# Electrolysis-Induced Myocardial Dysfunction
## A Novel Method for the Study of Free Radical Mediated Tissue Injury

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Oxygen-derived free radicals and other oxidizing species are thought to be involved in inflammation and ischemic tissue injuries. Recently, oxygen-derived free radicals also have been implicated in tissue injury of the myocardium subjected to ischemia/reperfusion. The purpose of this investigation was to determine if electrolysis of a physiological buffer would serve as a source of free radicals, and if these radicals would lead to alterations in myocardial function. Isolated Langendorff-perfused rabbit hearts perfused with buffer subjected to a 20 mA D.C. current for 2 min demonstrated significant increases in coronary perfusion pressure (37 ± 6 mmHg), left ventricular end diastolic pressure (41 ± 7 mmHg), and loss in left ventricular developed pressure (35 ± 5%). The free radical scavengers, superoxide dismutase and a combination of tryptophan plus glycine, were effective in protecting the hearts from the effects of electrolysis. The presence of free radicals was semiquantitated with a radical–luminol chemiluminescent assay. In this assay a variety of radical scavengers and antioxidants were effective (i.e., dimethyl sulfoxide, nitro blue tetrazolium, ascorbate, superoxide dismutase, 1, 3-diphenyisobenzofuran, and glycine, catalase), whereas mannitol and tryptophan were not effective. The data indicate that electrolysis of a physiological buffer produces a milieu containing several reactive oxygen species or free radicals that have the potential to produce alterations in a biological system. This method has the advantage over existing protocols for the generation of radicals in that it is a blood-free and an enzyme-free system.

**Key Words:** Electrolysis; Free radicals; Myocardial dysfunction; Radical scavengers

## INTRODUCTION

Oxygen-derived free radicals have been proposed as mediators of tissue injury in a variety of disease states. Temporary interruption of blood flow to heart muscle results in damage that has been presumed to occur during the period of ischemia and is thought to be due to a depletion of adenosine triphosphate (ATP). Another

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possibility is that the injurious effects actually occur during reperfusion and are due to free radical generation (Fridovich, 1979; Meerson et al., 1982). Oxygen metabolites such as superoxide anion radical ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$), and hydrogen peroxide ($H_2O_2$) have been implicated as mediators of the injury sustained by myocardial tissue subjected to ischemia and reperfusion (Rao and Mueller, 1983; Jolly et al., 1984; Werns et al. 1985). Reactive oxygen radicals also have been shown to be possible mediators in catecholamine-induced cardiomyopathy (Singal et al., 1982).

There is a paucity of data demonstrating the direct effects of free radicals on myocardial tissue. One approach has been the introduction of a $\cdot O_2^-$ radical-generating system (purine + xanthine oxidase) into a physiological buffer perfusing a rabbit septal preparation (Burton et al., 1984). This $\cdot O_2^-$ radical-generating system produced ultrastructural alterations but no significant functional alterations. Superoxide dismutase (SOD; $\cdot O_2^-$ selective scavenger) was effective in preventing the ultrastructural changes, thus implicating the involvement of $\cdot O_2^-$ in these changes. In the same study, a $\cdot OH$ radical-generating system ($Fe^{3+}$-transferrin + purine + xanthine oxidase) produced alterations in both ultrastructure and function of the myocardium; SOD was effective in this system as well. Hess and coworkers (1984) demonstrated that a $\cdot O_2^-$ radical-generating system (xanthine + xanthine oxidase) caused isolated myocardial sarcoplasmic reticulum to lose its ability to sequester calcium. Superoxide dismutase, but not mannitol ($\cdot OH$ scavenger), was effective in preventing the $\cdot O_2^-$ radical-mediated alterations in calcium transport.

McCord and Fridovich (1969) designed an experiment that involved the electrolytic generation of $\cdot O_2^-$ radical. Electrolysis was performed in an aprotic solvent to ensure a more stable $\cdot O_2^-$ radical that was capable of reducing ferricytochrome-C. Recently, Lamb and Webb (1984) demonstrated that electrical field stimulation of isolated rat tail and dog coronary arteries inhibited contractile responses to norepinephrine and potassium. Several radical scavengers were effective in reversing the loss of contractile responses, thus implicating a possible role of oxygen-free radicals in the alteration of vascular reactivity.

The primary goal of the present investigation was to design and characterize a new and unique blood-free system for the generation of free radicals. Evidence will be presented that demonstrates the electrolytic production of free radicals in a physiological buffer, and which shows that radicals formed under such conditions can cause alterations in the coronary circulation and myocardial function of a rabbit, isolated, Langendorff-perfused heart preparation.

## MATERIALS AND METHODS

Male New Zealand White rabbits (1.0–1.5 kg) were used in the present investigation. Hearts were removed quickly from rabbits stunned by a blow to the head and bled via the carotid arteries. The hearts were immediately attached to a modified Langendorff heart perfusion apparatus via the aorta and perfused in a retrograde manner. Perfusion was maintained by a Masterflex peristaltic pump at a constant flow of 25 ml/min. The perfusion medium was a modified Kreb's–Henseleit buffer

of the following composition (mM): NaCl (118); KCl (4); $MgCl_2 \cdot 6H_2O$ (1.2); $NaHCO_3$ (25); $KH_2PO_4$ (1.1); $CaCl_2 \cdot 2H_2O$ (2.4); glucose (5); and *l*-glutamic acid (5) prepared in double-distilled deionized water. The buffer was maintained at a pH of 7.4, constant temperature of 31°C (±0.5°), and continuously oxgenated (95% $O_2$, 5% $CO_2$). Hearts were electrically paced with bipolar platinum electrodes attached to the left atria (150 impulses/min, 5 ms duration at 2× threshold voltage) using a Grass SD-5C stimulator.

### Physiological Measurements

Aortic perfusion pressure (PP) was measured with a pressure transducer (Statham P-23 ID) attached to a side arm of the aortic cannula. The pulmonary artery was cannulated, and the effluent was collected for determination of coronary flow. Isovolumic left ventricular pressure (LVP) measurements were made with a saline-filled latex balloon connected to a pressure transducer inserted into the left ventricle by way of the left atrium. Perfusate that entered the left ventricle was drained via a vent in the apex of the heart. The first derivative of LVP, ± dP/dT, was electronically derived and recorded. The epicardial electrogram (EPI–ECG) was also recorded with electrodes positioned above the aorta and at the apex of the heart. All physiological measurements were recorded on a Grass polygraph (Model 79D) and simultaneously stored on magnetic tape. A typical recording of the performance of an isolated rabbit heart perfused under the conditions just described is illustrated in Figure 1. There were no significant alterations in coronary PP, LVP, ± dP/dT, or EPI–ECG over the course of a 2-hr perfusion.

