Malondialdehyde and Glutathione Production in Isolated Perfused Human and Rat Hearts

Maarten Janssen, Johan F. Koster, Egbert Bos, Jan Willem de Jong

A considerable number of studies show that oxygen-derived free radicals can cause injury related to cardiac ischemia/reperfusion. Various free radical sources, like the mitochondrial respiratory chain, arachidonic acid, leukocytes, and endothelial xanthine oxidoreductase, could play a role. McCord postulated that, during myocardial ischemia, xanthine dehydrogenase is converted to xanthine oxidase, which forms superoxide radicals upon reperfusion.

Superoxide dismutase converts superoxide to hydrogen peroxide, which can react with iron to form the highly cytotoxic hydroxyl radical (Haber-Weiss reaction). The latter can initiate the peroxidation of polyunsaturated fatty acids in cell membranes and thus impair or destroy cell function. Since catalase activity in myocardial tissue is very low, glutathione peroxidase seems to be crucial in detoxifying hydrogen peroxide via reduced glutathione (GSH). The excess of oxidized glutathione (GSSG) inside the cell is converted back to GSH by glutathione reductase or actively transported out of the cell.

Direct detection of oxygen-derived free radicals is almost impossible because of their instability and consequent short half-life. Electron spin resonance or spin trapping provides a new direct method to measure free radical formation. Indirect probing can be done by measuring compounds associated with the detoxification, by assaying products generated, or by applying free radical scavengers.

GSSG levels in tissue and coronary effluent rise during lack of oxygen. Likewise malondialdehyde (MDA), one of the many products of lipid peroxidation, can be demonstrated. GSSG and MDA are therefore useful as indexes of myocardial oxidative stress.

Various authors have reported MDA formation during coronary angioplasty procedures and pacing stress testing with ischemic periods of a few minutes, contrasting the negative reports for rabbit and rat hearts with ischemic periods up to 60 minutes. These contradictory results could be due to three factors: errors by interfering blood constituents during in vivo measurement of MDA, (2) the use of the aspecific thiobarbituric acid assay for MDA, and (3) species differences. The second hypothesis is based on the observation that studies reporting MDA production use the thiobarbituric acid assay whereas the negative findings are obtained with a more specific method.

To circumvent the possible influence of blood components, the isolated buffer-perfused heart is a useful
model for investigating the effects of ischemia/reperfusion. To shed light on the MDA controversy described above, we subjected human and rat hearts to temporary ischemia and measured MDA, GSH, and GSSG in coronary effluent and tissue samples. To validate the MDA detection method, we perfused rat hearts with cumene hydroperoxide, which is known to induce MDA formation.20 Our data show that oxidative stress caused by a 20-minute period of ischemia followed by reperfusion does not result in MDA release in isolated human and rat hearts. A novel finding is the absence of MDA in normoxic human myocardial tissue, whereas rat hearts contain sizable amounts, which do not increase after ischemia/reperfusion.

**Materials and Methods**

**Human Heart Perfusions**

Hearts (n=8) were removed from patients undergoing cardiac transplantation for end-stage heart failure due to ischemic heart disease or dilated cardiomyopathy. They were arrested in situ with ice-cold St. Thomas’ Hospital cardioplegic solution,21 removed, and transported within approximately 15 minutes in gassed (95% O2–5% CO2) ice-cold cardioplegic solution to the laboratory. For details about the perfusion system, see Smolen’ski et al.22 Briefly, the hearts were perfused in the Langendorff mode with a roller pump. The perfusion fluid consisted of a modified Tyrode’s buffer23 containing glucose and dextran (40 000 D, Isodex, Pharmacia, Uppsala, Sweden). It was oxygenated with 95% O2–5% CO2 using a baby oxygenator. Retrograde aortic perfusion started 30 to 50 minutes after cardiac arrest. Blood was washed from the hearts for 10 minutes, and then recirculation was started with 1.0 to 1.5 L perfusion fluid. After a 30-minute control period, the hearts were subjected to 20 minutes of no-flow ischemia, followed by a 30-minute reperfusion period. Arterial and venous samples were collected at regular intervals. Biopsies (0.1 g) were taken from multiple locations of the left ventricle with a Tru-cut needle (Travenol Laboratories, Deerfield, III) before the onset of the preparation period and after 30 minutes of reperfusion; they were immediately frozen with liquid nitrogen.

