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Role of Oxidative Stress in Transition of Hypertrophy to Heart Failure

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Objectives. **In an attempt to define the role of increased oxidative stress in the transition from compensatory hypertrophy to heart failure, this study examined the effects of long-term vitamin E therapy on the occurrence of heart failure subsequent to chronic pressure overload in guinea pigs.**

Background. **Hyperfunctional heart hypertrophy has been shown to be accompanied by an increase in the endogenous antioxidant reserve, whereas congestive heart failure is accompanied by a decrease in this reserve. The effects of vitamin E, a naturally occurring antioxidant, on the development of heart failure from a hypertrophic stage were examined.**

Methods. **The ascending aorta in guinea pigs was coarcted. For vitamin treatment, slow-release pellets were implanted at the time of the operation. The animals were assessed at 10 and 20 weeks for hemodynamic function, myocardial structure, antioxidant agents and oxidative stress.**

Results. **Banding of the ascending aorta in guinea pigs resulted in hyperfunctional hypertrophy at 10 weeks, which was followed by congestive heart failure at 20 weeks. Hypertrophied hearts showed decreased oxidative stress, as evidenced by a higher oxidation-reduction (redox) state and less lipid peroxidation, whereas the failure stage was characterized by increased oxidative stress. Supplementation of animals with timed-release vitamin E tablets resulted in an increased myocardial content of the vitamin, and the banded animals did not develop any signs of heart failure at 20 weeks. Hemodynamic function at 20 weeks in these vitamin E–treated animals was also better maintained. The myocardial reduced glutathione/oxidized glutathione ratio of vitamin E–treated animals at 20 weeks was higher and lipid peroxidation was less compared with the untreated animals. Ultrastructural abnormalities were significantly less in the vitamin E–treated hearts compared with the untreated failing hearts at 20 weeks.**

Conclusions. **An improved myocardial redox state with vitamin E therapy, coupled with the modulation of the development of heart failure, may indicate a pathophysiologic role for increased oxidative stress in the pathogenesis of heart failure. This study suggests the potential therapeutic value of long-term antioxidant treatment in modulating or preventing the pathogenesis of heart failure.**

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Congestive heart failure has been defined in many different ways, and many of these definitions imply it to be "the pathophysiological state in which an abnormality of cardiac function is responsible for failure of the heart to pump blood at a rate commensurate with the demands of the metabolizing tissues" (1). Apparently, congestive heart failure is a common end point of many abnormal cardiac conditions, including hypertrophy. When compensatory mechanisms that become activated during heart hypertrophy fail, congestive heart failure is manifested, and this is associated with a grave prognosis. Heart failure subsequent to cardiac hypertrophy remains a major clinical problem, and the progression of a well-compensated hypertrophied heart to that of a decompensated stage is poorly understood.

It has been shown that an increase in oxidative stress due to an increase in free radicals or a relative deficit in the endogenous antioxidant reserve, or both, can cause contractile dysfunction (2–4), and this has been suggested as one of the contributing factors in the transition of compensated heart hypertrophy to the decompensated stage (5). In this regard, hyperfunctional heart hypertrophy due to increased cardiac work load is associated with increased endogenous antioxidant enzyme activities (6–8). These hypertrophied hearts have also been found to be less vulnerable to ex vivo as well as in vivo oxidative stress conditions induced by free radicals, hypoxia-reoxygenation, ischemia-reperfusion injury and doxorubicin-induced cardiotoxicity (6,7,9,10). In the hypertrophied hearts, reduced oxidative stress was indicated by an increase in the ratio of reduced glutathione to oxidized glutathione as well as a decrease in lipid peroxidation (7,8). In contrast, heart failure due to a variety of conditions has been shown to be associated with increased oxidative stress, as indicated by reduced antioxidants, a depressed oxidation-reduction (redox) state and increased lipid peroxidation (7–11). Increased pulmonary pentane excretion in heart failure patients (12,13) is also viewed as

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an index of free radical injury and lipid peroxidation (13). Although these data from animal as well as patients experiments suggest a role for increased oxidative stress in the pathogenesis of heart failure, a cause and effect relation remains to be established.

Vitamin E is a lipid-soluble, naturally occurring antioxidant that helps stabilize biologic membranes by interrupting free radical chain reactions, as well as by reducing lipid peroxidation (14). It has been shown to reduce free radical–mediated injury in in vivo conditions (15,16), decrease platelet adhesiveness (17) and limit infarct size (18). Furthermore, animals put on a vitamin E–deficient diet were more prone to catecholamine- as well as adriamycin-induced cardiomyopathies, and both of these conditions are known to be mediated by free radical action (19,20). In the present study, the effects of long-term antioxidant therapy involving vitamin E on the occurrence of heart failure subsequent to a chronic pressure overload in guinea pigs were examined to define the role of oxidative stress in the transition process from compensated hypertrophy to heart failure.