Left ventricular function was assessed by generating Starling function curves. These curves were generated by inflating the intraventricular balloon in 0.1-ml increments and measuring the LVP and left ventricular end diastolic pressure (LVEDP) over the range of LVEDPs from 0 to 35 mmHg.

### Electrolysis of the Physiological Buffer

Electrolysis was performed by placing platinum wire electrodes into the inflow tract just above the heart (inset, Figure 2). The anode was 14.5 cm above the heart, and the cathode was 17.5 cm away. A constant 20 mA DC current generated by a Grass stimulator (SD-5C) and a Grass Constant Current Unit (CCU-1A) was applied to the physiological buffer for 2 min. A glass bubble trap (4.5 ml capacity) designed to prevent gas bubbles from entering the heart was placed above the aorta.

### Experimental Protocol

The hearts were allowed to equilibrate for 45 min at a LVEDP of 10–15 mmHg after isolation and instrumentation. Preelectrolysis measurements of left ventricular function were made by varying the LVEDP from 0 to 35 mmHg (as described above). The LVEDP was returned to 10 mmHg, and the hearts were allowed to stabilize for 10 min. The hearts were then perfused with Kreb's buffer subjected to a constant current of 20 mA for 2 min. The current was turned off, and the hearts were perfused with normal buffer for 30 min. This treatment represented the control electrolysis group. A second group of hearts was subjected to treatment with the radical scav-

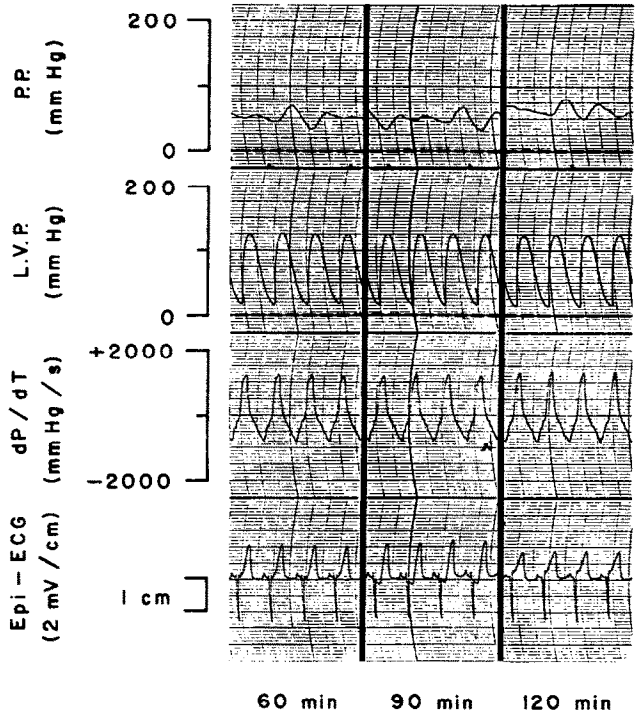



**FIGURE 1. A typical tracing of the function of a 2-hr control heart demonstrating no significant change in PP, LVP, dP/dT, or EPI–ECG is shown. The dotted lines denote baselines for each channel.**

engers, SOD, 100 U/ml, catalase (CAT, 150 u/ml), or trytophan + glycine (TRP + GLY, 5 mM each). The scavengers were added to the perfusate 10 min before electrolysis and were present throughout the electrolysis and the postelectrolysis phases of the experiment.

### Detection of Free Radicals

Determination of the radical species generated by the electrolysis of a physiologic buffer solution was carried out in conjunction with Dr. Rao utilizing a modified luminol assay for radical detection (Merenyi and Lund, 1980; Misra and Squatrita, 1982). Luminol ($LH_2$) reacts with free radicals (·R) in the following manner:

$$LH_2 + \cdot R \rightarrow \cdot LH$$

$$\cdot LH + \cdot O_2^- \rightarrow LOO^- \text{ (endoperoxide)}$$

$$LOO^- \text{—dissociates} \rightarrow L_2 + AP^* \text{ (aminophthalate dianion) (active state)}$$

$$AP^{*2} \text{—decomposes} \rightarrow AP^2 \text{ (inactive)} + h\nu \text{ (light)}.$$

The pH optimum for the reaction is 9.8–12.0. The ability of the luminol assay to detect radicals was standardized with an enzymatic system in which the generation