In a pilot experiment, perfusion took place 30 minutes with buffer supplemented with 0.5 mmol/L cumene hydroperoxide (Merek, Amsterdam, The Netherlands) to induce lipid peroxidation.

**Rat Heart Perfusions**

Adult male Wistar rats (400 to 450 g) were anesthetized intraperitoneally with 60 mg/kg pentobarbital (Nembutal, Sanofi, Paris, France) in accordance with guidelines of the American Physiological Society. Hearts were removed, arrested in ice-cold saline, and retrogradely perfused with a modified Tyrode’s buffer oxygenated with 95% O2–5% CO2 at 72 mm Hg and 37°C. Cannulation of the aorta was done <1 minute after arrest. Coronary flow was measured electromagnetically (Skalar, Delft, The Netherlands). Hearts were perfused in a nonrecirculating mode for 30 minutes, and then recirculation was started with a total volume of 100 mL. At this point, the hearts were divided into three groups. Control hearts (n=9) were recirculated for 60 minutes. In the ischemic group (n=6), hearts were subjected to 20 minutes of ischemia, followed by 30 minutes of reperfusion. The third group (n=6) was perfused for 30 minutes with buffer supplemented with 0.5 mmol/L cumene hydroperoxide to induce lipid peroxidation.

To check the influence of a prolonged transportation and preparation period (compare with human hearts), two additional groups (n=7) of rat hearts were investigated. Hearts were removed from the thorax as described above and immediately flushed for 3 minutes with gassed (95% O2–5% CO2) ice-cold St. Thomas’ Hospital cardioplegic solution.21 The organs, submerged in the cardioplegic solution, were put on melting ice for 40 minutes. Then they were connected to the perfusion system and subjected to the protocols (cardioplegic control and cardioplegic/ischemic groups) described above.

In the groups mentioned above, samples were collected every 5 minutes. Because of the minimal arteriovenous differences, release was calculated from the increase in catabolite concentration divided by the time interval. Because of the lag time in the recirculating system, sampling was omitted during the first few minutes of reperfusion. At the end of the experiments, hearts were freeze-clamped at liquid nitrogen temperature.

To investigate if GSSG release reflected production or increased washout, we subjected hearts (n=6) to 20 minutes of ischemia after a 30-minute stabilization period and perfused them for 1 minute before they were freeze-clamped.

**Glutathione Determination**

Effluent samples were deproteinized with 4% HClO4 (final concentration), centrifuged at 10 000g for 2 minutes, and neutralized with 6 mol/L KOH and 2 mol/L K2CO3. Tissue was crushed in liquid nitrogen and deproteinized with 5% sulfosalicylic acid.24 After centrifugation at 10 000g for 2 minutes, the supernatant fluid was neutralized with 6 mol/L KOH and 2 mol/L K2CO3. Total glutathione (GSH+GSSG) and GSSG were analyzed according to Ceconi et al.25 on a double-beam spectrophotometer (model U-2000, Hitachi, Tokyo, Japan). Contents were expressed per gram protein to circumvent weight problems due to (1) cumene hydroperoxide–induced loss of tissue water and (2) edema formation in isolated human hearts. The detection limit of GSSG in perfusion fluid was 0.01 nmol·min⁻¹·g wet wt⁻¹.

**MDA Determination**

Tissue samples, crushed in liquid nitrogen, and effluent samples were deproteinized with 4% HClO4 (final concentration), centrifuged at 10 000g for 2 minutes, and neutralized with 6 mol/L KOH and 2 mol/L K2CO3. MDA was assayed by high-performance liquid chromatography (HPLC) according to Bull and Marnet,26 with some modifications. A µBondapak C18 (10–µm particle diameter, Waters-Millipore, Milford, Mass), preceded by a Supelguard LC18 guard column (5–µm particle diameter, Supelco, Bellafonte, Pa), was eluted with 20% acetonitrile (vol/vol) in 1.5 mmol/L myristyltrimethylammonium bromide and 20 mmol/L sodium phosphate buffer (pH 7.2). Detection was done at 267 nm with a forward optical scanning detector (Spectra-Physics Analytical, San Jose, Calif). Peaks were identified by comparison of retention times and spectra (multiwave-
length scan) of the standard and the samples. MDA standard was prepared by hydrolysis of 1,1,3,3-tetraethoxypropane (Merck, Darmstadt, Germany), according to Largilliere and Melancon. The detection limit was 4 nmol/g protein for tissue and 0.02 nmol·min⁻¹·g wet wt⁻¹ for effluent.

