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B-180 Med Labs
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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q. Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

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Glucose Metabolism and Cancer Therapy

by

Iman Ahmad

B-180 Medical Laboratories
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

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<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
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<tbody>
<tr>
<td>CAT, catalase</td>
<td>$^1$O$_2$, singlet oxygen</td>
</tr>
<tr>
<td>CoQ, coenzyme Q</td>
<td>ROS, reactive oxygen species</td>
</tr>
<tr>
<td>G-6PDH, glucose-6phosphate dehydrogenase</td>
<td>RNS, reactive nitrogen species</td>
</tr>
<tr>
<td>GR, glutathione reductase</td>
<td>SOD, superoxide dismutase</td>
</tr>
<tr>
<td>GPx, glutathione peroxidase</td>
<td>TCA, tricarboxylic acid</td>
</tr>
<tr>
<td>GSH, glutathione</td>
<td></td>
</tr>
<tr>
<td>MAPK, mitogen activated protein kinase</td>
<td></td>
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<tr>
<td>NAC, N-acetyl cysteine</td>
<td></td>
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<td>NADP$^+$, nicotinamide adenine dinucleotide phosphate</td>
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Abstract

Oxidative stress results when the balance between the production of reactive oxygen species (ROS) overrides the antioxidant capability of the target cell. Cancer cells have low level of main antioxidant enzymes and exhibit a defective respiratory mechanism that leads to increase in steady state levels of hydroperoxide production. In this regard glucose metabolism appears to be an integral component of cellular peroxide detoxification pathways. The current review shows the involvement of ROS in the process of carcinogenesis, and how ROS can be involved in cancer treatment by glucose deprivation. Although the source of the increased prooxidant production is currently unknown, the review presented about glucose deprivation studies on cancer cells led to the proposal that mitochondrial hydroperoxide formation causes oxidative stress and cytotoxicity during glucose deprivation.

Introduction

Mammalian cells continuously produce reactive oxygen species (ROS) through different metabolic pathways. ROS are molecules that contain oxygen and have higher reactivity than ground-state molecular oxygen. These species include not only the oxygen radicals (like $\mathrm{O}_2^-$, $\cdot\mathrm{OH}$, and peroxyl radicals), but also non-radical molecules like
H$_2$O$_2$ and $^1$O$_2$. Superoxide is formed during the reduction of O$_2$ by the mitochondrial electron transport system [1]. Eukaryotic cells are equipped with an antioxidant system capable of converting ROS to H$_2$O via different cytosolic enzymes. Oxidative stress results when the balance between the production of ROS overrides the antioxidant capability of the target cell. It is generally thought that low levels of ROS are not harmful to cells and indeed even perform useful functions; high levels of ROS are detrimental through reactions with and modifying cellular protein, lipid, and DNA which results in altered target cell function. The accumulation of oxidative damage has been implicated in both acute and chronic cell injury inducing possible participation in the formation of cancer. Acute oxidative injury may produce selective cell death or sublethal injury, such as mutations, chromosomal aberrations or carcinogenesis [2,3]. In contrast, chronic oxidative injury may lead to a non-lethal modification of normal cellular growth control mechanisms. Cellular oxidative stress may modify intracellular communications, protein kinase activity, membrane structure and function, and gene expression, and result in modulation of cell growth [3]. It has been found that cancer cells demonstrate altered metabolism when compared to normal cells [4-6]. Warburg [4] suggested that cancer was a metabolic disease in which respiration was damaged and anaerobic fermentation was increased resulting in a malignant phenotype. Weber [5-6] suggested that cancer cells exhibit increased rate of pentose phosphate pathway activity that is characterized by an increase in glucose-6-phosphate dehydrogenase. On the other hand, Oberley et al. [8] suggested that tumor cells have increased steady state levels of O$_2^\cdot$ and H$_2$O$_2$ associated with aberrant respiration, which causes damage and inactivate signaling pathway leading to malignant phenotype. Therefore, it appears that tumor cells have an aberrant respiration, which is compensated by an increase in glycolysis and pentose phosphate
cycle activity as a compensatory mechanism to protect themselves from an increase in the steady state level of H$_2$O$_2$ [4-6]. The generation of O$_2^•$ and H$_2$O$_2$ within the mitochondria may pose a threat to the cancer cell mitochondria themselves since they are nearly always low in antioxidant enzymes. Glucose metabolism has been shown to be involved with cellular sensitivity to oxidative stress mediated by hydroperoxide, presumably via the formation of pyruvate and NADPH [13]. Since mitochondrial metabolism would be the preferred route of energy production during glucose deprivation, it is reasonable to hypothesize that glucose deprivation would create a metabolic state where O$_2^•$ and H$_2$O$_2$ concentrations were increased and peroxide scavenging via pyruvate as well as NADPH dependent reactions was decreased. This would be expected to result in a condition of metabolic oxidative stress characterized by increased levels of pro-oxidant production and increased levels of oxidized glutathione.

