Hydrogen peroxide does not. Depletion of GSH stores in both cytosol and mitochondria, as discussed by [2], showed that the inhibition of nitric oxide synthesis with N-nitro-L-arginine methyl ester hydrochloride or the peroxynitrite scavenger uric acid did not. Depletion of GSH stores in both cytosol and mitochondria enhanced the susceptibility of HepG2 cells to primary rat hepatocytes to 5% O2 exposure. However, this sensitization was abrogated by preventing mitochondrial ROS generation by complex I and II inhibitors. Moreover, selective mGSH depletion by \((R,S)\)-3-hydroxy-4-pentenoate that spared cytosol GSH levels sensitized rat hepatocytes to hydroxyl peroxide because of enhanced ROS generation. GSH restoration by GSH ethyl ester or by blocking mitochondrial electron flow at complex I and II rescued \((R,S)\)-3-hydroxy-4-pentenoate-treated hepatocytes to hypoxia-induced cell death. Thus, mGSH controls the survival of hepatocytes during hypoxia through the regulation of mitochondrial generation of oxidative stress.

In conditions of limited O2 supply, cells adapt and survive because of the existence of specific sensors that allow them to acclimate to the deprivation of oxygen and to recover from ischemic conditions. One of the best-characterized responses to hypoxia is the activation of hypoxia-inducible factor (HIF), a transcription factor that is central for the cellular adaptation to oxygen limitation as it is known to up-regulate genes involved in angiogenesis and glycolysis (1). HIF is a heterodimer comprising an \(\alpha\)-regulated \(\alpha\) subunit (HIF1\(\alpha\)) and a constitutively expressed \(\beta\) subunit (2–4). However, in conditions in which reoxygenation ensues oxygen deprivation (e.g. ischemia/reperfusion), the ROS stimulation then arises from both mitochondrial and extramitochondrial sources (5–6).

GSH is a major and versatile cellular antioxidant that is found mainly in cytosol where it is synthesized from its constituent amino acids and in mitochondria where it plays a key protective role against oxidant-induced cell death (11, 12). Because of its antioxidant function hypotha, hypoxia would be expected to reduce intracellular GSH stores. For instance, recent findings reported a reduction in cellular GSH in hypoxic human embryonic kidney 293 and Hep3B cells that required mitochondrial generation of ROS (13). Moreover, previous studies reported the decrease of hepatocellular GSH stores by hypoxia through various mechanisms ranging from enhanced GSH efflux to impaired synthesis (14–17). Furthermore, carbon monoxide has been reported to decrease the GSH/GSSG ratio in rat brain mitochondria (18).

Thus, although the regulation of intracellular GSH levels by hypoxia is known (13–18), the specific role that mitochondrial GSH (mGSH) plays in the survival of cells during hypoxia has not been reported to the best of our knowledge. Therefore, the aim of the present study was to examine the consequences of mGSH depletion during hypoxia on the regulation of ROS generation and survival of hepatocytes. Although the abrogation of mitochondrial ROS production rescued HepG2 cells or primary rat hepatocytes from hypoxia-induced cell death despite GSH depletion in both cytosol and mitochondria, using \((R,S)\)-3-hydroxy-4-pentenoate (HP), which is transformed in the mitochondria of hepatocytes into a Michael acceptor resulting in selective mGSH depletion with the sparing of cytosol...
Mitochondrial GSH and Hypoxia

GSH (19, 20). We provide evidence for a critical role of mGSH in determining the susceptibility of hepatocytes to hypoxia through control of mitochondrial oxidative stress.

MATERIALS AND METHODS

Reagents and Antibodies—GSH, GSH ethyl ester (GSEHE), buthionine-sulfoximine (BSO), diethylmaleate (DEM), antimycin A, rotenone, thioninfluroacetone (TTFA), diphenyleneiodonium, cyclosporin A, sodium orthovanadate, sucrose, and Igepal CA-360 were obtained from Sigma. Human recombinant TNF-α (44 units/mg of protein) was from Promega, ATP and dithiothreitol were purchased from Fisher Scientific Co. 2′,7′-Dichlorofluorescin diacetate was obtained from Molecular Probes (Eugene, OR). Caspase-3 inhibitor (Ac-DEVAD-CHO) and genistein were from Calbiochem.