Methods

Procedure. Male guinea pigs (25 ± 3 days old) weighing 250 ± 25 g were used in the study. Pressure overload on the heart was imposed by surgically narrowing the ascending aorta, which has been shown to result in hypertrophy that progresses into heart failure (21). Briefly, the animals were anesthetized with a single intraperitoneal injection of methohexital sodium (35 mg/kg) and placed on positive-pressure ventilation. Thoracotomy was performed between the second and third intercostal space. The ascending aorta was cleared from the adhering tissues. A steel wire (16-gauge steel needle) was placed along the aorta, and a 3-0 silk thread was gently tied around the wire as well as the aorta just above the coronary ostia. The wire was then withdrawn, the chest was closed and an antibiotic powder (Cicatrin, Burroughs Wellcome, Canada) was applied topically. The surgical procedure was carried out under sterile conditions. The postoperative mortality rate was <10%. Sham-operated animals were treated in an identical manner, except that no band was placed around the aorta. The procedure resulted in a marginally constricting band with a cross-sectional area (including the lumen as well as the aortic tissue) of 3.6 ± 0.02 mm^2.

Hemodynamic and other measurements. The animals were anesthetized with an intraperitoneal injection of methohexital (35 mg/kg), and a miniature-tipped pressure transducer catheter (model PR 249, Millar Instruments) was inserted into the right carotid artery and then advanced into the left ventricle. Left ventricular peak systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and aortic pressure were recorded on a precalibrated multichannel dyanograph (Beckman Instruments). Steady state readings were taken 15 min after catheterization. After hemodynamic assessment, the animals were killed and their hearts were removed for further analysis. To obtain the ventricular weight, the atria and other adhering tissues were removed. Left ventricular wall thickness was measured in heart slices midway between the apex and the base with an ocular micrometer at four random but diametric points. Wall thickness was also measured in paraffin sections from perfusion fixed hearts containing procainamide to ensure relaxed state fixation of the muscle (22). The results from two procedures were not different.

Vitamin E supplementation. Animals in both the sham as well as the banded groups were further divided in two subgroups. One subgroup in each category received vitamin E implants and the other did not. Guinea pigs were implanted with subcutaneous, timed-release vitamin E (synthetic alpha-tocopherol) pellets (Innovative Research of America). Each pellet was composed of vitamin E, in lactose, cholesterol and methyl cellulose matrix, designed to release a uniform dose of ~6 mg (6 IU) of the vitamin per day. The pellets were placed in the posterior aspect of the neck at the time of thoracic surgery. Animals were then randomly picked at 10 weeks for various studies, and the remainder were used at 20 weeks.

Vitamin E analysis. Hearts were freeze clamped and stored in liquid nitrogen until analyzed by the high performance liquid chromatography method described by Lang et al. (23). Heart tissue was weighed and homogenized in 1 ml of 0.1 mol/liter aqueous sodium dodecyl sulfate and 2 ml of water. The sample was transferred to a 10-ml test tube and fitted with a Teflon-lined screw cap. The homogenizer was rinsed with 2 ml of reagent alcohol, which was combined with the homogenate. The mixture was vortexed for 30 s and 2 ml of hexane was added. The tubes were capped and vortexed for 2 min and then centrifuged for 5 min at 1,000*g* to separate the layers. One milliliter of the hexane layer was transferred to a small vial and dried by nitrogen. The residue was redissolved in 0.5 ml of reagent alcohol. The samples were analyzed by high performance liquid chromatography immediately after preparation. Different components of the Beckman Gold high performance liquid chromatography system used included a model 116 programmable solvent delivery system, a model 166 programmable detector module, an Altex injector system, and a Brownlee Silica Spheri-5 (25 cm × 4.6 mm) column. The mobile phase consisted of 1:9 (vol/vol) methanol/reagent alcohol containing 20 mmol/liter of lithium perchlorate. The solvent flow rate was 1.0 ml/min and the injected volume was 20 μl. Using an ultraviolet detector at 275 nm, the area under the peak was recorded and compared with an external standard of alpha-tocopherol (Sigma).

