4 ±0.5	28 ±4

* Values significantly different from controls ($P < 0.05$).

among groups and combining values in banded animals did not result in a significant difference from that of nonbanded animals. As reported by Buccino et al. (16) right ventricular hydroxyproline concentration exceeded that of the left ventricle in nonbanded animals. However, no significant change in hydroxyproline concentration in the right or left ventricle occurred after banding or unbanding. Neither β or α elastic constants varied significantly among groups or between banded and nonbanded groups.

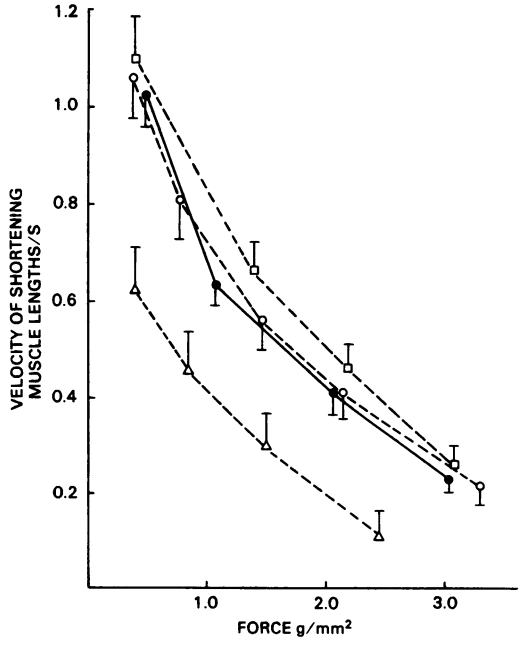

FIGURE 1 Force-velocity relations in the nonbanded (●), 52-wk banded (○), 76-wk banded (△), and unbanded (□) groups. Values represent mean±1 SD.

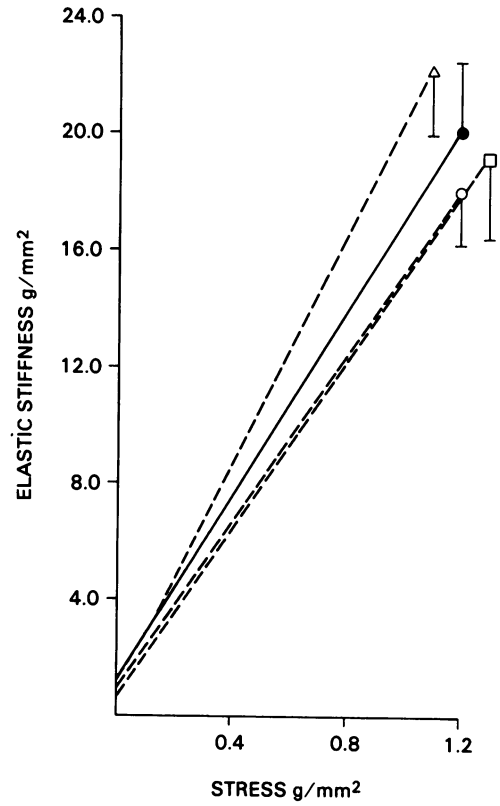

FIGURE 2 Elastic stiffness-stress relations in nonbanded (●), 52-wk banded (○), 76-wk banded (△), and unbanded (□) groups. Symbols represent mean values±1 SD at Lmax.

Elastic stiffness-stress relations are illustrated in Fig. 2. This relationship in 52-wk banded and unbanded animals were quite similar to that of nonbanded animals. In contrast, stiffness in the 76-wk banded group became increasingly greater than that of the other groups as stress increased, although statistically the slope is not significantly different from that of the other groups. However, at Lmax, elastic stiffness was significantly greater ($P < 0.05$) in the 76-wk banded group than in the other groups.