Cell Culture and Incubation—The human hepatoblastoma cell line HepG2 was obtained from the European Collection of Animal Cell Cultures (Salisbury, Wilts, UK). Cells were cultured in Dulbecco's modified Eagle's medium containing high glucose levels, supplemented with 10% heat-inactivated (56 °C, 30 min) fetal bovine serum, 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Rat hepatocytes were prepared from Sprague-Dawley rats (250 g) by collagenase perfusion and were cultured as described previously (20, 21). Cells were maintained at 37 °C in a humidified incubator containing 21% oxygen and 5% carbon dioxide (referred to as normoxic conditions). Hypoxic conditions were attained by exposure to 2% or 5% oxygen (not shown) thus further validating published observations (7–9, 13). Furthermore, we examined the effect of inhibition of mitochondrial electron flow at distinct respiratory complexes on the stimulation of ROS in both cell types (Fig. 1, E and F). Further diphenyleneiodonium, which blocks flavin-linked enzymes including respiratory complex I (28), reduced the generation of ROS in hypoxic cells, whereas antimycin A, which blocks electron flow at the Q cycle within complex III, enhanced ROS formation caused by 5% O2 (Fig. 1E). Consistent with previous findings (8), because it was recently reported that rat liver mitochondria generated NO contributing to hypoxia-mediated oxidative stress (29), we examined the effect of l-NAME or uric acid, a peroxynitrite scavenger (30), on the stimulation of ROS, as DCF fluorescence is also indicative of peroxynitrite (31). l-NAME failed to prevent ROS stimulation by 5% O2 in primary hepatocytes and uric acid did not prevent the ROS stimulation caused by 5% O2. In contrast, MnTBAP, a superoxide anion scavenger, abrogated ROS generation during hypoxia (Fig. 1F). Furthermore, allopurinol (200 μM), a xanthine oxidase inhibitor, did not prevent the ROS stimulation caused by 5% O2 (not shown). Thus, these findings established that 5% O2 causes a burst of superoxide anion generated mainly from the mitochondria in both HepG2 cells or rat hepatocytes and that hepatoma HepG2 cells appear more resistant than primary cells to the oxidative stress induced by hypoxia. Moreover, we observed increased nuclear levels of HIF1α and enhanced DNA binding of NF-κB in HepG2 or primary hepatocytes subjected to 5% O2 incubation by ROS-dependent and independent mechanisms (not shown) thus further validating published observations (7–9) on the role of ROS in hypoxia-induced gene expression.