Antioxidant enzymes and lipid peroxidation assays. Superoxide dismutase activity in the hearts was determined by the method previously described by Marklund (24). The hearts were homogenized (1:10) in 50 mmol/liter of Tris-HCl, pH 8.20, containing 1 mmol/liter of diethylenetriamine pentaacetic acid. The homogenate was centrifuged at 20,000*g* for 20 min. Superoxide dismutase activity in the supernatant was determined by following the inhibition of pyrogallol autooxidation. The assay is highly reproducible, and the standard curve was linear up to 250 μg of protein with a correlation coefficient of 0.998 (8). Glutathione peroxidase (GSHPx) activity was deter-

Table 1. Ventricle and Body Weight Changes in Guinea Pigs at 10 and 20 Weeks of Aortic Banding, With and Without Vitamin E Treatment

Animal Group	VW (g)	BW (g)	VW/BW × 1,000	LVWT (mm)
10-wk group				
Sham	1.92 ± 0.06	760 ± 14	2.52 ± 0.04	4.5 ± 0.2
Banded	2.57 ± 0.13*	751 ± 16	3.42 ± 0.05*	6.1 ± 0.3*
Sham+VE	1.96 ± 0.11	769 ± 15	2.55 ± 0.07	4.4 ± 0.2
Banded+VE	2.55 ± 0.13*	734 ± 19	3.47 ± 0.05*	6.1 ± 0.2*
20-wk group				
Sham	2.34 ± 0.12	928 ± 26	2.52 ± 0.05	4.7 ± 0.2
Banded	3.63 ± 0.14*†	854 ± 31	4.25 ± 0.06*†	5.3 ± 0.1*†
Sham+VE	2.25 ± 0.13	935 ± 29	2.40 ± 0.06	4.6 ± 0.4
Banded+VE	3.87 ± 0.17*	895 ± 23	4.32 ± 0.09*	6.0 ± 0.3‡*

*$p < 0.05$ versus corresponding sham control animal. †$p < 0.05$ versus corresponding value in 10-week group. ‡$p < 0.05$ versus untreated control animal in same group. Data are expressed as mean value ± SE for 8 to 10 animals. BW = body weight; LVWT = left ventricular wall thickness; VE = vitamin E; VW = ventricular weight.

mined in whole heart by a method previously described by Paglia and Valentine (25). The hearts were homogenized in (1:10) 75 mmol/liter of phosphate buffer, pH 7.0. The homogenate was centrifuged at 20,000*g* for 25 min and the supernatant was aspirated and assayed for total cytosolic GSHPx activity (8). For the study of catalase activity, the hearts were homogenized in (1:10) 50 mmol/liter of potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 40,000*g* for 30 min. The activity in the supernatant was determined as described by Clairborne (26). The lipid peroxide content in hearts was studied by determining the thiobarbituric acid reactive substances as described previously (8).

Glutathione assay. The concentration of total glutathione (reduced glutathione [GSH] plus oxidized glutathione [GSSG]) was measured in the myocardium by the glutathione reductase/5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) recycling assay (27). The rate of DTNB formation is followed at 412 nm and is proportional to the sum of GSH and GSSG present. Myocardial tissue was homogenized in 5% sulfosalicylic acid. The tissue homogenate was centrifuged for 10 min at 10,000*g*. The supernatant was stored at 4°C until assayed. GSSG alone was measured by treating the sulfosalicylic acid supernatant with 2-vinylpyridine and triethanolamine. The solution was vigorously mixed and the final pH of the solution was determined to be between 6 and 7. After 60 min, the derivatized samples were assayed as described previously in the DTNB–GSSG reductase recycling assay. GSH values were calculated as the difference between total (GSSG plus GSH) and GSSG concentrations. Values are expressed as nanograms per mg of tissue weight.

Ultrastructure. Four hearts from each group were perfusion fixed for 15 min and processed for electron microscopic examination using the techniques previously described (22). Briefly, midmyocardial pieces (4 to 6 mm in size) were obtained from four different areas of the left ventricular free wall between the midregion and the apex of the heart. These prefixed tissue pieces were immersed in 0.1 mol/liter phosphate buffer, pH 7.4, containing 3% glutaraldehyde and were further cut into cubes <1 mm in size. The aldehyde fixation was continued for additional 2 h. The tissues were washed for 1 h in the previously described phosphate buffer containing 0.05 mol/liter of sucrose. Postfixation was performed in 2% OsO_4 for 2 h, followed by a routine procedure for embedding in Epon. Ultrathin sections, placed on Formvar-coated grids, were stained with uranyl acetate and lead citrate and were examined by electron microscopy.

Protein and statistical analysis. Proteins were determined by the method of Lowry et al. (28). Data are expressed as the mean value ± SEM. Individual means were compared using the unpaired Student *t* test. One-way analysis of variance was performed to determine differences between groups, and the Bonferroni post-hoc test was performed to compare individual means. Values of $p < 0.05$ were considered significant.