**Protein Assay**

Tissue protein was assayed with Coomassie brilliant blue (Bio-Rad Laboratories, Munich, Germany) according to Bradford, using bovine serum albumin as the standard.

**Statistics**

All data represent mean±SEM. Rat and human effluent data were analyzed by two-tailed analyses of variance (least significant differences and Tukey’s test, respectively). Hemodynamic data and tissue measurements were analyzed with Student’s paired t test. A value of P<.05 was considered a significant difference.

**Results**

**General**

The weight of the human hearts was 480±62 g (n=8). Because of the perfusion, the weight increased 25% (P<.05). The hearts beat spontaneously within 5 minutes after connection to the perfusion system. The preischemic and postsischemic heart rate was 52±6 beats per minute; average flow was 1.0 mL·min⁻¹·g wet wt⁻¹. This relatively low coronary flow seems to be adequate, since baseline lactate production was virtually absent (data not shown). Early during reperfusion, flow did not increase. The heart perfused with cumene hydroperoxide stopped beating after 25 minutes of drug infusion.

Rat heart weight (1.48±0.07 g, n=41) was similar before and after the experiments. During the stabilization period, hearts had a rate of 254±9 beats per minute; the postsischemic rate was 12% less (P=NS). Hearts stopped beating 20 minutes after the start of cumene hydroperoxide infusion.

In rat hearts, coronary flow was initially 10.5±0.5 mL·min⁻¹·g⁻¹. In the control groups, flow decreased little in the course of the experiment. In the groups subjected to transient ischemia, flow increased 32% and 47% (P<.005) early during reperfusion in the ischemic and treated ischemic groups, respectively, and decreased subsequently toward baseline. In hearts perfused with cumene hydroperoxide, flow increased 49% (P<.005 versus baseline), declining quickly after 20 minutes to 30% of the initial flow (P<.05 versus baseline).

**Glutathione in Human Heart Effluent and Tissue**

Baseline arterial and venous GSH concentrations in the ischemic/reperfused hearts did not differ significantly. Early during reperfusion, GSH release increased rapidly to 0.43±0.22 nmol·min⁻¹·g⁻¹ (P<.05 versus baseline, Fig 1). Efflux decreased toward baseline after 10 minutes of reperfusion. GSSG was undetectable in the control period. After 4 minutes of reperfusion, the release amounted to 0.24±0.12 nmol·min⁻¹·g⁻¹ (P<.05 versus baseline). GSSG efflux ceased after 5 minutes (Fig 1).

Postischemic myocardial GSH content was 50% lower than the control value (P<.05, Table 1). GSSG increased 28% in ischemic tissue when compared with control tissue (P=NS). The ratio between GSH and GSSG in control hearts (42±2) decreased twofold in ischemic hearts (P<.05). There seems to be an imbalance between the decrease in tissue GSH and the

![Figure 1](http://circres.ahajournals.org/doi/figure/10.1161/01.CIR.80.2.1034)

**Table 1. Decrease in Human Heart Glutathione Content due to Ischemia/Reperfusion**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Ischemia/Reperfusion</th>
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<tbody>
<tr>
<td>GSH, μmol/g protein</td>
<td>25.3±4.5</td>
<td>12.8±1.5*</td>
</tr>
<tr>
<td>GSSG, μmol/g protein</td>
<td>0.61±0.12</td>
<td>0.84±0.24</td>
</tr>
<tr>
<td>Ratio</td>
<td>42.0±2.5</td>
<td>20.5±3.4*</td>
</tr>
</tbody>
</table>

GSH indicates reduced glutathione; GSSG, oxidized glutathione; and ratio, GSH/GSSG. Values are mean±SEM (n=7).

Hearts were removed from patients undergoing cardiac transplantation. They were arrested with cold cardioplegia and subsequently perfused according to the Langendorff procedure. After baseline perfusion for 30 minutes, hearts were subjected to normoxic perfusion for another 30 minutes (control) or to 20 minutes of global ischemia and 30 minutes of reperfusion (ischemia/reperfusion). Tissue biopsies were taken before the ischemic period and after reperfusion. The data indicate that ischemia/reperfusion lowers the cardiac GSH but does not increase GSSG content.