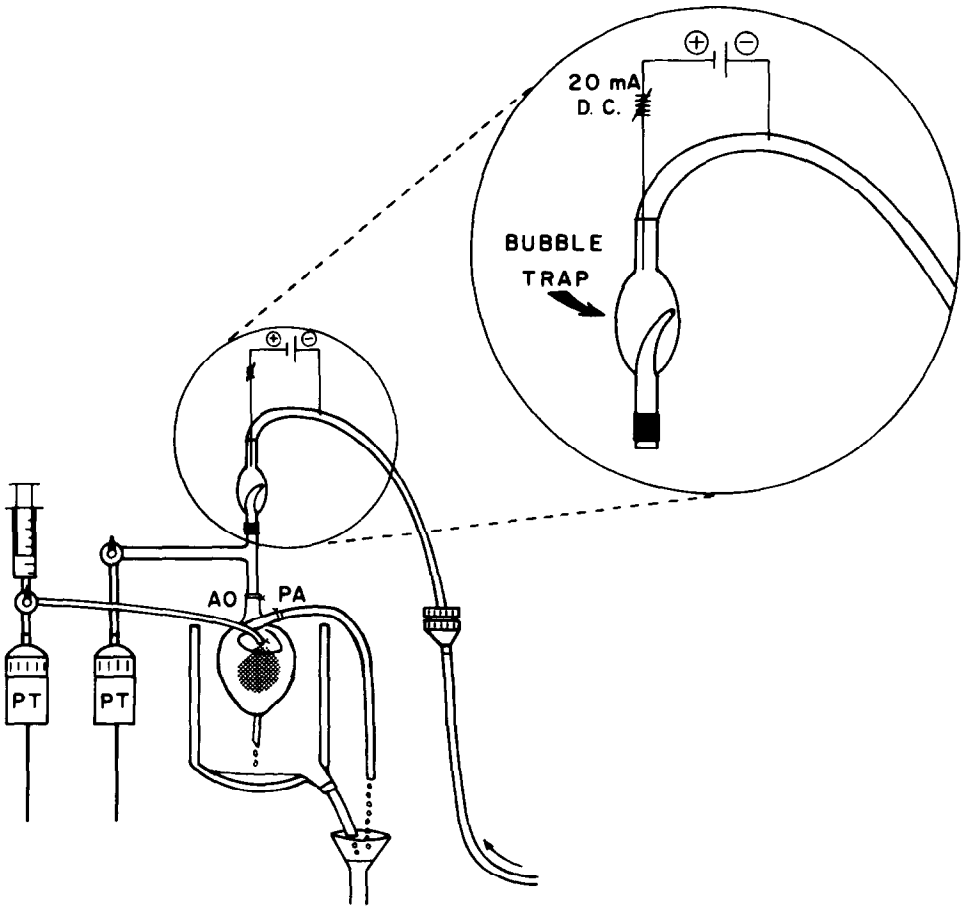



**FIGURE 2. Schematic diagram of the electrolysis apparatus is shown. The insert represents the modification of the Langendorff perfusion apparatus to provide for electrolysis of the buffer. Symbols: + = anode; − = cathode, aorta = AO, pulmonary artery = PA; and pressure transducer = PT. The shaded area within the left ventricle represents the saline-filled latex balloon.**

of free radicals could be quantified. The quantification of luminol luminescence was obtained with the following reaction:

$$\text{hypoxanthine} + \text{xanthine oxidase} \rightarrow \text{urate} + \cdot O_2^-.$$

The generation of $\cdot O_2^-$ was quantified by the reduction of ferricytochrome-C.

These experiments were performed without the isolated heart in order to identify the radicals being produced by electrolysis of a physiological buffer solution. A 50-ml aliquot of physiological buffer (the volume perfusing the heart during the 2-min period of electrolysis) was stirred continuously while being subjected to a period (2 min) of electrolysis. At the end of the time interval, two aliquots (1 ml each, pH

7.4) were placed immediately in cuvettes containing 200 μl of luminol (25 μM final concentration), shaken, and the luminescence was determined with LKB Luminometer (Model 1250). The pH of the reaction mixture was 10.25–10.33. Sampling time was less than 1 sec, and luminescence was determined in less than 15 sec. The intensity of the luminescence was recorded as a millivolt response on an LKB single-channel strip recorder. A 450-mV response with the luminol assay was equivalent to 12 ± 0.5 nM/ml of $\cdot O_2^-$ ($\bar{X}$ ± *SEM*, $n$ = 6) determined by the reduction of ferricytochrome-C.

### Chemicals and Enzyme Preparations

The chemicals and enzyme preparations used in these experiments were: luminol (Fisher); SOD (Boerhinger–Mannheim, specific activity = 5,000 units/mg, 95–98% Cu-Zn SOD); hypoxanthine (Sigma, 2); xanthine oxidase (Cal Biochem, specific activity = 1.03 units/mg); ferricytochrome-C (Sigma, Type VI); dimethyl sulfoxide (DMSO, Crown Zellerbach); GLY (Sigma); 1,3-diphenylisobenzofuran (DPBF, Aldrich); Ascorbate (Sigma); mannitol (Sigma), nitro blue tetrazolium (Sigma); and TRP (Sigma).

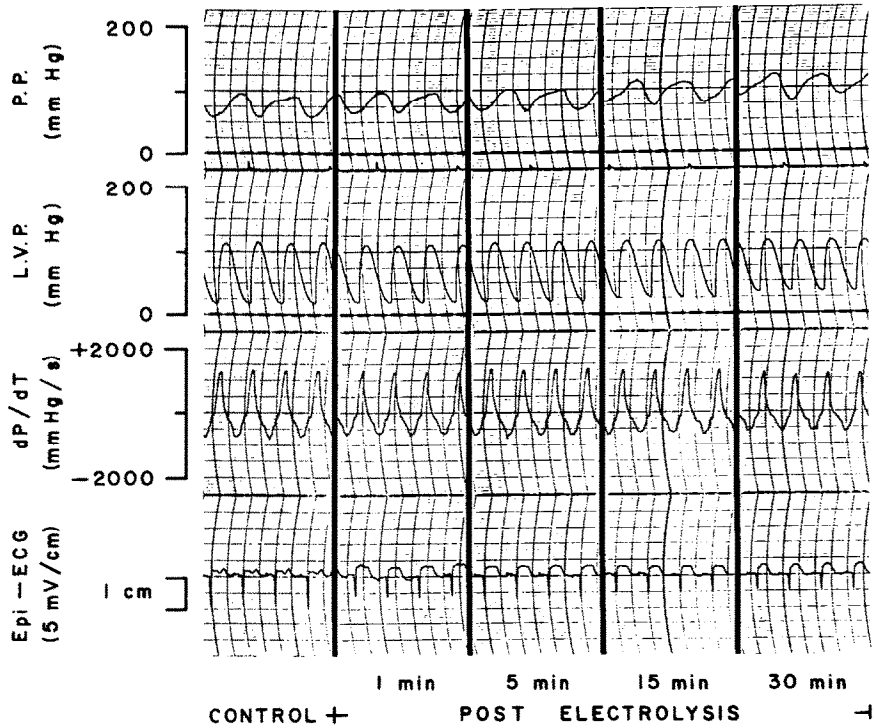



**FIGURE 3. Typical tracings of a heart perfused with buffer subjected to electrolysis, which demonstrates increases in PP and LVEDP and decreases in both + and − dP/dT are shown. The elevation in the S-T segment of the EPI–ECG should be noted.**

### Statistical Analysis

All groups were analyzed statistically by one-way analysis of variance. Statistical significance between groups was determined by Student–Newman–Keuls multiple range test where the level of significance was at least $p < 0.05$. All values were expressed as the mean ± SEM.