In this review several areas of literature that point to the involvement of O$_2^•$ and other oxygen species in the process of carcinogenesis and cancer will be reviewed. Data summarizing what is known about the variation in antioxidant enzymatic levels in tumor tissue will be presented. The role of glucose metabolism in cancer protection against increased level of ROS will be discussed. Finally, this study will focus on glucose deprivation studies in different human tumor cells that would be expected to result in increased level of pro-oxidant production as a way of treating cancers, and hypothesizing that mitochondrial H$_2$O$_2$ is the prooxidant.

The literature cited would be introduced in order to support the following suppositions upon which this review is based:

1) The decrease in tumor cell antioxidant enzyme activity is an important biochemical difference between tumor and normal cells.
2) The loss of naturally occurring antioxidant enzyme activity in tumor cells and reduced rates of respiration due to "damage" to the respiratory mechanism could lead to an increased flux of free-radicals produced from electron transport chain associated phenomena. We hypothesize that tumor cells were thought to compensate for this defect by increasing glycolysis, and pentose phosphate pathway.

3) Glucose deprivation induces cytotoxicity and oxidative stress in a variety of transformed human cell types.

4) Transformed and untransformed human cells are differentially susceptible to glucose deprivation-induced cytotoxicity and oxidative stress.

5) Glucose deprivation-induced cytotoxicity and oxidative stress are dependent upon O$_2$ metabolism.

6) Mitochondrial H$_2$O$_2$ formation causes oxidative stress during glucose deprivation.

**Superoxide production in tumor cells**

The mitochondrial respiratory chain consists of a series of redox catalysts (pyridine nucleotide, flavoproteins, iron sulfur proteins, ubiquinone and cytochromes) which when assembled constitute the mitochondrial inner membrane. These respiratory carriers are arranged according to their redox potentials. The mitochondria have long been known to be one of the major cellular sources of O$_2^-$ ions. 1-2% of the electrons that traverse the respiratory chain ends up as stray O$_2^-$ ions [1]. Much of the original research into ROS production from mitochondria suggested that the majority of these O$_2^-$ originate from complex III [15-16]. However recent work clearly demonstrates that complex I also plays a major role in the production of O$_2^-$ in mitochondria [17]. In
addition, Ishii et al. have suggested based on their work with nematodes that $O_2^\cdot $ may also arise from complex II [18]. Clearly there are a number of potential sources of ROS within the mitochondrial respiratory enzyme chain. Almost, all of these studies have shown that normal mitochondria produces $O_2^\cdot$ by utilizing different electron transport chain inhibitors as, antimycin A, which is known to inhibit the transfer of electrons from cytochrome b to coenzyme Q (CoQ), myxthiaazole, which prevents the transfer of electrons from the reduced CoQ to to the FeS (III) (the iron sulfur protein) and then to cytochrome c [19], and rotenone which is specific blocker of NADH-dehydrogenase activity in the mitochondria electron transport chain of site I [21] (Figure 1) [Modified from 21].