Results

Assessment of hypertrophy. The animals were examined for body weight, ventricle weight and left ventricular wall thickness 10 and 20 weeks after the surgical procedure (Table 1). Ventricle weight in both banded groups was significantly higher than their respective control animals. Ventricle to body weight ratio increased by 36% at 10 weeks postoperatively and by 68% at 20 weeks. Left ventricular wall thickness was increased by 35% at 10 weeks, although this difference between sham and banded animals declined to 13% at 20 weeks. There were no clinical signs of congestive heart failure in the banded animals at 10 weeks. Vitamin E treatment did not have significant effect on these variables in the 10-week group. However, in the 20-week banded group, left ventricular wall thickness in the vitamin E–treated group was significantly higher than in the untreated banded group.

At 20 weeks, untreated banded animals showed evidence of dyspnea, an enlarged abdomen and cyanosis of the limbs and ear pinnae. These animals were also torpid and, at death, showed hydrothorax, ascites and congested liver and lungs. The mortality rate at 20 weeks in the banded group was 55%.

Table 2. Effects of Vitamin E Treatment on Hemodynamic Function in Guinea Pigs 10 and 20 Weeks After Operation

	AoP (mm Hg)		LVP (mm Hg)	
Animal Group	Syst	Diast	Peak Syst	End Diast
10-wk group				
Sham	76.4 ± 3.2	58.3 ± 2.1	77.5 ± 2.2	5.2 ± 1.1
Banded	94.6 ± 2.1*	75.6 ± 2.4*	138.4 ± 1.4	5.7 ± 1.4
Sham+VE	74.4 ± 2.6	60.4 ± 3.3	75.9 ± 3.2	5.4 ± 2.3
Band+VE	91.8 ± 4*	64.6 ± 2.4	137.6 ± 4.4*	6.3 ± 1.2
20-wk group				
Sham	76.7 ± 3.4	54.3 ± 1.2	80.4 ± 2.1	5.4 ± 0.4
Banded	64.5 ± 2.2*†	51.1 ± 2.3†	68.5 ± 2.2*†	11.2 ± 2.1*†
Sham+VE	78.4 ± 3.1	59.4 ± 4.3	80.4 ± 2.1	5.4 ± 0.2
Band+VE	85.2 ± 2.1‡	66.2 ± 4.2	131.7 ± 9.1‡*	6.1 ± 1.1‡

*$p < 0.05$ versus corresponding sham control animal. †$p < 0.05$ versus corresponding value in 10-week group. ‡$p < 0.05$ versus untreated control animal in same group. Data are expressed as mean value ± SE for six to eight experiments. AoP = aortic pressure; Diast = Diastolic; LVP = left ventricular pressure; Syst = systolic; VE = vitamin E.

There was significantly less mortality (31%) in the banded animals treated with vitamin E, and the survivors of this group at 20 weeks showed no clinical signs of heart failure. Thus, dyspnea and cyanosis of the extremities were not observed and, at death at 20 weeks, no edema was seen in the chest or abdomen of the vitamin E–treated animals.

Hemodynamic assessment. Animals were examined for left ventricular function (LVSP, LVEDP) and aortic pressures at 10 and 20 weeks after the operation (Table 2). At 10 weeks, LVSP increased significantly. At 20 weeks of banding, LVSP decreased significantly, and as a result there was a very small pressure gradient from the ventricle to the aorta. Although no change was observed in LVEDP at 10 weeks, the pressure was increased significantly at 20 weeks. Both systolic and diastolic aortic pressures were significantly higher in the 10-week group. In the 20-week banded animals, systolic blood pressure was significantly lower than that in sham control animals, and there was no difference in the diastolic blood pressure values. Hemodynamic function was not influenced significantly in vitamin E–treated banded animals at 10 weeks. However, in the 20-week vitamin E–treated group, LVSP, LVEDP and blood pressure readings were maintained, and these values were comparable to those of the untreated 10-week banded group.

Oxidative stress. *Antioxidant enzymes.* In an earlier study of this experimental model, it was reported that superoxide dismutase (SOD) and GSHPx activities increased at 10 weeks in the banded animals (8). These findings were confirmed in the present study. In the untreated banded animals, myocardial SOD and GSHPx activities were significantly less in the 20-week group than in the 10-week group (Table 3). Catalase activity did not change in any of the groups. Vitamin E treatment did not have significant effect on antioxidant enzyme activities in either the 10- or 20-week banded group (Table 3). Thus, SOD and GSHPx activities within the 10- and 20-week groups with or without vitamin E treatment were not different from each other.

Vitamin E content. The vitamin E content in the hearts was measured by quantitating the alpha-tocopherol content by high performance liquid chromatography. The myocardial vitamin E content was not different in untreated sham control and banded animals at 10 and 20 weeks after the operation (Table 4). Supplementation of vitamin E resulted in more than a twofold increase in the 10-week sham group and a fivefold increase in the 20-week sham group (Table 4). The vitamin E content was also increased in the 10- and 20-week banded groups, but the gain was much less as compared with that seen in the sham groups.