## RESULTS

### Electrolysis and Heart Function

Hearts perfused with buffer subjected to electrolysis for 2 min demonstrated a gradual decline in heart function over a postelectrolysis period of 30 min. This decline in function was characterized by an increase in coronary artery PP and LVEDP and a decrease in both positive and negative dP/dT (Figure 3). Figure 3 also illustrates that there were two types of alterations in the EPI–ECG of the hearts: 1) an elevation in the S–T segment; and/or 2) a loss of QRS voltage amplitude. It was observed that the alteration in EPI–ECG was manifested immediately after electrolysis, whereas there was a delay of 8–10 min before an alteration in PP or LVEDP was detected.

Panels A of Figures 4 and 5 demonstrate that there was a significant increase in PP (37 ± 6 mmHg) and LVEDP (41 ± 7 mmHg), respectively. When Starling function

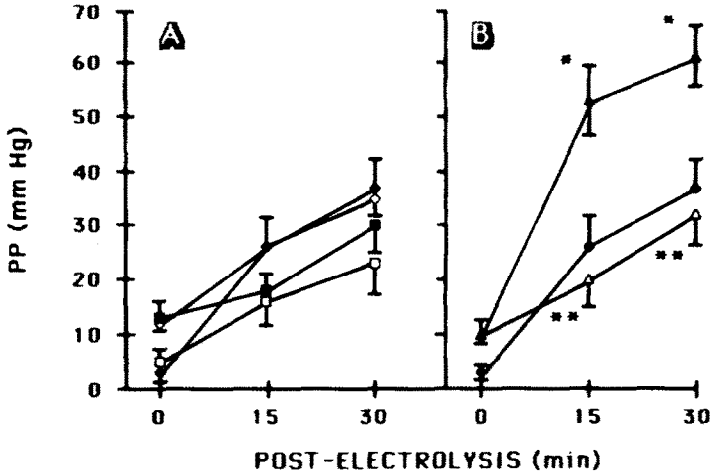



**FIGURE 4. The effects of electrolysis on PP are illustrated. A. Control electrolysis and the effects of free radical scavengers: CAT, SOD, and TRP + GLY. B. $H_2O_2$ enhancement of electrolysis and the effect of the $H_2O_2$ scavenger, CAT. * Denotes a significant difference at the level $p < 0.05$ when $H_2O_2$-treated hearts were compared to control electrolysis (panel A). ** Denotes a significant difference at the level $p < 0.05$ when CAT-treated hearts were compared to their respective $H_2O_2$-treated, electrolysis hearts. Each point represents the mean ± SE. ◆ electrolysis ($n = 6$); ◇ electrolysis + CAT (150 U/ml, $n = 5$); ■ electrolysis + SOD (100 U/ml, $n = 4$); □ electrolysis + TRP (5 mM) + GLY (5 mM) ($n = 4$); ▲ electrolysis + $H_2O_2$ ($1 \times 10^{-5}$ M, $n = 8$); △ electrolysis + $H_2O_2$ + CAT ($n = 4$).**

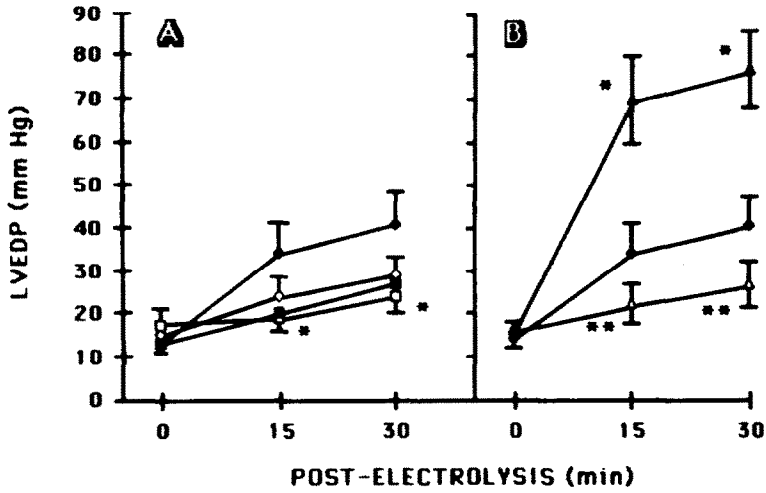



**FIGURE 5. The effects of electrolysis on LVEDP are illustrated. A. Control electrolysis and the effects of free radical scavengers: CAT, SOD, TRP + GLY. B. $H_2O_2$ enhancement of electrolysis and the effect of the $H_2O_2$ scavenger, CAT. * Denotes a significant difference at the level $p < 0.05$ when SOD, TRP + GLY, and $H_2O_2$-treated hearts were compared to control electrolysis hearts. ** Denotes a significant difference at the level $p < 0.05$ when the CAT-treated hearts were compared to the $H_2O_2$-treated electrolysis hearts. Each point represents the mean ± SE. ◆ Electrolysis ($n = 6$); ◇ electrolysis + CAT (150 U/ml, $n = 5$); ■ electrolysis + SOD (100 U/ml, $n = 4$); □ electrolysis + TRP (5 mM) + GLY (5 mM) ($n = 4$); ▲ electrolysis + $H_2O_2$ ($1 \times 10^{-5}$ M, $n = 8$); △ electrolysis + $H_2O_2$ + CAT ($n = 4$).**

relationships were determined it was observed that there was a significant reduction in ventricular function 30 min postelectrolysis. Figure 6 (panel A) illustrates the depressed ventricular function (LVDP) at a LVEDP of 15 mmHg in hearts subjected to 2-min electrolysis. Control values for PP, LVEDP, and LVDP were 45 ± 8 mmHg, 11 ± 3 mmHg, and 108 ± 6 mmHg, respectively ($n = 19$).

### Effects of Free Radical Scavengers

The addition of CAT, SOD, or TRP + GLY to the perfusate produced varying degrees of protection of hearts perfused with a buffer that was subjected to electrolysis. There was an observed protection against the rise in LVEDP and loss of ventricular function; however there was no significant protection in regard to the increase in coronary artery PP. The free radical scavengers produced no adverse effects when hearts were perfused for 1 hr in the presence of each scavenger.

Figure 5 (panel A) illustrates that SOD and the combined administration of TRP + GLY produced significant protection against the rise in LVEDP. The administration of TRP + GLY was the only treatment significantly effective in preventing the loss of LVDP (Figure 6, panel A). The administration of TRP alone did not provide any protection of the hearts from the effects of electrolysis (data not shown).