DISCUSSION

The major finding of this study is that hydroxyproline, a measure of collagen, increases proportionately to muscle mass as pressure-induced hypertrophy develops and decreases proportionately as hypertrophy recedes. These findings are at variance with several experimental studies involving mechanically induced concentric hypertrophy in which collagen concentration was in-

creased (16–19) but are similar to those from other laboratories (11, 12, 14, 27) including our own (15).

The reason we suggest for these different results is conjectural but we believe the manner in which the load is applied is a major factor. Most studies demonstrating increased collagen concentration in mechanically induced concentric hypertrophy used ascending aortic banding (17, 18) or severe pulmonary artery constriction of adult animals (16) in which the increase in afterload is marked and abrupt. Those studies in which collagen concentration was normal used abdominal aortic constriction (11, 12, 14, 27) in which pressure rises over several days (28) or, as in our previous study, mild banding of growing animals (15) in which the load also develops relatively slowly. Cutilleta et al. (21) who reported increased collagen concentration and lack of regression of collagen after regression of hypertrophy also used ascending aortic constriction.

The importance of loading conditions on the connective tissue response is apparent from the study of Bishop and Melsen (29), who observed myocardial necrosis and fibrosis in adult cats after marked pulmonary artery constriction, but not in cats with congenital pulmonary valve stenosis. Thus, we believe that with less severe initial loading conditions and more slowly developing hypertrophy, collagen increases only in a manner sufficient to maintain a normal relationship to the muscle mass. Furthermore, when hypertrophy in such hearts regresses so does the collagen formed during hypertrophy. In contrast, if the load is of such severity as to produce myocardial necrosis, irreversible connective tissue replacement occurs.

An exception to the above is the report of Cooper et al. (19) that right ventricular hydroxyproline concentration increased in kittens with slowly developing pulmonary artery constriction. Their kittens were 7–8 wk of age at the time of banding, which is younger than the growing animals used in previous studies using slowly developing hypertrophy, and it may be a factor in the differing results.

Studies in other forms of slowly developing concentric hypertrophy, the Goldblatt kidney model and the spontaneously hypertensive rat, also have produced widely disparate results with myocardial collagen concentration reported to increase (27), decrease (14), or to remain unchanged (13, 20). Regression of hypertrophy in these models has been associated with increased (13) and normal collagen concentration (20). As emphasized by others (30, 31), factors in addition to increased afterload may affect the myocardial changes that occur in these models and comparison to other models of pressure-induced hypertrophy may be inappropriate.

It has been proposed that the connective tissue matrix of the heart is an important determinant of passive stiffness (32). If this hypothesis is correct, our findings combined with those of others indicate that it is disproportionate and not proportionate changes in collagen and muscle mass that alter stiffness. We found normal passive elastic constants in hearts with increased total hydroxyproline but normal hydroxyproline concentrations; whereas stiffness is increased in hearts in which hydroxyproline concentration is elevated (18). Furthermore, our results suggest that increased passive stiffness, which has been observed in pressure-induced hypertrophied ventricles (33) i.e., chamber stiffness, is not due invariably to alterations in intrinsic muscle stiffness, a conclusion reached previously by others (34). Our results also underscore the importance of the elastic stiffness-stress relationship in defining the "operational" stiffness of muscle as emphasized by Mirsky and Parmley (24). Elastic stiffness increased more rapidly with increasing stress in our 76-wk banded animals than in the other groups and at Lmax the difference was statistically significant. Thus, whereas little difference in stiffness would occur among these hearts at low stress levels, a striking difference would occur if the muscles were operating near the peak of their length-tension curves.