*P<.05 vs control.
amount found during 30 minutes of reperfusion in the effluent: Tissue GSH+GSSG content decreased 700 nmol/g wet wt, whereas the total amount released in 30 minutes was only 10 nmol/g.

After the start of cumene hydroperoxide infusion, effluent GSH and GSSG release increased rapidly to 0.8 and 0.3 nmol·min⁻¹·g wet wt⁻¹, respectively. These values—somewhat higher than those after ischemia (compare with Fig 1)—decreased little during the remaining perfusion period. At the end of the experiment, tissue GSH and GSSG levels were 7.95 and 0.70 nmol/g protein, respectively. These levels were slightly lower than those observed after ischemia/reperfusion (compare with Table 1).

Glutathione in Rat Heart Effluent and Tissue

Control and cardioplegic control rat hearts released no GSH and GSSG. GSH efflux increased rapidly to 5.4±0.8 and 0.8±0.2 nmol·min⁻¹·g wet wt⁻¹ in the ischemic (Fig 2A) and cardioplegic/ischemic (Fig 2B) group, respectively (P<.001). In the ischemic group, GSSG efflux amounted to 1.0±0.3 nmol·min⁻¹·g wet wt⁻¹ at 10 minutes of reperfusion (P<.05 versus baseline, Fig 2A). In the cardioplegic/ischemic group, the pattern of GSSG release was similar to that in the ischemic group; however, the values were five times lower (P<.05; compare Fig 2A and 2B).

Infusion of cumene hydroperoxide in normoxic hearts caused a biphasic release of both GSH and GSSG (Fig 3). GSH release was of the same order of magnitude as in rat hearts made ischemic (compare with Fig 2A). GSSG efflux was five times higher than in human and ischemic rat hearts, approximately 10 nmol·min⁻¹·g⁻¹ (see Figs 1 and 2A). After 15 minutes of infusion, there was a second burst of GSH and GSSG release, coinciding with arrest of the hearts and a decrease in flow.

Tissue GSH content was 35% lower in the ischemic group than in the control group (P<.05, Table 2). GSSG

![Graphs showing release of reduced glutathione (GSH) and oxidized glutathione (GSSG) in isolated rat hearts.](http://circres.ahajournals.org/)

**Fig 2.** Bar graphs show the release of reduced glutathione (GSH) and oxidized glutathione (GSSG) in isolated rat hearts. Data were obtained after 20 minutes of no-flow ischemia (A) or after 40 minutes of cardioplegia plus 20 minutes of no-flow ischemia (B). The data indicate that signs of oxidative stress were reduced in hearts that underwent long-term hypothermic cardioplegia. Values are mean±SEM (n=6 to 7). Baseline values (B) before the onset of ischemia were not detectable (ND, <0.01 nmol·min⁻¹·g wet wt⁻¹). Note the difference in scale of the y axes. *P<.05 vs baseline.
content was similar in these two groups. The ratio between GSH and GSSG in the control group (71±7) decreased 34% in the ischemic group (P<.005 versus the control group). The hearts subjected to 40 minutes of cardioplegia showed a different pattern (Table 2): GSH content of the control group showed a 32% (P<.05) decrease due to cardioplegia. Cardioplegia with and without ischemia/reperfusion did not affect the GSH/GSSG ratio (Table 2).

Tissue GSH and GSSG content in the ischemic group at 1 minute of reperfusion was 11.5±1.1 and 0.22±0.08 μmol/g protein, respectively. These are similar to those at 30 minutes (Table 2).

Hearts subjected to cumene hydroperoxide contained 3.9±0.8 μmol GSH/g protein, i.e., three times lower than GSH in control hearts (P<.05). GSSG content was higher than in control hearts (0.45±0.11 μmol/g protein, *P<.05). The ratio of GSH to GSSG had decreased by a factor of 6 (P<.005).

In rat hearts, an imbalance also existed between loss of tissue glutathione and total efflux, although it was less pronounced than in human preparations. Rat heart content decreased by 400 nmol/g wet wt, whereas GSH+GSSG release in 30 minutes was 200 nmol/g.

**MDA in Effluent and Tissue of Both Species**

No MDA was detected in the human arterial or venous samples during the whole period of perfusion and reperfusion (detection limit, 0.02 nmol·min⁻¹·g wet wt⁻¹). In addition, we could not detect any MDA in control or ischemic/reperfused human tissue samples (detection limit, 4 nmol/g protein). MDA efflux could not be measured because of an interfering peak that coincided with MDA. This peak was not present in the effluents of the ischemic hearts and must be related to the peroxide stress. Tissue MDA amounted to 14 nmol/g protein at the end of drug infusion (n=1).