![Figure 1](image-url)  
*Figure 1.* Scheme showing the sites of $O_2^\cdot$ production in the mitochondrial respiratory chain [Modified from 21].
Many studies have been done on the production of $O_2^{\bullet-}$ in tumor cells. Almost all these studies have shown that tumor cells produce $O_2^{\bullet-}$, by utilizing electron transport chain inhibitors or uncouplers in order to increase $O_2^{\bullet-}$ production [22-23]. However, Docampo et al. have studied $O_2^{\bullet-}$ production in intact mitochondria from mouse sarcoma 180 cells in the presence of NADH without respiratory chain inhibitors [24]. They found that the tumor did indeed produce $O_2^{\bullet-}$. Dinescu-Romalo and Mihai have performed a study of $O_2^{\bullet-}$ production in several tissues from normal and Guerin T$_8$ ascites tumor-bearing rats without inhibitors [25]. They found that the tissues from tumor bearing animals produced $O_2^{\bullet-}$ at a much higher rate than the same tissues from non-tumor bearing animals. The results of all of these studies on $O_2^{\bullet-}$ production in tumor cells seems to suggest that tumor cells mitochondria are capable of producing $O_2^{\bullet-}$. The mitochondria of malignant human tumor cells have been shown to exhibit histological pleomorphism when compared to the mitochondria from normal human cells [26]. This pleomorphism is manifested in the abnormal arrangements of the mitochondrial cristae, mitochondrial hypertrophy, and the fragmentation of mitochondria observed with an electron microscope.

**Protection mechanism**

It is well proven that ROS will be present in high amounts when cells are exposed to any form of stress [33]. These ROS include non-organic molecules, such as $O_2^{\bullet-}$, $H_2O_2$ and $^\bullet OH$, as well as organic molecules such as alkoxy and peroxyl radicals. ROS are continuously generated during oxidative metabolism. In order to avoid damage caused by ROS, mechanisms exist which remove ROS or prevent the generation of ROS [29-30]. For example, the removal of $O_2^{\bullet-}$ and $H_2O_2$ prevents the generation of $^\bullet OH$, which are formed by the iron-catalyzed Fenton reaction or by Haber-Weiss reaction [31-32], and
are the most reactive species within the ROS family. The mechanism of removal of $O_2^{-}$ and $H_2O_2$ is as follow [33]:

![DIagram](image)

Many studies have shown that cancerous cells have lower MnSOD and catalase activity and that glutathione peroxidase activity is variable in different tumor tissues [11]. How then cancer cells remain viable?

Since oxygen free radicals are produced by mitochondrial membrane bound electron transport chain and mitochondria have been shown to be structurally abnormal in almost all malignant tumor cells and exhibit low level of antioxidant enzymes, it is reasonable to increase their glycolysis and pentose phosphate pathway as a compensatory mechanism to protect themselves via the formation of pyruvate and NADPH. In this regard glucose metabolism appears to be an integral component of cellular peroxide detoxification pathways, which is explained in the following section [13,27-28].

**Glucose metabolism**

Carbohydrates serve as the primary source of energy in the cell and carbohydrate metabolism is central to all metabolic processes, which can be accomplished either anaerobically or aerobically, resulting in the synthesis of ATP. After the formation of glucose-6-phosphate, the major pathways of glucose metabolism include glycolysis and
pentose phosphate cycle [7,21]. Glycolysis results in the formation of pyruvate, and the pentose phosphate cycle results in the formation of NADPH [7,21]. Pyruvate is an important junction point in glucose metabolism and a substrate for the formation of acetyl-CoA, an entry point to the TCA cycle and mitochondrial oxidative phosphorylation [7,21,34]. As early as 1904, it was shown that \( \text{H}_2\text{O}_2 \) causes a rapid non-enzymatic and stoichiometric decarboxylation of pyruvate to acetic acid, \( \text{H}_2\text{O} \) and \( \text{CO}_2 \) [35]:

\[
\text{H}_3\text{C} - \text{C} - \text{C} - \text{OH} + \text{H}_2\text{O}_2 \rightarrow \text{H}_3\text{C} - \text{C} - \text{OH} + \text{H}_2\text{O} + \text{CO}_2
\]

pyruvate \hspace{1cm} \text{acetic acid}

It was recently discovered that mammalian cells secrete pyruvate as an antioxidant defense to \( \text{O}_2 \) radicals [36], and other hydroperoxides [27]. NADPH, besides being the source of reducing equivalents for the glutathione/glutathione peroxidase/glutathione reductase system, has also been shown to participate in the metabolic decomposition of \( \text{H}_2\text{O}_2 \) and organic hydroperoxides [28]. Therefore, in addition to the well-known role of glucose metabolism in energy production, it appears to be involved with cellular sensitivity to oxidative stress mediated by hydroperoxides as by products of oxidative metabolism presumably via the formation of pyruvate and NADPH [13]. It has been shown that increasing glucose concentrations in the tissue culture media render CHO cells resistant to \( \text{H}_2\text{O}_2 \)-induced cytotoxicity [13]. A schematic diagram showing the role of glucose metabolism as an energy source and detoxifying pathway of \( \text{H}_2\text{O}_2 \) is shown in Figure 2.
Recently, it has been discovered that simply removing glucose from the cell culture medium (glucose deprivation) induces cytotoxicity and oxidative stress in human tumor cells [37-39]. It also appears that transformed human cell types may be more susceptible to glucose deprivation induced cytotoxicity and oxidative stress, relative to normal human cell types [37,39].

**Glucose deprivation studies**

Glucose is necessary for survival of cancer cells from pro-oxidants that are produced during respiration. Lee et al. showed that glucose deprivation causes cytotoxicity in the MCF-7/ADR human multidrug-resistant breast carcinoma cell line, as shown in Figure 3 [38].
Also, it was shown that glucose deprivation-induced cytotoxicity was inhibited by adding glucose into the medium, supporting that survival was dependent on the concentration of glucose. Glucose deprivation-induced cytotoxicity in this model system was found to be preceded by activation of MAPK as determined by ERK-2 phosphorylation [38]. Several researchers have shown that ERK-2 is activated via phosphorylation of tyrosine and threonine residues [40-41]. Moreover, it has been shown that there is a rapid activation of several other signal transductions pathways such as, Lyn kinase (a src family kinase), c-Jun N-terminal kinase (JNK), MEK, Raf, PKC, and Ras [14,39,45-46]. Furthermore, in glucose-deprived MCF-7/ADR cells, it was shown that there was an increase in the DNA-binding activity of AP-1 transcription factor as well as an increase in the angiogenic factor, basic fibroblast growth factor (bFGF) [14,44]. Finally, the involvement of mitochondrial metabolism in glucose deprivation–induced cytotoxicity might be supported by the protection of these same cell line from glucose deprivation-induced cytotoxicity by the over-expression of the mitochondrial protein, Bcl-2 [44]. These results show that glucose deprivation of these human tumor cell line results in cytotoxicity, activation of signal transduction, and increased gene expression. Metabolic oxidative stress was suggested to

![Figure 3. Effect of addition of glucose on glucose deprivation-induced cytotoxicity in MCF-7/ADR cells. Cell monolayers were washed free of glucose, and then placed into media containing the indicated concentrations of glucose. Cell survival after treatment with glucose-free medium (■) or various concentrations (0.001-1 mM) of glucose-supplemented medium for various intervals (up to 16 h) [38].](image)
be the mechanism by which glucose deprivation causes cytotoxicity and alterations in signal transduction as evidenced by a significant increase in the steady state level of oxidized glutathione (GSSG) induced by 4 h of glucose deprivation on the same human tumor cell line as shown in Table 1[38].