RESULTS

Hypoxia Stimulates Mitochondrial ROS Generation in HepG2 Cells and Rat Hepatocytes—Although the stimulation of ROS during hypoxia predominantly from mitochondria has been already described, we used this paradigm to specifically address the role of mGSH in the survival of hepatocytes to hypoxia. Furthermore, because previous findings showed a differential tolerance between hepatoma cells and primary hepatocytes to anoxia (27), we compared the ROS stimulation and survival of HepG2 cells and rat hepatocytes cultured under 2% and 5% O2. First we validated the source of ROS generated by hypoxia in hepatocytes. As seen, hypoxia stimulated a ROS generation in both HepG2 cells and primary rat hepatocytes with respect to normoxic incubation in a time-dependent fashion that increased with the severity of oxygen deprivation (Fig. 1, A and B). Furthermore, primary rat hepatocytes were more sensitive to hypoxia than HepG2 cells as reflected by the ROS stimulation and loss of survival observed at 2% O2 exposure (Fig. 1, C and D). Moreover, because previous studies demonstrated that the predominant source of ROS during hypoxia was the mitochondrial electron transport chain (7–9, 13), we examined the effect of inhibition of mitochondrial electron flow at distinct respiratory complexes on the stimulation of ROS in both cell types (Fig. 1, E and F). Furthermore diphenyleneiodonium, which blocks flavin-linked enzymes including respiratory complex I (28), reduced the generation of ROS in hypoxic cells, whereas antimycin A, which blocks electron flow at the Q cycle within complex III, enhanced ROS formation caused by 5% O2 (Fig. 1E), consistent with previous findings (8). Because it was recently reported that rat liver mitochondria generated NO contributing to hypoxia-mediated oxidative stress (29), we examined the effect of l-NAME or uric acid, a peroxynitrite scavenger (30), on the stimulation of ROS, as DCF fluorescence is also indicative of peroxynitrite (31). l-NAME failed to prevent ROS stimulation by 5% O2 in primary hepatocytes and uric acid did not prevent the ROS stimulation caused by 5% O2. In contrast, MnTBAP, a superoxide anion scavenger, abrogated ROS generation during hypoxia (Fig. 1F). Furthermore, allopurinol (200 μM), a xanthine oxidase inhibitor, did not prevent the ROS stimulation caused by 5% O2 (not shown). Thus, these findings established that 5% O2 causes a burst of superoxide anion generated mainly from the mitochondria in both HepG2 cells or rat hepatocytes and that hepatoma HepG2 cells appear more resistant than primary cells to the oxidative stress induced by hypoxia. Moreover, we observed increased nuclear levels of HIF1α and enhanced DNA binding of NF-κB in HepG2 or primary hepatocytes subjected to 5% O2 incubation by ROS-dependent and independent mechanisms (not shown) thus further validating published observations (7–9) on the role of ROS in hypoxia-induced gene expression.

GSH Depletion Sensitizes HepG2 Cells to 5% O2—To evaluate the role of GSH on survival during exposure to 5% O2, GSH levels were depleted in HepG2 cells during hypoxia. Initially we verified that, consistent with the effect on ROS generation, 5% O2 depleted GSH in both cytosol and mitochondrial compartments of HepG2 cells (Fig. 2A). However, when cells were incubated with DEM (an α-β unsaturated carbonyl electrophile, which is conjugated with GSH by GSH S-transferases) during the hypoxic period followed by BSO (a potent and specific inhibitor of the rate-limiting enzyme in GSH biosynthesis, γ-glutamylcysteine synthetase) to prevent any potential GSH...
recovery caused by the rapid induction of γ-glutamylcysteine synthetase by DEM (32), GSH levels in either compartment were severely depleted compared with the levels found in normoxic or hypoxic untreated cells (Fig. 2A). Although this approach caused a significant increase in ROS formation (Fig. 2B), the survival of normoxic cells did not decrease (Fig. 2C). However, when this strategy was used during the exposure of cells to 5% O₂ this translated into enhanced ROS formation and a significant loss of viability (Fig. 2, B and C). The role of GSH in the control of cell survival was further verified by treatment of cells with GSHEE during exposure of cells to 5% O₂ that caused the replenishment of GSH stores in both cytosol and mitochondria (Fig. 2A). GSHEE attenuated ROS generation and protected DEM plus BSO-treated HepG2 cells from 5% O₂-induced cell death (Fig. 2, B and C). In addition, rotenone plus TTFA diminished the enhanced ROS generation in DEM plus BSO-treated HepG2 cells, thus protecting them from 5% O₂-induced necrotic and apoptotic death.

Selective mGSH Depletion Determines the Survival of Rat Hepatocytes to 5% O₂—To examine specifically the role of mGSH on the sensitivity of hepatocytes to hypoxia, we selectively depleted the mitochondrial pool of GSH by HP during the exposure of rat hepatocytes to 5% O₂. HP is transformed in mitochondria into a Michael acceptor (CH₂=CH-CO-) that reacts with GSH in the matrix resulting in its depletion (19, 20). The strategy for mGSH depletion by HP is