Control and ischemic/reperfused rat hearts, with or without cardioplegia, released no detectable MDA. Cumene hydroperoxide infusion caused a rapid increase in MDA release, reaching a maximum of 13.2±1.5 nmol·min⁻¹·g wet wt⁻¹ after 20 minutes of perfusion (Fig 4).

Tissue MDA content determined in rat hearts is shown in Fig 5. Tissue MDA content in ischemic/reperfused rat myocardium at the end of 30 minutes of reperfusion was similar to control values, as was MDA in the cardioplegic groups. Cardioplegia induced an insignificant decrease in MDA content, both in control and ischemic/reperfused hearts. MDA content in rat hearts perfused with cumene hydroperoxide increased 3.6 times (P<.01 versus control hearts).

**Discussion**

**Glutathione as Indicator of Oxidative Stress**

Tissue GSH content after ischemia/reperfusion decreases as a result of a combination of washout and conversion to GSSG, thereby decreasing the ratio of tissue GSH to GSSG.**29** GSSG release after ischemia is an important indicator for oxidative stress.**29,30** We observed GSH and GSSG release from human and rat hearts after

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**TABLE 2. Decrease in Rat Heart Glutathione due to Cardioplegia and/or Ischemia/Reperfusion**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without Cardioplegia</th>
<th>After Cardioplegia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Isch/Rep</td>
</tr>
<tr>
<td>GSH, μmol/g protein</td>
<td>14.5±1.3</td>
<td>9.4±0.7*</td>
</tr>
<tr>
<td>GSSG, μmol/g protein</td>
<td>0.22±0.03</td>
<td>0.23±0.03</td>
</tr>
<tr>
<td>Ratio</td>
<td>70.9±7.3</td>
<td>47.1±8.2*</td>
</tr>
</tbody>
</table>

Isch/Rep indicates ischemia/reperfusion; GSH, reduced glutathione; GSSG, oxidized glutathione; and ratio, GSH/GSSG. Values are mean±SEM (n=6 to 9).

Two groups of hearts received 40 minutes of cardioplegia before the start of the perfusion, and two groups did not. After baseline perfusion for 30 minutes, hearts were subjected to normoxic perfusion for another 30 minutes (control) or to 20 minutes of global ischemia and 30 minutes of reperfusion (Isch/Rep). Hearts were freeze-clamped at the end of the perfusion. The data indicate that Isch/Rep lowers the cardiac GSH but does not increase GSSG content. In addition, the data show that cardioplegia induces loss of GSH.

*P<.05 vs control without cardioplegia.
ischemia (Figs 1 and 2), with changes in tissue contents in accordance with literature data. This release of GSSG reflects a production, because tissue GSSG levels are comparable after 1 and 30 minutes of reperfusion. Tissue content does not increase, because GSSG is actively transported from the cell. Our isolated human heart data are in agreement with the clinical results of Ferrari et al.\cite{Ferrari}

Because of cardioplegia, rat hearts lost large amounts of glutathione (Table 2). This could explain the relatively low release from the human hearts, which were also subjected to cardioplegia.

We cannot rule out the possibility that the diseased human hearts used differ in scavenging ability and behavior from normal hearts. In this respect, it is noteworthy that hypertrophied rat hearts have higher glutathione peroxidase activities.\cite{Hypertrophied}

In our experiments, there was an apparent loss of GSH, as evidenced by the imbalance between GSH+GSSG release and decrease of tissue content. Others attributed a similar finding to the formation of glutathione S-conjugates,\cite{S-conjugates} mixed disulfides, or dipeptides\cite{Dipeptides} during reperfusion.

**MDA Detection**

MDA, generated during lipid peroxidation, can react with thiobarbituric acid, forming a colored complex, but so do various other compounds. Several reports confirm that the colorimetric assay, based on this principle, is not a reliable index for lipid peroxidation.\cite{Colorimetric, Assay}

With these studies in mind, we have opted for the direct quantitation of MDA by HPLC.\cite{HPLC} A novel finding is the absence of MDA in human myocardial tissue and the lack of MDA release after a considerable ischemic period.