![Table 1](image)

<table>
<thead>
<tr>
<th></th>
<th>Total glutathione</th>
<th>GSH</th>
<th>GSSG</th>
<th>Ratio GSH/GSSG</th>
</tr>
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<tbody>
<tr>
<td>+Glucose</td>
<td>9.1 ± 2.3</td>
<td>6.9 ± 1.9</td>
<td>1.1 ± 0.3</td>
<td>6.3</td>
</tr>
<tr>
<td>−Glucose</td>
<td>17.7 ± 3.7a</td>
<td>10.9 ± 1.5a</td>
<td>3.4 ± 1.8a</td>
<td>3.2</td>
</tr>
</tbody>
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*a Significantly different from +glucose control (p < 0.05). Errors represent ±1 S.D. of the mean of three separately collected samples.

Although, there is an increased in GSH synthesis in glucose deprived MCF-7/ADR cells in an attempt to counteract the increased production of intracellular pro-oxidants, but it is clear that the increase in GSH content is not sufficient to counteract the pro-oxidant production as evidenced by the cytotoxicity and accumulation of GSSG. It has been shown previously that ERK-1/ERK-2 were activated by oxidants (such as H₂O₂) and suppressed by reductants [42-43]. This led to the hypothesis that reductants such as NAC could suppress the activation of MAPK induced by glucose deprivation in MCF-7/ADR. This hypothesis was supported by the findings that NAC inhibited the prolonged activation of ERK-2 [38], and the sustained activation of Lyn kinase and JNK [39] (Figure 4).
Moreover, the addition of 1mM of NAC completely protected the cells against glucose deprivation-induced cytotoxicity in MCF-7/ADR cells as shown in Figure 5 [38].
There are several possibilities for how NAC could inhibit oxidative stress during glucose deprivation. One of these possibilities could be by increasing the intracellular cysteine pools, which could directly scavenge radicals as well as provide vital substrates (as cysteine and γ-GC), for increased synthesis of glutathione (Figure 6) [39].

The major findings of these work is that glucose deprivation induced oxidative stress appears to be account for the cytotoxicity and alterations in signal transduction in MCF-7/ADR cells, and that prooxidant production is the cause for that oxidative stress, which, was measured by using the oxidation-sensitive probe (Table 2) [38]. The increases in mean fluorescence intensity of the probe are interpreted to indicate increases in intracellular pro-oxidant production, and hyroperoxide involvement is inferred by suppressing fluorescence with hyroperoxide scavenging, such as pyruvate [38].
However, the source of the increased prooxidant production caused by glucose deprivation is currently unknown, but several observations support intracellular hydroperoxide production by mitochondrial metabolism may be involved [38].

Spitz et al. showed that glucose deprivation-induced cytotoxicity and oxidative stress occurs in several human transformed cell lines such as SV40 human transformed fibroblasts (IMR90 SV40, GM00637G), and human colon cancer cells (HT-29), and it is not limited to a single type of transformed human cell type (MCF-7/ADR) (Figure 7) [37].

![Figure 7](image_url)

**Figure 7.** Clonogenic cell survival data showing the differential susceptibility of untransformed (IMR90, GM00037F, Aorta) and transformed (IMR90 SV40, GM00637G, HT29) human cell types to glucose deprivation-induced cytotoxicity. Asterisks indicate the significant differences between +Glu and −Glu from each cell line (p < 0.05) [37].