Fig. 1. Differential susceptibility of HepG2 cells and rat hepatocytes to hypoxia. HepG2 cells (A) or primary rat hepatocytes (B) were cultured under 2 or 5% O₂ for various periods of time, and ROS generation was determined by DCF fluorescence. Data were expressed as the percentage of DCF fluorescence from normoxic cells (21% O₂) incubated for the same period of time. Survival of HepG2 cells (C) or rat hepatocytes (D) under 2 or 5% O₂ incubation was determined by LDH release. E, HepG2 cells were incubated in the presence of rotenone (Rot, 2.5 μM), antimycin A (AA, 10 μM), diphenyleniodonium (DPI, 10 μM), or TTFA (2 μM) for the last 4 h of the 72-hour period in normoxic or hypoxic conditions (5% O₂), and ROS generation was measured from DCF fluorescence. F, primary rat hepatocytes were treated with MnTBAP (50 μM), l-NAME (1 mM), or uric acid (1 mM) for the 24-hour exposure to 5% O₂ to measure ROS generation. In addition hepatocytes were treated with rotenone plus TTFA (R/T, 15 μM/20 μM) for the last 4 h of the 24-h period of 5% O₂ exposure. Results are given as a mean ± S.E. of four independent experiments.

*, p < 0.05 versus normoxia; **, p < 0.05 versus hypoxia (72 h, 5% O₂).
As seen, HP depleted mGSH in hypoxic hepatocytes by 60–70%, whereas cytosol GSH was unaffected (Fig. 5B). The pool of mGSH remained depleted for up to 8–10 h following HP exposure (not shown). This treatment during normoxia was accompanied by an increased generation of ROS that was insufficient to cause cell death, indicating that mGSH depletion per se is not cytotoxic as reported previously (19–21). However, when hepatocytes were treated with HP during 5% O₂, ROS generation was enhanced (2–5-fold) over time and was correlated with a progressive loss of viability compared with normoxic HP-treated cells or hypoxic untreated hepatocytes (Fig. 5, C and D). In contrast to this effect in hepatocytes, the levels of mGSH from HepG2 cells following exposure to HP (1–5 mM) were 85–93% of untreated cells, indicating that HP failed to deplete the mitochondrial pool of GSH in this cell line (not shown). In addition, consistent with the suppression of ROS stimulation, rotenone plus TTFA protected rat hepatocytes from hypoxia despite HP treatment (Fig. 6, A and B). Moreover, restoration of GSH by GSHEE rescued mGSH-depleted hepatocytes from hypoxia-induced oxidative stress and cell death (Fig. 6, A and B). Thus, these findings clearly demonstrated the relevance of mGSH in the control of survival during hypoxia rendering hepatocytes susceptible to 5% O₂ after its depletion.

DISCUSSION

It is known that hypoxia stimulates ROS generation, and the contribution of mitochondria to this process may vary depending on whether reoxygenation follows the hypoxic phase. Although hypoxia/reoxygenation has been shown to stimulate a burst of ROS of mitochondrial and extramitochondrial origin (e.g. xanthine oxidase) (10), hypoxia is known to stimulate mitochondria to generate ROS, as reported, in different cell types cultured under 1.5–5% O₂ (7–9, 13, 33, 34). We showed in this study that in both HepG2 cells and primary rat hepatocytes the main source of superoxide anion during 5% O₂ incubation is the mitochondrial electron transport chain. Thus, inhibition of mitochondrial electron flow at complexes I and II significantly ameliorated the stimulation of ROS by hypoxia in both cell types, whereas MnTBAP suppressed it. Moreover, diphenyleneiodonium, known to block electron flow at complex I (28), prevented ROS generation by 5% O₂. Previous observations, however, indicated that NO produced in rat liver mitochondria contributed to oxidative stress after hypoxia (29), and the generation of a superoxide anion in the presence of NO is known to yield peroxynitrite. However, we observed that treatment of hepatocytes with L-NAME or uric acid, a peroxynitrite...
scavenger (30), did not prevent ROS generation caused by 5% O₂. Furthermore, consistent with these findings, we did not detect changes in the synthesis of NO determined as the release of NO₂/H₂O₂, the stable breakdown product of NO (35), in normoxic versus hypoxic (5% O₂) rat hepatocytes (not shown), thus further discarding the contribution of stimulated NO in the oxidative stress induced by hypoxia. Thus, it seems that the bulk of superoxide anion generated by 5% O₂ in the absence of reoxygenation derives from the flavin mononucleotide group of complex I from reversed electron transfer as suggested recently (33). In addition, complex III of the respiratory chain has been confirmed as a major source of ROS generation, and the inhibition of mitochondrial respiration is known to stimulate ROS production due to the increased half-life of the intermediates of the electron transport chain at complex I and III that are capable of generating superoxide anion (36, 37).