Table 3. Myocardial Antioxidant Enzyme Activities at 10 and 20 Weeks of Banding of Ascending Aorta in Guinea Pigs With and Without Vitamin E Treatment

	10 Weeks		20 Weeks	
Antioxidant Enzyme	−VE	+VE	−VE	+VE
SOD (U/mg protein)	18.0 ± 3.1	16.2 ± 2.2	11.0 ± 2.1*	10.1 ± 4.2
GSHPx (nmol/mg protein)	58.3 ± 3.3	53.1 ± 4.1	40.9 ± 5.3*	35.6 ± 3.1*
Catalase (U/mg protein)	44.9 ± 2.4	40.5 ± 4.3	39.9 ± 3.1	35.2 ± 4.3

*Significantly different ($p < 0.05$) from the corresponding value in the 10-week group. Data are expressed as mean value ± SE for five to six experiments. GSHPx = glutathione peroxidase; SOD = superoxide dismutase; −VE = without vitamin E; +VE = with vitamin E.

Table 4. Myocardial Alpha-Tocopherol, Glutathione and Thiobarbituric Acid Reactive Substances at 10 and 20 Weeks of Banding of Ascending Aorta in Guinea Pigs With and Without Vitamin E Treatment

Myocardial Content	No. of Expts	10 Weeks				20 Weeks			
		Without VE		With VE		Without VE		With VE	
		Sham	Banded	Sham	Banded	Sham	Banded	Sham	Banded
Alpha-tocopherol (μmol/g wet wt)	5–6	0.38 ± 0.01	0.43 ± 0.05	1.04 ± 0.1*	0.78 ± 0.02*†	0.37 ± 0.01	0.46 ± 0.06	2.08 ± 0.11*‡	0.99 ± 0.04*†§
GSH (ng/mg wet wt)	5–8	218 ± 10	354 ± 14§	280 ± 8*	350 ± 7§	358 ± 15‡	267 ± 10‡§	349 ± 10	321 ± 13*
GSSG (ng/mg wet wt)	6–8	55 ± 6	42 ± 5	38 ± 7	42 ± 4	62 ± 10	116 ± 8‡§	51 ± 4	58 ± 5*
GSH/GSSG ratio		4	8.4§	7.4*	8.5	5.8	2.3‡§	6.9	5.5*
TBARS (nmol/g wet wt)	6–7	81 ± 12	61 ± 5§	68 ± 6	58 ± 7	81 ± 8	82 ± 9‡	65 ± 5	67 ± 4*

*$p < 0.05$ versus untreated control animal in the same group. †$p < 0.05$ versus corresponding vitamin E (VE)-treated sham control animal. ‡$p < 0.05$ versus corresponding value in 10-week group. §$p < 0.05$ versus corresponding sham control animal. Data are expressed as mean value ± SE for number of experiments (Expts). GSH = reduced glutathione; GSSG = oxidized glutathione; TBARS = thiobarbituric acid reactive substances.

Redox state. There was a significant age-dependent increase in the GSH content in the 20-week sham control animals. GSH activity increased by 62% in the 10-week banded group and decreased by 25% in the 20-week banded group as compared with their respective sham control animals (Table 4). The GSSG content at 10 weeks was not significantly different in banded and control animals (Table 4). However, an increase >100% in the GSSG content was seen in the 20-week banded group as compared with the control animals. The redox ratio (GSH/GSSG) was significantly increased in the 10-week banded group and was decreased in the 20-week banded group as compared with their respective sham control animals (Table 4). Vitamin E treatment increased the GSH content in the 10-week sham treated group, whereas the 10-week banded treated group showed no change. In the 20-week banded treated group (Table 4), GSH was higher as compared with respective untreated control animals. The GSSG content was decreased significantly in the 20-week banded vitamin E–treated animals as compared with the 20-week untreated group (Table 4). Vitamin E treatment significantly improved the redox ratio in the 20-week banded group (Table 4).

Lipid peroxidation. Thiobarbituric acid reactive substances (TBARS) were 25% less in the 10-week banded group than in its sham control group (Table 4). There was no difference in the myocardial TBARS content between the 20-week control group and the banded group. However, TBARS in the 20-week banded group were significantly greater than in the 10-week banded hearts. Vitamin E treatment in banded animals significantly reduced lipid peroxidation at 20 weeks as compared with the untreated group (Table 4).