### Table II

<table>
<thead>
<tr>
<th>Survival&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fluorescence intensity&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Glucose</td>
<td>100</td>
</tr>
<tr>
<td>– Glucose</td>
<td>20</td>
</tr>
<tr>
<td>– Glucose + 10 mm glutamate</td>
<td>95</td>
</tr>
<tr>
<td>– Glucose + 1 mm pyruvate</td>
<td>80</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent survival is normalized to the respective control (+Glu).<br>
<sup>b</sup>Mean F.I. of 25,000 cells analyzed by flow cytometry. Mean F.I. was not different in glucose-deprived and glucose competent cells when labeled with the non-oxidation sensitive dye (C-369) showing that changes in F.I. are indicative of changes in prooxidant production [38].
Furthermore, transformed cells appear to be more susceptible to glucose deprivation-induced cytotoxicity and oxidative stress as compared to their normal matched pair cells as shown in Figure 7 [37]. This supports the speculation that cancer cells exhibit a defect in oxidative metabolism, which could be used as a therapeutic advantage when trying to kill cancer cells and spare the normal tissues. Moreover, it was shown that glucose deprivation-induced cytotoxicity in transformed cell line (GM00637G), is dependent upon the metabolism of O$_2$ [37]. Since most of O$_2$ metabolism occurs in the mitochondria, this result is consistent with the speculation that a defect in transformed cell’s respiratory mechanism may lead to an increase in the (ROS) responsible for glucose deprivation-induced cytotoxicity and oxidative stress.

**Summary**

All oxygen metabolizing cells are thought to produce a relatively low level of prooxidants, which is thought to be not harmful to cells and indeed even perform useful functions. On the other hand, cancer cells have a defective respiratory mechanism that leads to increase in steady state levels of hydroperoxide production. As a result, metabolism of glucose could be up regulated to produce more pyruvate and NADPH to compensate for this defect. In this regard glucose deprivation can be used as a therapeutic anticancer that will be expected to result in a condition of metabolic oxidative stress characterized by increased levels of prooxidant production. Although, the source of the increased prooxidant production is currently unknown, the review presented above led us to propose that mitochondrial H$_2$O$_2$ formation causes oxidative stress during glucose deprivation which is going to be studied by different experiments.
Future studies

During glucose deprivation, steady state levels of intracellular pro-oxidant (presumably H$_2$O$_2$) appear to increase immediately in cancer cells [38]. This suggests that H$_2$O$_2$ are being produced by ongoing metabolic processes and that the metabolic decomposition of these pro-oxidants is compromised by the removal of glucose, probably via a decrease in intracellular NADPH and pyruvate (Figure 2) [37]. It was hypothesized that pro-oxidant production is occurring via mitochondrial electron transport chain activity, because in the absence of glucose, mitochondrial metabolism (fatty acids and amino acids) would be the preferred route of energy production [21]. Therefore, it would be expected that glucose deprivation result in a condition of metabolic oxidative stress characterized by increased levels of pro-oxidant production, that leads to increases levels of oxidized glutathione and cytotoxicity [38].

Hypothesis

*Mitochondrial H$_2$O$_2$ formation causes oxidative stress and cytotoxicity in human tumor cells during glucose deprivation.*

To test this hypothesis three main areas will be researched: 1) Mitochondrial involvement in glucose deprivation-induced cytotoxicity and oxidative stress. 2) Pro-oxidant formation causes cytotoxicity and oxidative stress during glucose deprivation. 3) H$_2$O$_2$ is the pro-oxidant.

Specific aim #1: Mitochondria is involved in glucose deprivation-induced cytotoxicity and oxidative stress.

Many studies have shown that tumor cells produce O$_2$. by utilizing electron transport chain inhibitors in order to increase O$_2$. production [22,23]. The results of all of these studies on O$_2$. production in tumor cells seem to suggest that tumor cells
mitochondria are capable of producing $\text{O}_2^-$. Therefore, using these inhibitors (Antimycin A, Myxthiazole, Rotenone) during glucose deprivation will be expected to accelerate cytotoxicity and oxidative stress in human tumor cells from 48 h to 2 or 4 h, and that antimycin A would be expected to have the greatest effect due to its site of action as mentioned in the background.

To accomplish this goal, the human tumor cell line to be utilized in this study will include HT-29 colon carcinoma. This cell line was chosen based on preliminary results indicating that it demonstrates some degree of sensitivity to glucose deprivation-induced cytotoxicity [37]. This cell line will be deprived of glucose in the presence of the electron transport chain inhibitors for 2 to 4 hs. GSSG content will be used as an index of oxidative stress, and clonogenic cell survival will be used as an indicative of cytotoxicity. We expect more cytotoxicity and more GSSG accumulation with the use of antimycin A during glucose deprivation as compared to other inhibitors. These inhibitors then will show the involvement of mitochondria in glucose deprivation induced-cytotoxicity and oxidative stress.