Our data indicated that the inhibition of electron transfer at complexes I/II with rotenone plus TTFA did not completely block the rise in ROS during hypoxia, suggesting alternative ROS sources. For instance, complex III has been reported to be responsible for the ROS generation during hypoxia in Hep3B cells and isolated rat liver mitochondria (7, 8), and hence electrons released from the ubisemiquinone site of complex III may contribute to the generation of ROS during hypoxia. In addition, Walford et al. (30) observed that ROS generation in bovine aortic endothelial cells by NO donors was potentiated by hypoxia in wild or ρ 0 cells, indicating the contribution of extra-mitochondrial sources. Indeed, these authors observed that indomethacin, which in addition to blocking cyclooxygenases has been shown to decrease NAD(P)H oxidase activity (38), reduced hypoxia potentiation of ROS by NO donors (30).

This study addressed the role of GSH, particularly in mitochondria, on the susceptibility of hepatocytes to hypoxia-induced oxidative stress. Consistent with the burst of ROS generation, it has been shown that hypoxia depletes GSH stores (14–16) and that carbon monoxide decreases GSH/GSSG in rat
brain mitochondria (18). In agreement with these findings we showed that 5% O_2 results in GSH depletion in both cytosol and mitochondrial compartments. However, the depletion seen during 5% O_2 exposure was insufficient to cause cell death in either HepG2 cells or primary hepatocytes in agreement with previous findings. For instance, hepatoma cell lines have been reported to resist the exposure to various ranges of hypoxia (1.5–5% O_2) (7–9), and previous studies showed that 3% O_2 did not cause toxicity in cultured rat hepatocytes despite GSH depletion because of inactivation of methionine adenosyltransferase activity (15). The present study examined for the first time the impact of mGSH depletion on the survival of hepatocytes during exposure to hypoxia. This aim was addressed by two approaches. The abrogation of mitochondrial ROS generation by rotenone plus TTFA rescued both HepG2 cells and primary rat hepatocytes from hypoxia-induced oxidant cell death. 

**Fig. 3.** GSH depletion by DEM plus BSO sensitizes rat hepatocytes to hypoxia. Rat hepatocytes were treated under hypoxia with DEM plus BSO as described in Fig. 2 for HepG2 cells, and then hepatocytes were fractionated into cytosol and mitochondria to determine GSH levels (A). In some cases, rotenone plus TTFA (15 mM + 20 mM) or GSHEE (2 mM) were added during the last 4 h of the 24-h hypoxic period to measure cell viability by leakage of lactate dehydrogenase into the culture medium (B). Control cytosol GSH and mGSH of rat hepatocytes were 33.7 ± 6 and 7.5 ± 0.9 nmol/mg of protein, respectively.