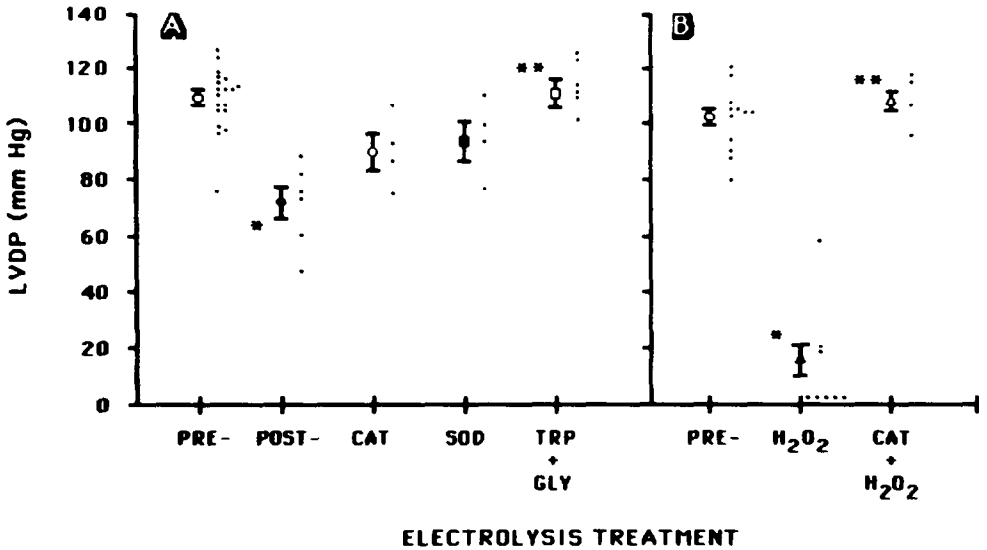



**FIGURE 6. The effects of electrolysis on ventricular function (left ventricular developed pressure) at 15 mmHg LVEDP are shown. A. Control electrolysis and the effects of free radical scavengers: CAT, SOD, and TRP + GLY. B. $H_2O_2$ enhancement of electrolysis and the effect of the $H_2O_2$ scavenger, CAT. * Denotes a significant difference at the level $p < 0.05$ when compared to preelectrolysis function. ** Denotes a significant difference at the level $p < 0.05$ when the scavenger-treated hearts were compared to the postelectrolysis hearts. Each point represents the mean ± SE. ○ Preelectrolysis; ◆ post-electrolysis; ◇ postelectrolysis + CAT (150 U/ml); ■ postelectrolysis + SOD (100 U/ml); □ postelectrolysis + TRP (5 mM) + GLY (5 mM); ▲ postelectrolysis + $H_2O_2$ ($1 \times 10^{-5}$ M); △ postelectrolysis + $H_2O_2$ + CAT.**

### Hydrogen Peroxide Potentiation of Electrolysis and Protection by Catalase

Figure 7A illustrates that the addition of $H_2O_2$ ($1 \times 10^{-5}$M) to the perfusate for 5 min before electrolysis had no effect on heart function. Electrolysis in the presence of $H_2O_2$ resulted in a significant enhancement of the effects (i.e., increases in PP and LVEDP and loss of LVDP) observed to occur with electrolysis alone (Figures 4B, 5B, 6B, 7A). This enhancement was characterized by a 100% increase in PP and LVEDP above the values obtained with electrolysis in the absence of $H_2O_2$. Figure 6B also illustrates that LVDP was decreased by 90% in which five of eight hearts lost all contractile function. Catalase was able to inhibit the $H_2O_2$-induced enhancement of all the recorded parameters (Figures 4B, 5B, 6B, 7B). Control values in these experiments for PP, LVEDP, and LVDP were 50 ± 3 mmHg, 13 ± 2 mmHg, and 102 ± 6 Hg, respectively ($n = 12$).

Figure 8 A and B illustrates that hearts perfused with buffer containing $H_2O_2$ and subjected to electrolysis demonstrated histologic evidence of tissue injury. Figure 8B demonstrates that hearts perfused with buffer subjected to electrolysis in the presence of $H_2O_2$ manifested a pattern of wavy, attenuated myofibers. Increases in

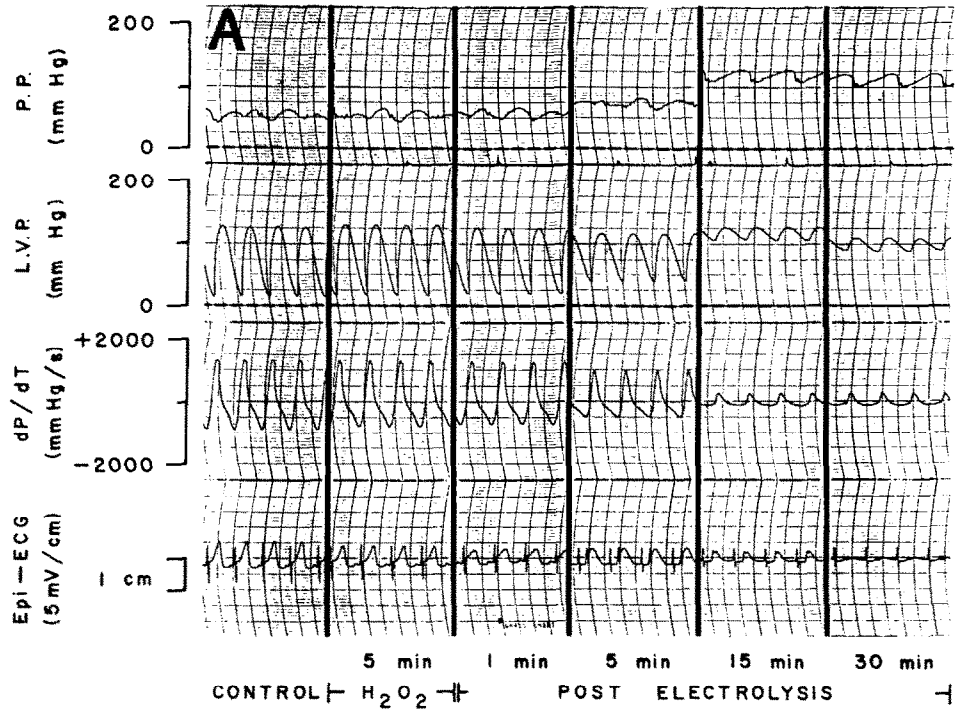
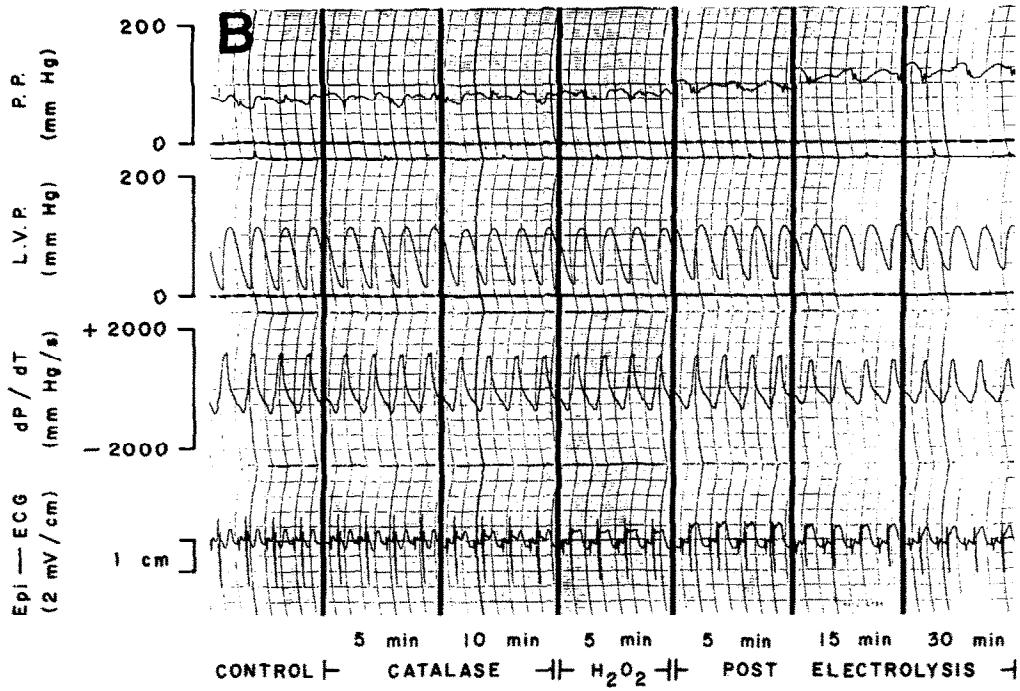