**Specific aim #2: Pro-oxidant production causes cytotoxicity and oxidative stress during glucose deprivation.**

Lee *et al.* determined intracellular pro-oxidant production in MCF-7/ADR cells deprived of glucose for 2 h using the oxidative sensitive probe (C-400, 10 µg/ml) and flow cytometry [38]. They showed that mean fluorescent intensity is greater in the glucose deprived cells relative to the glucose competent cells. This probe indicates increases in intracellular pro-oxidant production.

In aim #2, the work done in aim #1 will be repeated using oxidation-sensitive (C-400, 10 µg/ml) and oxidation-insensitive (C-369, 10 µg/ml) fluorescent dyes. The oxidation-
insensitive dye acts as a control for changes in uptake, ester cleavage and efflux, so that any changes in fluorescence seen with the oxidation-sensitive dye will be directly attributed to changes in pro-oxidant production. We expect an increase in the mean fluorescent intensity in the glucose deprived cells relative to the glucose competent cells using oxidative probe and no changes in using insensitive probe; which indicates an increase in the intracellular pro-oxidant production that is responsible for cytotoxicity and oxidative stress seen during glucose deprivation in aim #1; and that this increase will be expected to be higher with the use of antimycin A.

**Specific aim #3: H$_2$O$_2$ is the pro-oxidant.**

The role of H$_2$O$_2$ in glucose deprivation-induced cytotoxicity and oxidative stress will be studied. As stated many studies have shown that tumor cells produce O$_2$•$^-$ [22,23], so by using transduction method, O$_2$•$^-$ can be converted into H$_2$O$_2$ and also can specifically be removed. Therefore, by using adenovirus-containing cDNA of MnSOD and mitochondrial targeted catalase will indicate the role of H$_2$O$_2$ in glucose deprivation-induced cytotoxicity and oxidative stress. The work done in aim #1 will be repeated using only antimycin A and MnSOD alone and in combination with adenovirus catalase with the proper controls. Using AdMnSOD, H$_2$O$_2$ formation will increase and expected to result in more oxidative stress and cytotoxicity during glucose deprivation. While catalase cotransfection would be expected to result in a removal of H$_2$O$_2$ and enhanced survival. This increase (during catalase cotransfection) and decrease (during MnSOD transfection) in H$_2$O$_2$ can be measured using electron paramagnetic resonance in the presence of spin trap DMPO. We expect to see a nice spectrum for DMPO/OH as an indication of H$_2$O$_2$ in AdMnSOD transfected cells while a disappearance of that spectrum in catalase cotransfected cells.
However, the inability of AdCAT to rescue cells from killing and oxidative stress during glucose deprivation, suggest the involvement of other pro-oxidant species (RNS) besides the ROS. Nitric oxide is produced during the oxidation of L-arginine to citrulline catalyzed by nitric oxide synthase (NOS). The recent findings of a mitochondrial enzyme (mNOS) in the inner mitochondrial membrane by Giulivi et al. [7,20], and by Ghadoufar and Richter [47] supports the idea of a physiological role for •NO in mitochondrial respiration. To test the effects of the endogenous •NO production on glucose deprivation-induced cytotoxicity and oxidative stress, the HT-29 cells will be supplemented with the inhibitor of NOS N(G)-monomethyl-L-arginine (NMMA) and deprived of glucose in the presence of antimycin A. The experiment then will be analyzed for survival and GSSG content as described before and compare the results with the control group.
References


35. Holleman MAF. (1904) Note on the action of oxygenated water on α-ketoacids and 1,2, dicetones. Recl Trav Chi Pays-Bas Belg. 23:169-172.