**Fig. 4.** Assessment of cell death by flow cytometry. Hypoxic HepG2 cells were exposed to 5% O_2 for 72 h without (A), with DEM/BSO treatment for the last 4 h (B) or 8 h (C) of the 72-h hypoxic period, or with DEM/BSO for 8 h in the presence of rotenone plus TTFA (D). Cells were collected, washed twice, stained with propidium iodide and fluorescein isothiocyanate-conjugated annexin V, and analyzed by flow cytometry. The figure is representative of three independent experiments showing similar results.
death despite severe GSH depletion in both cytosol and mitochondria. Although this approach suggested the relevance of mGSH rather than that of cytosol GSH in the susceptibility of HepG2 cells to hypoxia-induced mitochondrial ROS generation, it was not definitive proof for the vital role of mGSH. Using HP to selectively deplete mGSH in primary rat hepatocytes with the sparing of cytosol GSH (19–21), we showed that HP-treated hepatocytes became susceptible to 5% O2-induced ROS generation, and this sensitivity was prevented if mitochondrial ROS generation was abolished by rotenone plus TTFA or upon GSH replenishment by GSHEE. However, as opposed to primary hepatocytes, HP did not decrease the levels of GSH in mitochondria in HepG2 cells. Although the reason for this differential outcome between primary hepatocytes and HepG2 cells is not fully understood, it may be because of the inability of mitochondria from hepatoma cells to convert HP into the Michael acceptor 3-oxo-4-pentenoate that is then conjugated with GSH (19–21). Interestingly, lower hydroxybutyrate dehydrogenase activity has been reported in hepatoma cells (39), and whether or not the hydroxybutanoate NAD\(^+\) oxidoreductase responsible for the mitochondrial biotransformation of HP into 3-oxo-4-pentenoate is functional in HepG2 remains to be established. Thus, the present study widens the protective role of mGSH against oxidant cell death induced by tumor necrosis factor-\(\alpha\) or sphingolipids (12, 20, 21, 40).

Consistent with previous findings (27), we show that HepG2 cells appear to be more resistant than primary rat hepatocytes to the oxidative stress induced by hypoxia. Although as discussed above the mitochondrial pool of GSH is of relevance in this response, the resistance of HepG2 to hypoxia is not determined by higher mGSH levels in these cells, as the mGSH levels in both cell types are similar (6–7 nmol/mg of protein). Rather, it appears that mitochondria from hepatoma cells are less capable of generating a superoxide anion than primary cells in response to hypoxia (Fig. 1). In line with this, many tumor cells including hepatoma cell lines display a lower state 3 respiratory rate and oxidative phosphorylation than normal liver, which is accounted for by their dependence on glycolysis for energy production (27, 41, 42).

Our data indicate a threshold for ROS increase to inflict cell death in both HepG2 cells and rat hepatocytes. Because the major source of ROS under hypoxia was mitochondria, this
threshold may relate to the threshold for mGSH depletions to stimulate ROS generation and cell death (43). Although mGSH concentration is high, and moderate mGSH decrease may not impact negatively on ROS generation and cell death, the depletion of mGSH below a critical level would compromise adequate elimination of reactive species and cell survival, particularly in conditions of stimulated ROS generation from the mitochondrial electron transport chain. For instance, under complex III inhibition by antimycin A, stimulated hydrogen peroxide formation increased exponentially when mGSH was depleted to below 2 nmol/mg of protein (24), which may correspond to the $K_m$ (3 mm) of GSH peroxidase for GSH (44). Thus, although the depletion of mGSH below a critical level may translate to life-threatening accumulation of ROS during hypoxia, a minor contribution of cytosol GSH in the susceptibility to hypoxia cannot be completely ruled out.

Finally, our present findings, although expected, may have important pathological implications. For instance, a hypoxic environment is known to promote tumor growth and survival (1, 2), and because mGSH is shown here to be important for the susceptibility of hepatoma cells to oxygen deprivation, its depletion may constitute a promising strategy to sensitize tumor cells to hypoxia. Furthermore, hypoxia is known to contribute to alcohol-mediated hepatocellular injury (45). Under physiological conditions, the oxygen tension within the liver is ~65 mm Hg in the perportal area and falls to ~35 mm Hg in the perivenous zone (46, 47), and the oxidative metabolism of alcohol increases the rate of oxygen uptake accentuating the oxygen gradient existing in the portal to the central venous end of the liver sinusoid. In addition, chronic alcohol feeding is known to deplete mGSH particularly in the perivenous zone (48) that would be expected to contribute to the sensitization of hepatocytes to tumor necrosis factor-α (20, 21, 49), as well as to hypoxia. Thus, our data illustrate the synergism between mGSH depletions and hypoxia contributing to the recognized susceptibility of perivenous hepatocytes to the damaging effects of alcohol.