Ultrastructure. Sham control hearts showed normal ultrastructure, including compact myocytes, myofibrils having the typical appearance of A and I bands, T tubules and a sarcoplasmic reticulum system (Fig. 1). Mitochondria in the control hearts were generally normal with a moderately dense matrix. The Golgi membranes were seen in the polar regions of the nucleus. There were no signs of intracellular or interstitial edema. The ultrastructural details of myocytes in 10-week banded hearts appeared normal and were not different from

Figure 1. Electron micrograph of a control guinea pig heart demonstrating normal ultrastructure: sarcomeres (S), T tubules (**arrowheads**) and mitochondria (M). **Bar** = 1 μm.

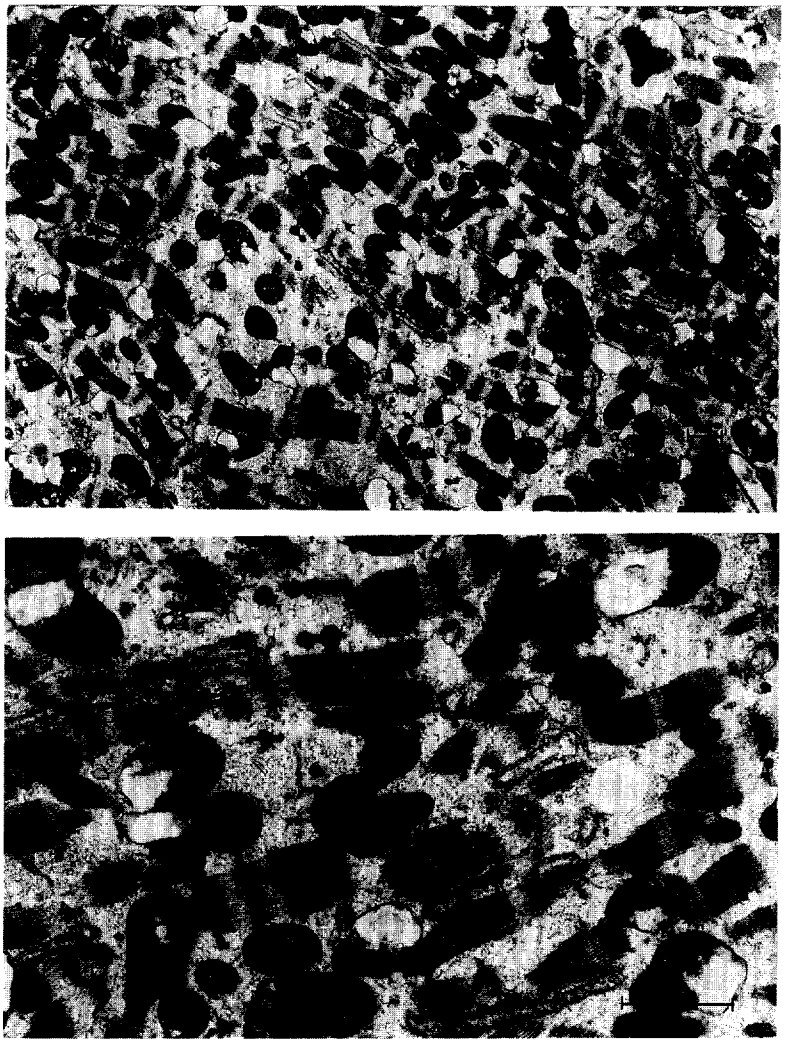

Figure 2. Electron micrographs from a 20-week failing guinea pig heart. **Top,** Ultrastructural injury is characterized by the loss of myofibrils, mitochondrial damage, intracellular edema and loss of a typical sarcomere arrangement. **Bottom,** Vacuolization as well as mitochondrial damage in greater detail. **Bars** = 1 μm.

the sham control hearts. However, some deposition of collagen fibers around the blood vessels as well as the interstitial spaces was observed. Hearts from the 20-week banded group did not show a normal ultrastructure owing to increased vacuolization as well as mitochondrial injury (Fig. 2A). Loss of contractile elements, as well as intracellular edema, was apparent. At a higher magnification, mitochondrial injury was characterized by a dropout of cristae membranes (Fig. 2B). Treatment with vitamin E improved the ultrastructure of cardiomyocytes in the 20-week banded group (Fig. 3). Vacuolization, mitochondrial injury as well as intracellular edema appeared to be less in the 20-week banded group treated with vitamin E (Fig. 3). Although complete protection of the ultrastructure was not seen with vitamin E treatment, there was an obvious reduction in the injury as compared with the 20-week banded untreated hearts.