**FIGURE 7. Typical tracings are shown of a heart perfused with buffer subjected to the following. A. $H_2O_2$ + electrolysis; which illustrates the enhancement of the increases in PP and LVEDP and the decrease in dP/dT. B. Catalase + $H_2O_2$ + electrolysis, which illustrates preservation of all physiological changes.**

the interstitial spaces were also observed. Hearts perfused with normal buffer (no electrolysis and $H_2O_2$) were observed to contain normal-appearing myofibers and interstitial spaces (Figure 8A).

### Luminol Detection of Free Radicals

Electrolysis of the Kreb's buffer with a 20 mA constant current for 2 min produced a 30.8 ± 3 mV chemiluminescent response equivalent to 821 ± 80 pM/ml of free radicals (Table 1). Figure 9 illustrates that a number of free radical scavengers were effective in reducing or inhibiting the interaction of the radical species with luminol. Selective scavengers of $\cdot O_2^-$ (SOD) and $H_2O_2$ (CAT) produced inhibition of the response by 76 ± 2% and 30 ± 5%, respectively ($X$ ± SEM, $n$ = 3). Other radical scavengers, DMSO (·OH), GLY (HOCL), DPBF ($^1O_2$), and ascorbate (nonselective), were effective inhibitors of the radical–luminol chemiluminescence. Mannitol and TRP (not shown), ·OH radical scavengers, were ineffective as inhibitors of the chemiluminescence. These data indicate that the effectiveness of the combination of TRP + GLY was due to GLY alone. Figure 9 also illustrates that the electrolytic production of radicals in a physiologic buffer is dependent upon the presence of $Cl^-$ ions,




determined by the substitution (equivalent ionic strengths) of all chloride-containing chemicals in the buffer, i.e., NaCl, KCl, $MgCl_2$, and $CaCl_2$, with those containing sulfate. Chloride ions or other halides are necessary for the first oxidation step in the transfer of electrons from the platinum anode to the buffer solution.

## DISCUSSION

This investigation provides evidence that demonstrates that electrolysis of a physiological buffer solution leads to the production of free radicals. These free radicals can initiate events that produce alterations in coronary artery and myocardial function. Rabbit isolated hearts perfused (modified Langendorff) with buffer subjected to 2 min of electrolysis exhibited significant increases in PP and LVEDP and decreases

**TABLE 1 Quantification of Free Radical production**[a]

| CONDITION | mV | $O_2^-$ |
|---|---|---|
| Hypoxanthine + xanthine oxidase | 450 ± 10 | 12 ± 1 nM/ml ($n$ = 6) |
| Electrolysis | 31 ± 3 | 821 ± 80 pM/ml ($n$ = 20) |

[a] All values represent $\bar{X}$ ± SEM.