Acknowledgments—We thank Drs. Xavier Rome (Universitat de Barcelona) for assistance in FACS experiments and Ramon Massaguer and Helena Eixarc (Merck Barcelona) for their contribution. We appreciate the excellent technical assistance of Susana Nunez in many aspects of the work.

REFERENCES

1. Semenza, G. L. (2000) Biochem. Pharmacol. 59, 47–53
2. Wenger, R. H. (2002) FASEB J. 16, 1151–1162
3. Pugh, C. W., and Ratcliffe, P. J. (2000) Nat. Med. 9, 677–684
4. Safran, M., and Kaelin, W. G. Jr. (2003) Trends Cell Biol. 13, 279–285
5. Epstein, A. C., Gleadle, J. M., and McNeill, L. A. (2001) J. Clin. Invest. 111, 779–783
6. Bruck, R., and McNeill, L. A. (2001) Science 294, 1357–1360
7. Chandel, N. S., Maliepe, E., Goldwasser, E., Mathieu, C. E., Simon, M. C., and Schumacker, P. T. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11715–11720
8. Chandel, N. S., McClintock, D. S., Feliciano, C. E., Wood, T. M., Melendez, J. A., Rodriguez, A. M. and Schumacker, P. T. (2000) J. Biol. Chem. 275, 25130–25138
9. Chandel, N. S., Tryza, W. C., McClintock, D. S. and Schumacker, P. T. (2000) J. Immunol. 165, 1013–1021.
10. Jaeschke, H., and Mitchell, J. R. (1989) Biochem. Biophys. Res. Commun. 160, 140–147
11. Fernandez-Checa, J. C., Kaplowitz, N., Garcia-Ruiz, C., Colell, A., Miranda, M., Mari, M., Ardite, E., and Morales, A. (1997) Am. J. Physiol. 273, G7–G17
12. Fernandez-Checa, J. C. (2000) Biochem. Biophys. Res. Commun. 264, 471–479
13. Mansfield, K. D., Simon Celeste, M., and Keith, S. (2004) J. Appl. Physiol. 97, 1358–1366
14. Strubelt, O., Younes, M., and Li, Y. (1992) Pharmacol. Toxicol. 70, 280–284
15. Avila, M. A., Carretero, M. V., Rodriguez, E. N., and Mato, J. M. (1998) Gastroenterology 114, 364–371
16. Khan, S., and O’Brien, P. J. (1997) Biochem. Biophys. Res. Commun. 238, 320–322
17. Tribble, D. L., Jones, D. P., and Edmonson, D. E. (1988) Mol. Pharmacol. 34, 413–420
18. Zhang, J., and Piantadosi, C. (1992) J. Clin. Investig. 90, 1193–1199
19. Shaw, X., Jones, D. P, Hashmi, M., and Anders, M. W. (1993) Chem. Res. Toxicol. 6, 75–81
20. Garcia-Ruiz, C., Colell, A., Mari, M., Morales, A., Calvo, M., Enrich, C., and Fernandez-Checa, J. C. (2003) J. Clin. Investig. 111, 197–208
21. Colell, A., Garcia-Ruiz, C., Miranda, M., Ardite, E., Mari, M., Morales, A.,...
Corrales, F., Kaplowitz, N., and Fernandez-Checa, J. C. (1998) Gastroenterology 115, 1541–1551
22. Lluis, J. M., Colell, A., Garcia-Ruiz, C., Kaplowitz, N., and Fernandez-Checa, J. C. (2003) Gastroenterology 124, 708–724
23. Paris, R., Morales, A., Sanchez-Reyes, A., Garcia-Ruiz, C., and Fernandez-Checa, J. C. (2002) J. Biol. Chem. 277, 48870–48876
24. Garcia-Ruiz, C., Colell, A., Morales, A., Kaplowitz, N., and Fernandez-Checa, J. C. (1995) Mol. Pharmacol. 48, 825–834
25. Roman, J., Gimenez, A., Lluis, J. M., Gasso, M., Rubio, M., Caballeria, J., Pares, A., Rodes, J., and Fernandez-Checa, J. C. (2000) J. Biol. Chem. 275, 14684–14690