**MDA as Free Radical Indicator**

MDA formation, assessed by the thiobarbituric acid assay, was significant in humans during pacing stress testing\cite{Pacing} and coronary angioplasty.\cite{Angioplasty} Both interventions disturb the cardiac oxygen supply/demand balance for short periods, ie, minutes. In view of the discrepant literature data described in the introduction, we subjected isolated hearts from humans and rats to ischemia/reperfusion. Under these conditions, MDA was undetectable (\(<0.02 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{g wet wt}^{-1}\)) in the cardiac effluent of both species during reperfusion, regardless of the use of cardioplegia; this finding confirms the (rat heart) data of Julicher et al.\cite{Julicher} Under physiological conditions, MDA was present in rat myocardial tissue (41 nmol/g protein), in accordance with literature data.\cite{MDAcontent, Humans}

The lack of MDA in human ischemic myocardial tissue could be explained in several ways. First, cardioplegia could have affected MDA content (we took our control biopsies just before ischemia). Second, the state of these diseased hearts may have interfered. We used hearts from patients undergoing transplantation for end-stage cardiac disease. Turkeys with cardiomyopathy are known to have lower lipid peroxidation.\cite{Cardiomyopathy} Nevertheless, our preliminary data indicate that cumene hydroperoxide can induce MDA formation in human heart, but the tissue value remained 10 times lower than that in rat hearts. Third, species-dependent membrane susceptibility to lipid peroxidation could explain differences between human and rat MDA tissue content.\cite{Species}

Rat heart MDA content after ischemia/reperfusion in the noncardioplegic group showed a tendency to increase (Fig 5), in agreement with the data of Ceconi et al.\cite{Ceconi}

Subject to more drastic stress (cumene hydroperoxide), rat hearts released substantial amounts of MDA (Fig 4), as reported in earlier work.\cite{Hydroperoxide} The myocardial MDA content increased significantly in comparison with

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**Fig 4.** Bar graph shows the release of malondialdehyde (MDA) from rat hearts during peroxide infusion. After a stabilization period of 30 minutes, hearts were perfused for 30 minutes with 0.5 mmol/L cumene hydroperoxide in a recirculating manner. MDA was determined by high-performance liquid chromatography. The data suggest that substantial lipid peroxidation due to hydroperoxide infusion occurred. Data are expressed as mean±SEM (n=6). *P<.05 vs baseline.

**Fig 5.** Bar graph shows malondialdehyde (MDA) content of rat myocardial tissue subjected to cardioplegia, ischemia/reperfusion, or peroxide stress. After baseline perfusion for 30 minutes, control hearts were subjected to normoxic perfusion for another 30 minutes, and ischemic hearts were subjected to 20 minutes of global ischemia and 30 minutes of reperfusion. A series of normoxic hearts was infused for 30 minutes with cumene hydroperoxide (CumOOH). Two additional groups received 40 minutes of cardioplegia before the protocol for the control or ischemic hearts. Hearts were freeze-clamped at the end of the perfusion protocol. The data indicate that only a drastic stress such as CumOOH induced a significant rise in MDA. In contrast, cardioplegia lowered the MDA content. Values are mean±SEM (n=6 to 9). #P<.05 vs control.
control hearts (Fig 5). This suggests that MDA release from the heart or an increase in its myocardial content occurs only under extreme conditions like exposure to toxic hydroperoxide concentrations; then the endogenous scavenging systems apparently cannot cope with the large amounts of free radicals formed.

The absence of MDA formation in the isolated human heart preparations sheds doubt on the reports on MDA found in clinical studies. Until now, the source of free radicals was unclear. False-positive reports could be ascribed to the classic, aspecific, colorimetric thiobarbituric acid assay for MDA, which also measures other aldehydes.

**Effect of Cardioplegia on Glutathione and MDA Content**

Although cardioplegia followed by ischemia might be beneficial for postischemic functional recovery, restoration of blood flow paradoxically poses a threat to the myocardium. Concomitant with reoxygenation, superoxide radical formation, as well as washout of important cellular compounds, takes place.

We found that GSH levels decreased by a factor of 1.5 as a result of cardioplegia in control rat hearts (Table 2). This can be explained by two factors: (1) massive oxidative stress upon reperfusion with conversion of GSH to GSSG or (2) washout of large amounts of GSH. At the start of the perfusion, GSH release is extensive without detectable amounts of GSSG (unreported data). Therefore, we think that the second explanation is valid. The imbalance in human and rat hearts increased as a result of cardioplegia; it is probably caused by loss at the start of the perfusion. Thus, supplementation of a cardioplegic solution with GSH may have beneficial effects. In line with this hypothesis, Menasché et al. recently showed that the addition of N-acetylcysteine to cardioplegia improved hemodynamic recovery.