Previously we reported that right ventricular papillary muscle function declined briefly and then returned to normal in kittens studied at various periods up to 52 wk after pulmonary artery banding in a manner similar to that used in this study (15). In the present study, myocardial dysfunction was apparent after 76 wk of banding. In terms of Meerson's (35) three stages in experimental pressure-induced hypertrophy, our model is characterized initially by a short period of myocardial "damage" followed by a prolonged period of "stable hyperfunction" with the stage of "exhaustion" occurring between 52 and 76 wk after banding. The cause of this late decline in function is unclear but excessive proliferation of fibrous tissue is not a major factor, since collagen concentration was not significantly increased in our 76-wk banded animals. Furthermore, our studies demonstrate that removal of the pressure load late during the stage of "stable hyperfunction" will prevent or retard the stage of "exhaustion" and that collagen formation during the former stage does not become a self-perpetuating process. However, data from the 76-wk banded group must be interpreted with caution. Statistically, right ventricular hydroxyproline concentration, the β elastic constant, and the slope of the elastic stiffness-stress relationship in these banded animals were larger than in the other groups but the differences are insignificant statistically. We do not yet know whether this trend toward increased hydroxyproline concentration and passive stiffness will become significant with longer periods of banding. Most importantly we do not know whether the response to unbanding differs at this stage.

TABLE VI
Mean Values±1 SD in Muscles ≤ 1.5 mm²

					Hydroxyproline					
					Content		Concentration			
	n	RV/BW	X sec	PF	RV	LV	RV	LV	β	α
		g/kg	*mm²*	*g/mm²*	*mg*		*mg/100 g*		*g/mm²*	
Controls	8	0.59	1.2	4.9	2.9	7.3	1.3	0.7	15.7	0.08
		±0.09	±0.2	±0.8	±0.9	±2.7	±0.3	±0.2	±2.9	±0.04
52 wk	4	1.16	1.1	5.3	4.0	5.3	1.1	0.6	13.1	0.10
		±0.33	±0.2	±0.4	±1.1	±0.9	±0.2	±0.2	±3.3	±0.05
76 wk	3	1.24	1.1	2.9	4.1	5.6	1.3	1.0	18.3	0.03
		±0.12	±0.2	±0.8	±1.8	±5.9	±0.4	±0.2	±6.6	±0.02
Unbanded	4	0.75	1.0	4.8	4.1	8.9	1.4	0.8	16.5	0.08
		±0.17	±0.3	±0.3	±1.1	±3.2	±0.1	±0.2	±6.1	±0.06

For abbreviations see Table I.

Several other factors must be considered in the interpretation of our data. The cross-sectional area of the papillary muscles was larger than optimum and an undetermined degree of central hypoxia may have been present. We arbitrarily excluded muscles larger than 2.0 mm², which required us to discard the mechanical data from several banded animals. Had we limited this study to muscles 1.0 mm² or less we would have data from a total of only 10 animals. However, comparison of data from muscles ≤ 1.5 mm² (Table VI) yielded results similar to that using all data. We have assumed that larger than optimum muscles of comparable cross-sectional area have comparable degrees of core hypoxia.

One end of the papillary muscle is "damaged" and the extent to which such changes may affect mechanical function is unknown. Again, we have assumed that the extent of damage among muscles was comparable.

Hydroxyproline was not measured in papillary muscles from which the mechanical data was derived, thereby preventing a definitive conclusion regarding the relationship between changes in hydroxyproline and function. However, in our previous study, hydroxyproline concentration in both normal and hypertrophied papillary muscles was consistently less than that in the right ventricular free wall but the relationship of papillary muscle to free wall concentration was similar in hypertrophied and nonhypertrophied hearts (15).

We have equated changes in hydroxyproline to changes in collagen since this amino acid is found almost exclusively in collagen. However, the fraction of collagen composed of hydroxyproline may vary according to the type of collagen (36). We cannot exclude the possibility that collagen type changed during development or regression of hypertrophy but if so, this was not sufficient to alter passive stiffness.

Our conclusions regarding hydroxyproline apply only to myocardial hypertrophy produced by mechanical pressure loading, for it has been demonstrated that myocardial collagen responds differently to a variety of hypertrophic stimuli (11). Nevertheless, we have demonstrated that in this model changes in collagen are proportional to changes in muscle mass, both active and passive mechanical properties are preserved for long periods and when myocardial dysfunction does occur, it is not the result of excessive proliferation of fibrous tissue.

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