26. Mari, M., Bai, J., and Cederbaum, A. I. (2002) Free Radic. Biol. Med. 32, 73–83
27. Wissemann, D. H., Anundi, I., Lauchart, W., Viebahn, R., and Groot, H. (1991) Hepatology 13, 297–303
28. Liu, Y., Fiskum, G., and Schubert, D. (2002) J. Neurochem. 80, 780–787
29. Schild, L., Reinebeck, T., Reiser, M., Horn, T. F. W., Wolf, G., and Augustin, W. (2003) FASEB J. 17, 2194–2201
30. Walford, G. A, Moussignac, R. L., Schiavinato, A. W., and Leopold, J. A. (2004) J. Biol. Chem. 279, 49870–49876
31. Chen, Q., Vazquez, E. J., Moghaddas, S., Hoppel, C. L., and Lesniewski, E. J. (2003) J. Biol. Chem. 278, 36027–36031
32. St-Pierre, J., Buckingham, J. A., Reebuck, S. J., and Brand, M. D. (2002) J. Biol. Chem. 277, 44784–44790
33. Schumacker, P. T. (2002) Am. J. Physiol. 283, L918–L921
34. Dada, L. A., Chandel, N. S, Ridge, K. M., Pedemonte, C., Bertorello, A. M., and Sznajder, J. I. (2003) J. Clin. Investig. 111, 1057–1064
35. Morales-Ruiz, M., Lee, M. J., Zollner, S., Gratton, J. P., Scotland, R., Shiojima, S., Walsh, K., Hla, T., and Sessa, W. C. (2001) J. Biol. Chem. 276, 19672–19677
36. Chen, Q., Vazquez, E. J., Moghaddas, S., Hoppel, C. L., and Lesniewski, E. J. (2003) J. Biol. Chem. 278, 36027–36031
37. St-Pierre, J., Buckingham, J. A., Reebuck, S. J., and Brand, M. D. (2002) J. Biol. Chem. 277, 44784–44790
38. Umeki, S. (1990) Biochim. Biophys. Acta 1000, 559–564
39. Zhan, W. W., Churchill, S., Lindhal, R., and Churchill, P. (1989) Cancer Res. 49, 2435–2437
40. Garcia-Ruiz, C., Colell, A., Paris, R., and Fernandez-Checa, J. C. (2000) FASEB J. 14, 847–858
41. Feo, F. (1975) Biochim. Biophys. Acta 413, 116–134
42. Pedersen, P. L. (1978) Prog. Exp. Tumor Res. 22, 190–274
43. Fernandez-Checa, J. C., and Kaplowitz, N. (2005) Toxicol. Appl. Pharmacol., in press
44. Little, C., Olinescu, R., Reid, K. G., and O’Brien, P. J. (1970) J. Biol. Chem. 245, 3632–3638
45. French, S. W. (2004) Hepatol. Res. 29, 69–74
46. Israel, Y., Kalant, H., Orrego, H., Khanna, J. M., Vide, L., and Phillips, J. M. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1107–1141
47. Ji, S., Lemasters, J. J., Christenson, V., and Thurman, R. G. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 5415–5419
48. Garcia-Ruiz, C., Morales, A., Ballesta, A., Rodes, J., Kaplowitz, N., and Fernandez-Checa, J. C. (1994) J. Clin. Investig. 94, 193–201
49. Pastorino, J. G., and Hoek, J. B. (2000) Hepatology 31, 1141–1152
Critical Role of Mitochondrial Glutathione in the Survival of Hepatocytes during Hypoxia
Josep M. Lluis, Albert Morales, Carmen Blasco, Anna Colell, Montserrat Mari, Carmen Garcia-Ruiz and José C. Fernandez-Checa

J. Biol. Chem. 2005, 280:3224-3232.
doi: 10.1074/jbc.M408244200 originally published online November 16, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M408244200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 48 references, 16 of which can be accessed free at
http://www.jbc.org/content/280/5/3224.full.html#ref-list-1