Discussion

Compensated hypertrophy and heart failure. Several factors have been suggested to play a role in the transition from compensated heart hypertrophy to a decompensated stage, leading to the clinical symptoms of congestive heart failure. These include abnormalities in excitation-contraction coupling, calcium handling, contractile proteins, sympathetic support mechanisms and diastolic function (29,30). The exact cause of heart failure still remains elusive and may actually be a result of a combination of many factors. The present study provides evidence, for the first time, that vitamin E therapy delays the development of heart failure associated with the condition of increased oxidative stress. Furthermore, the beneficial effect afforded by vitamin E may be attributable to its antioxidant properties, as was suggested by both an improved redox state and reduced lipid peroxidation. Although vitamin E treatment improved the hemodynamic function as well as the survival rate, there was still a significant degree of hypertrophy and mortality in this group. There is no immediate and definitive explanation for the mortality in the vitamin E–treated group. Sudden death in a sustained chronic hypertrophy condition remains a possibility. Patients with hypertrophic cardiomyopathy show a higher incidence of sudden death, although the range of clinical spectrum in these individuals

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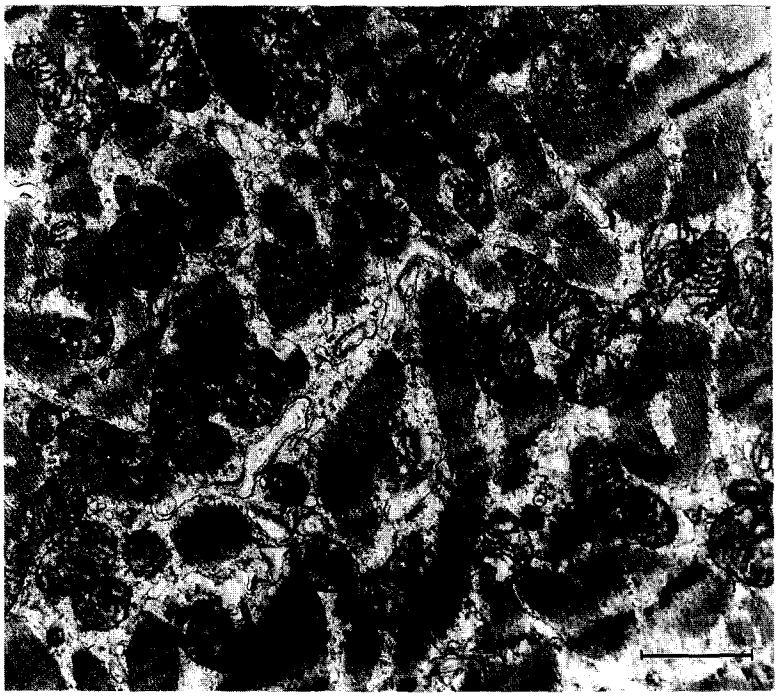

Figure 3. Electron micrograph from a 20-week banded, vitamin E–treated guinea pig heart. Vacuolization as well as mitochondrial damage is significantly less. Contractile elements as well as general cellular organization were better maintained in this group. **Bar** = 1 μm.

varies and may include the asymptomatic form to sudden death as an initial presentation (31).

In the present study, a chronic model of gradually increasing pressure overload was used to induce heart hypertrophy that progresses into failure (21). Hyperfunctional hypertrophy without heart failure at 10 weeks in this study was indicated by a symmetric increase in left ventricular wall thickness as well as increases in ventricle to body weight ratio, aortic pressure and LVSP with no change in LVEDP. Although total ventricle weight increased further at 20 weeks, left ventricular wall thickness was decreased. Thus, thinning of the left ventricular wall at 20 weeks suggests dilation or remodeling of the heart at this stage. Congestive heart failure in the 20-week banded group was indicated by left ventricular dilation, depressed LVSP, increased LVEDP, ascites and dyspnea. Depressed myocardial function, thinning of the left ventricular wall and ultrastructural changes in the myocardium, taken together, provide a strong indication of myocardial abnormalities. In any case, the stages of compensated hypertrophy at 10 weeks and congestive heart failure at 20 weeks in the banded groups were clearly established. An increase in endogenous antioxidant enzyme activities in the hypertrophied hearts, with a concomitant decrease in lipid peroxidation and an increase in the redox ratio, suggests that heart hypertrophy at 10 weeks was accompanied by a reduction in oxidative stress, although the opposite was true in the failing hearts at 20 weeks.

Oxidative stress and myocardial dysfunction. Despite the lack of difference in the myocardial vitamin E levels in the 10- and 20-week untreated banded groups, an increase in the oxidative stress in the 20-week banded group was suggested by several other observations made in this study. These included a significant decrease in the antioxidant enzyme activities, a decrease in the GSH/GSSG ratio and an increase in TBARS during heart failure in the 20-week banded group as compared with the nonfailing 10-week banded group. Although these observations suggest that increased oxidative stress and thus failure in the 20-week banded group were not due to vitamin E deficiency per se, vitamin E supplementation modulated these abnormalities.