Ischemia did not induce a decrease in GSH content in cardioplegic rat hearts (Table 2). In contrast, GSH levels in human hearts decreased (Table 1). We are unable to offer an explanation for this difference.

Hypothermic cardioplegia lowers the MDA content nonsignificantly in rat hearts (Fig 5). Ceconi et al. observed a decrease of MDA tissue content during ischemia; MDA returned to aerobic values after reperfusion of flow. These workers hypothesized that MDA levels might be closely related to oxygen tension. Since flow was absent after the cardioplegic period, oxygen levels, and therefore MDA content, could have decreased.

**Source of Free Radicals or of MDA**

In vivo chemotaxis of leukocytes to injured myocardial tissue, e.g., after myocardial infarction, is known to induce free radical formation.9-40 The severity of the myocardial injury is correlated with the serum MDA concentration.41 Such a mechanism, however, does not explain the immediate release of MDA after short ischemic periods. Also, platelets adhere to arterial lesions and atherosclerotic plaques, and MDA formation is closely related to prostaglandin synthesis by platelets.42 MDA formation observed during coronary angioplasty may originate from the atherosclerotic lesion, as balloon inflation crushes the plaque and adhering platelets. On the basis of literature data, we assume that xanthine oxidoreductase plays only a minor role in free radical generation in human hearts (for review, see References 23 and 43). However, Abadeh et al. found an inactive form of human milk xanthine oxidase that produces superoxide radicals through NADH oxidation by the flavin adenine dinucleotide group. Human myocardium could contain a similar xanthine oxidoreductase responsible for radical-induced damage.

On the basis of the tissue GSH content and release of GSSG, human and rat hearts seem to have a similar vulnerability for oxidative stress. However, other systems like the pentose-phosphate cycle or GSH transferases can also change GSH and GSSG levels inside the cell under ischemic conditions.

**Limitations of the Study**

GSH release may have been underestimated, since we were unable to collect samples every minute early during reperfusion in rat hearts. GSH could have been converted to GSSG during recirculation, resulting in an overestimation of the GSSG efflux data. GSH and GSSG can be taken up by the heart.45 Consequently, this would change tissue content and underestimate glutathione efflux. We are unable to assess from the experiments conducted to what extent these processes affected our results.

The increase in human heart weights evidences edema, despite the presence of the polymer dextran in the perfusion buffer. Dextran used in rat heart perfusions did not influence glutathione or MDA levels (data not shown). We are not sure whether edema influenced GSH and GSSG release.

As already mentioned, the explanted diseased human hearts may differ from normal hearts in their MDA-producing potential or scavenging ability. However, hearts of cardiac patients undergoing coronary angioplasty or other procedures could have changed to a similar, but smaller, extent.

**Conclusion**

After ischemia, isolated human and rat hearts released GSSG, which indicates that oxidative stress has occurred. Lipid peroxidation (as measured by MDA formation) took place in control rat hearts, decreased nonsignificantly after cardioplegia, but did not increase after ischemia/reperfusion. Substantial amounts of MDA were formed under extreme conditions, i.e., perfusion with cumene hydroperoxide. Then, scavenging systems seemed unable to cope with the amounts of free radicals formed. The novel finding that human hearts lack MDA under normoxic conditions and after ischemia possibly reflects a lower MDA-producing potential. MDA found in clinical studies could come from another source or could be due to the use of the aspecific thiobarbituric acid assay.

**Acknowledgments**

This study was supported by a grant from The Netherlands Heart Foundation (88.253). We are grateful to the Rotterdam Heart Transplantation Team for the supply of explanted human hearts; to R. Krams MD, PhD, for advice on the perfusion system; to R. Zuiderent, BSc, and Ms A.S. Nieukoop for improvement of the MDA determination; to R.D. Caljouw,
MD, and Ms E. Keijzer for technical assistance; and to Ms C.D.M. Poleon-Weghorst for secretarial help.

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Malondialdehyde and glutathione production in isolated perfused human and rat hearts.
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doi: 10.1161/01.RES.73.4.681

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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