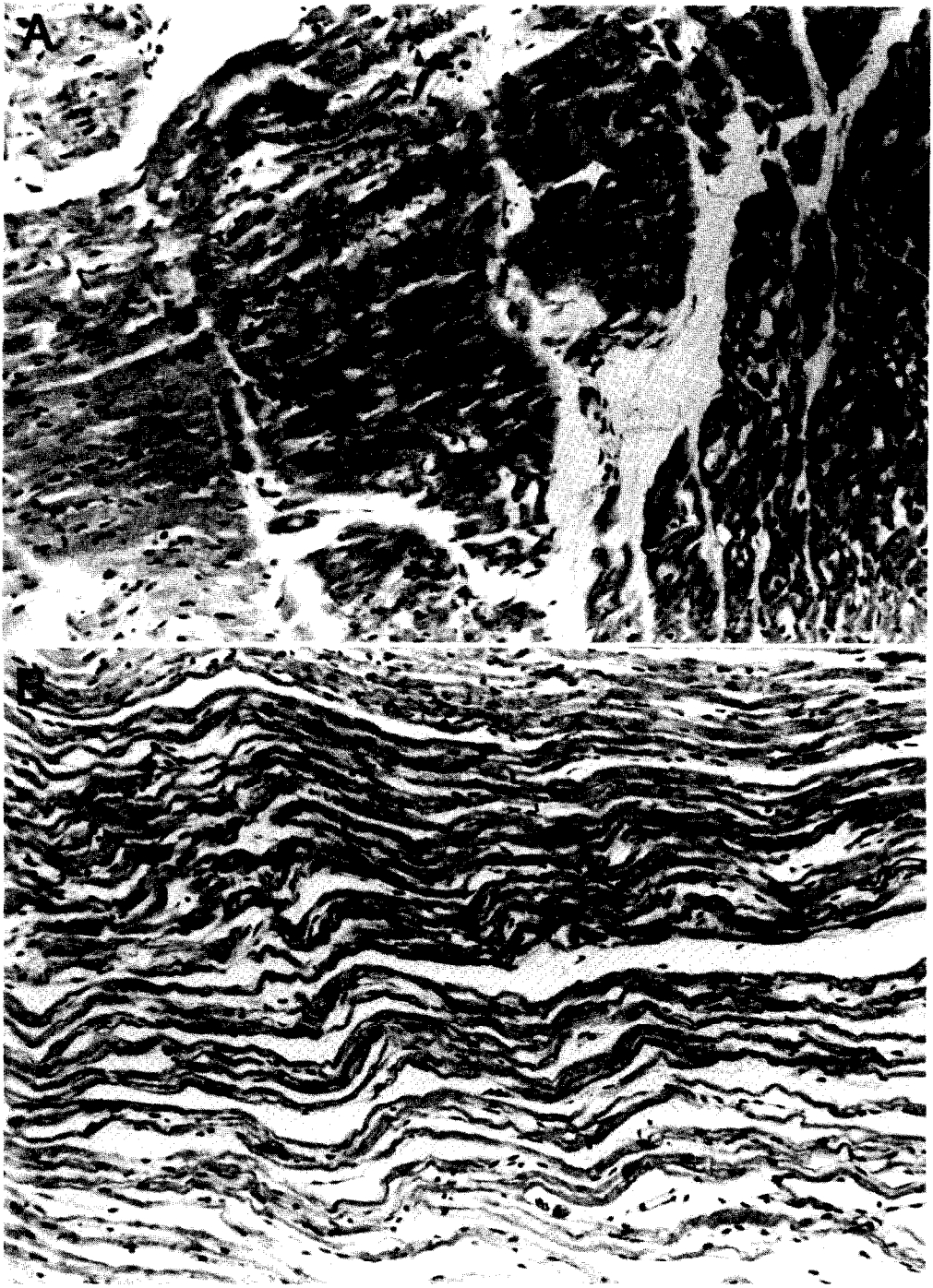



**FIGURE 8. The histological alterations of hearts perfused with buffer subjected to electrolysis + $H_2O_2$ are shown. Panel A represents a control heart perfused with normal buffer for 2 hr. Panel B represents a heart perfused with buffer subjected to electrolysis + $H_2O_2$. (Magnification = 188X).**

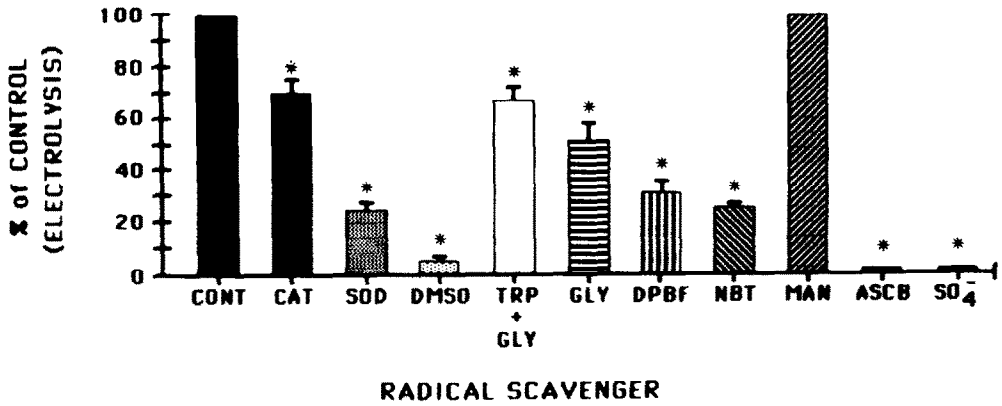



**FIGURE 9. Free radicals produced by electrolysis can be detected by the luminol–luminescence assay. A variety of free radical scavengers and their ability to inhibit the radical–luminol reaction are depicted in this figure. * Denotes a significant inhibition of the chemiluminescent response. Vertical lines represent the SEM. Abbreviations: CONT = control electrolysis; CAT = catalase (37 nM); SOD = superoxide dismutase (1.8 μM); DMSO = dimethyl sulfoxide (10 mM); TRP + GLY = tryptophan (5 mM) + glycine (5 mM); DPBF = 1,3-diphenylisobenzofuran (34 μM); NBT = nitro blue tetrazolium (10 μM); MAN = mannitol (12.5 mM); ASCB = ascorbate (0.6 mM); $SO_4^-$ = sulfate substitution for all $Cl^-$-containing chemicals. All values represent the means ± SEM of three determinations.**

in LVDP. These alterations were attenuated by the introduction of known radical scavengers to the buffer during electrolysis. Secondly, this investigation describes a simple and rapid method for the detection of radicals utilizing a luminol–chemiluminescence assay.

Placement of an anode and a cathode within a perfusion system, as illustrated in Figure 2, constitutes a simple electrolytic cell. Passing an electric current (20 mA, DC) through a physiological buffer (salt) solution initiates a series of chemical reactions. Oxidation of the chloride ion ($Cl^-$) and $H_2O$ occurs at the anode in the following manner:

$$2Cl^- \rightarrow Cl_2\ (g) + e^-$$

$$6H_2O \rightarrow O_2\ (g) + 4H_3O^+ + 4e^-.$$

The above two reactions are dependent upon the applied voltage, current density (amperes/centimeters squared of electrode surface) and temperature. The end result of the two equations is the generation of electrons ($e^-$), gaseous oxygen, and chlorine. The natural movement of electrons in an electrolytic cell is from anode to cathode. At the cathode $H_2O$ is reduced in the following reaction:

$$H_2O + e^- \rightarrow OH^- + H_2\ (g).$$

Conditions are now optimum for the initiation of free radical formation. Formation of oxygen-free radicals ($\cdot O_2^-$, $\cdot OH$, $^1O_2$) and $H_2O_2$ can now be postulated to occur

from an array of chemical reactions:

$$O_2 + e^- (aq) \rightarrow \cdot O_2^-$$

$$\cdot O_2^- + \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$$

$$H_2O_2 + \cdot O_2^- \rightarrow \cdot OH + OH^- + O_2$$

$$H_2O_2 + 2e^- + H^+ \rightarrow \cdot OH + H_2O$$

$$\cdot OH + \cdot O_2^- \rightarrow {}^1O_2 + OH^-.$$

The highly potent oxidizing species, hypochlorous acid (HOCl), may also be produced:

$$H_2O_2 + Cl^- + e^- \rightarrow HOCl + OH^-.$$

These reactions, resulting from electrolysis of the physiological buffer solution, would produce a milieu rich in radicals. These radicals would then have the potential to be presented to the circulation of the perfused rabbit heart and initiate the formation of secondary radicals. Both primary and secondary radicals could lead to lipid peroxidation, loss of membrane integrity, and propagation of irreversible cell damage (Freeman and Crapo, 1982). Addition of a free radical scavenger(s) could terminate this sequence of events by neutralizing the radical(s).

In this investigation, the presence of free radicals formed via electrolysis was determined using a luminol–chemiluminescence method. Luminol–chemiluminescence has been used by investigators to study phagocytic cell function (Seim, 1983; Shah et al., 1983) and as a detection system for oxygen-free radicals and radical intermediates (Merenyi and Lind, 1980; Lippman, 1980; Misra and Squatrito, 1982). As presented in Methods, radical(s) react with luminol and produce a luminol radical (·LH) that has a longer half-life (minutes) compared to micro- and milliseconds for the oxygen-free radicals. The ·LH reacts with a second radical ($\cdot O_2^-$) to produce an endoperoxide intermediate that undergoes dissociation into an aminophthalate dianion (active state). Aminophthalate dianion (active state) spontaneously decomposes to an inactive state, resulting in the emission of light (chemiluminescence).

Electrolysis of the physiological buffer produced a 30.8 ± 3 mV chemiluminescent response, which was equivalent to 821 ± 80 pM/ml of radicals when standardized in relation to a chemiluminescent response produced by an enzymatic radical generating system (Table 1). A variety of scavengers was effective in neutralizing the free radicals produced by electrolysis (Figure 9). The intracellular enzymatic radical scavengers, CAT and SOD, were effective in neutralizing the radical–luminol reaction, 30% and 76%, respectively. These two scavengers would implicate the presence of $H_2O_2$ and $\cdot O_2^-$. Classical ·OH-radical scavengers (DMSO, mannitol (MAN), TRY) exhibited varying degrees of effectiveness. Dimethyl sulfoxide was the most effective (95% inhibition), whereas MAN (12.5 mM) and TRY (5 mM, not shown) were essentially ineffective. These data raise some question as to the involvement of ·OH radical in this study. Dimethyl sulfoxide is much more potent as a scavenger of ·OH radical than either MAN or TRP. In most in vitro systems where MAN or TRP

have been shown to be effective scavengers their concentrations were 20–100 mM. Hess et al. (1984) demonstrated that MAN was not very effective when used alone to protect isolated cardiac sarcoplasmic reticulum from an enzymatic radical generating system at concentrations of 10–100 mM. In these same studies, however, if SOD was added to MAN there was an observed protection of sarcoplasmic reticular function (calcium uptake). These high concentrations of MAN also would have osmotic effects on an isolated heart preparation. In the present investigation, when MAN and TRP were administered in concentrations to limit osmotic effects to a minimum (12.5 mM and 5 mM, respectively), there was no observable protection of the hearts or inhibition of the radical–luminol chemiluminescence.

The hypochlorous acid scavenger, glycine, and the singlet oxygen scavenger, DPBF, were both effective inhibitors of the chemiluminescent response. The nonselective scavengers, nitro blue tetrazolium and ascorbate, were very effective; ascorbate, even at concentrations as low as 0.2 mM, inhibited the response by 100%. The luminol data would implicate the electrolytic production of a milieu of oxidizing species (i.e., $\cdot O_2^-$, $H_2O_2$, $^1O_2$, $\cdot OH$, and HOCl). Caution must be used, however, in interpreting the data derived from "selective" radical scavengers. Recently, Singh (1980) and Fantone and Ward (1982) discussed the possibility that selective radical scavengers under certain conditions can react with more than one radical species. These conditions also make it difficult to quantify the concentration of a specific radical within this mixture of radicals.

In the present investigation, hearts pretreated with the enzymatic radical scavengers, CAT and SOD, and a combination of TRY + GLY ($\cdot OH$ and HOCl scavengers, respectively) demonstrated varying degrees of protection. None of the treatments were significant in protecting against the changes in PP (Figure 4). The SOD and TRP + GLY treatments, however, were effective in preventing the increase in LVEDP (Figure 5). The administration of TRP + GLY was the only treatment significantly effective in preventing the loss of LVDP (Figure 6). The $H_2O_2$, a toxic oxidant, greatly enhanced the physiological alterations observed in response to electrolysis; CAT provided 100% protection from the $H_2O_2$-induced effects (Figures 4–6). The electrolysis-induced physiological alterations observed in this study are similar to those by Burton et al. (1984) who observed a loss in contractility (i.e., dT/dt), as well as ultrastructural alterations (vacuolization of myocytes and endothelial cells) of a rabbit isolated septal preparation perfused with an enzymatic-$\cdot OH$ radical generating system; SOD was found to protect in this preparation.

In summary, this investigation provides data that suggests that electrolysis of a physiological buffer induces alterations in the vascular and myocardial function of an isolated perfused rabbit heart. These alterations were similar to those observed in myocardial preparations exposed to radicals generated exogenously using a xanthine-xanthine oxidase generating system (Burton et al., 1984). Superoxide dismutase and the combination of TRP + GLY were effective in providing protection to hearts exposed to electrolyzed buffer, implicating the involvement of $\cdot O_2^-$ and HOCl in the observed physiological alterations. Detection of free radicals by the luminol assay supports this contention in that SOD and TRP + GLY were effective in reducing the chemiluminescence caused by the radical–luminol interaction.

In conclusion, electrolysis as a novel method for the generation of free radicals

in a blood-free system has been described. This new method allows for the study of the direct effects of free radicals on membranes and organ systems such as the heart. Under the proper conditions this method could possibly be used for the pharmacological development and detection of new radical scavengers.

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