The suggestion that a relative increase in oxidative stress was contributing to the pathogenesis of heart failure was supported by the fact that banded animals supplemented with vitamin E maintained better hemodynamic function at 20 weeks. Vitamin E is a naturally occurring antioxidant that helps stabilize biologic membranes (14). Polyunsaturated fatty acids in the membranes are highly susceptible to oxidation by endogenously produced free radicals. Vitamin E, because of its lipid solubility, is available in the lipid phase and is very effective in offering protection (14). The exact mechanism of vitamin E protection is unknown. However, vitamin E is considered to react directly with partially reduced forms of oxygen, yielding vitamin E radical and lipid hydroperoxides, which can be removed by phospholipase–GSHPx systems (32). This not only interrupts the radical chain reaction that propagates the peroxidation of membranes, but it may also have a sparing effect on the GSHPx system, thereby improving the GSH/GSSG ratio seen in the 20-week vitamin E–treated heart. Vitamin E has also been shown to be an effective antioxidant against free radical–induced injury in in vivo conditions (15,16,20). In contrast, animals put on a vitamin E–deficient diet were reported to be more prone to catecholamine- as well as adriamycin-induced cardiomyopathies, and both of these conditions are known to be mediated by free radical action

(19,20). In animals with vitamin E deficiency, myopathy of the heart muscle is frequently seen (33).

As a cosubstrate of glutathione peroxidase, GSH plays an essential role against oxygen free radicals and prevents the peroxidation of membrane lipids (34). This protective mechanism results in an increased rate of the intracellular, oxidized glutathione formation. The changes in glutathione status provide important information on the cellular oxidative events, tissue accumulation and release of GSSG, and this status has been used as a sensitive index of oxidative stress (2,34–36). An increased redox state seen in the 10-week banded group suggests less oxidative stress at this stage. However, during the heart failure stage, the redox state was decreased and the change was mainly due to increased GSSG accumulation. This suggests increased oxidative stress in the 20-week failing group. Vitamin E treatment significantly improved the redox ratio in the failing animals. By interrupting lipid radical chain reactions, vitamin E reduces lipid peroxides as well as the formation of GSSG. In this regard, a significant reduction in GSSG levels in vitamin E–treated hearts at 20 weeks was seen in this study.

Normally, endogenous antioxidants help protect the cell against free radical injury. However, these protective mechanisms may be compromised during the conditions of increased oxidative stress (2,5,36). Increased oxidative stress during the failure stage in the present study was indicated by a decrease in the GSH/GSSG ratio as well as by depressed activities of GSHPx and SOD. In this regard, increased production of free radicals has been shown in mitochondria isolated from hypertrophied as well as failing hearts (11,37). Lipid peroxidation in cardiomyopathic hearts was also reported to increase in hamsters (11).

Vitamin E and heart failure in patients. It has been shown that patients with ischemic heart disease have higher plasma lipid peroxide concentrations than control subjects (38). An increase in the breath pentane content in patients with chronic heart failure (13) as well as coronary artery disease suggests increased lipid peroxidation (12,39). Breath pentane was significantly lowered in chronic heart failure patients maintained on free radical scavengers (13). Inhibition of peroxidation by vitamin E might favorably influence the balance between peroxidative damage and the repair mechanisms of the body. Two separate studies provide evidence for an association between a high intake of vitamin E and a reduced risk of coronary artery disease in both men and women (40,41). Increased lipid peroxidation, as well as higher levels of pentane in exhaled air, has also been correlated with reduced levels of vitamin E (12,13). In a recent study, administration of different antioxidants, including vitamin E, in patients with acute myocardial infarction within 24 h after the onset of symptoms, was reported to improve several cardiac end points, including cardiac death (42). These data from humans provide a strong indication for the beneficial role of vitamin E as an antioxidant.

Because vitamin E treatment did not affect other myocardial enzymatic antioxidants in the present study, the beneficial effect is most likely the result of a direct antioxidant effect. Relatively less accumulation of vitamin E in the banded animals, at both 10 and 20 weeks, as compared with the sham control animals, may be a reflection of either altered metabolism or an increase in the utilization of vitamin E in these hearts. The latter possibility may be more plausible in the failing hearts that had reduced enzymatic antioxidant reserve.

Conclusions. A decrease in antioxidants and an increase in oxidative stress during the failure stage, as well as a delay in the pathogenesis of heart failure by vitamin E treatment, together indicate that heart failure may be the result of a mismatch between oxygen radical production and the available antioxidant reserve in the heart. The study suggests a potential beneficial role of antioxidant therapy in modulating the pathogenesis of heart failure.

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