State-of-the-Art: Hyperbaric Medicine for the Treatment of Carbon Monoxide Poisoning: Examining the Issues

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Thursday, May 25, 2000, 8:00-9:30 am

My assignment for this "State of the Art" discussion was to review the most recent basic science advances dealing with the theoretical benefits of hyperbaric oxygen (HBO_2) in carbon monoxide (CO) poisoning.

Scope of the problem: Carbon monoxide is one of many ubiquitous contaminants of our environment that requires prevention and control measures to insure adequate protection of the public health. The incidence of CO-related mortality and morbidity is similar worldwide, and CO may be responsible for over half of all fatal poisonings[1-4]. Carbon monoxide poisoning has been estimated to cause 40,000 persons per year to seeking medical attention at emergency departments in the United States[5,6].

Primary mechanism of toxicity: The toxicity of CO is based on its propensity for forming stable complexes with transition metals. The affinity of CO for hemoglobin is more than 200-fold greater than that of O_2 and formation of carboxyhemoglobin (COHb) is a recognized effect of CO exposure[7].

CO Pathophysiology: Hypoxia, ischemia, and more...: CO enters the body via the lungs and pulmonary cells may be injured by direct interactions, without need for delivery of CO by blood-borne hemoglobin. Elsewhere in the body, CO will be delivered by hemoglobin and concentrations found in perivascular and extravascular sites are estimated using calculations first described by Coburn[8]. The symptoms, signs and prognosis of acute CO poisoning correlate poorly with the level of COHb measured at the time of arrival at the hospital[4,9-13]. These observations have created two concerns. The first is that investigators must be extremely careful when attempting to study patient populations because determination of the severity of poisonings and, therefore, the efficacy of treatment, will be difficult. The second concern is that these clinical observations raise questions regarding the mechanisms of toxicity and suggest that it extends beyond the classical perspective regarding hemoglobin binding by CO.

Hypoxic stress: An elevated COHb can precipitate tissue hypoxia, and this stress appears to be responsible for fatalities, cardiac injuries, and the acute neurological abnormalities which develop, according to most studies, in approximately 14 % of survivors from serious CO poisoning[14-16]. However, both clinical and animal studies have failed to establish a correlation between elevated COHb levels and delayed neurological injuries, which is the most frequent form of CO-associated morbidity[4,9-13,17]. Recent studies indicate that between 23 and 47% of patients with CO poisoning develop impairments of concentration and learning, dementia, cog wheel rigidity, amnesia and/or depression, between 6 days and 3 weeks after poisoning[12,18-20]. These events occur despite rapid and appropriate emergency care followed by careful neuropsychological evaluations. CO poisonings that appear to be relatively mild also may cause subtle neurological dysfunction, which indicates that the clinical assessment of the severity of poisoning is unreliable[12,21-23]. The mechanism for delayed neurological injuries is unknown.

More on basic mechanisms- new information: Mysteries persist regarding the chemical interactions that have bearing on CO pathophysiology. Whereas physiological stresses of CO are classically related to competition between CO and O_2 for hemoproteins, recent attention has focused on interactions between CO and another small gaseous ligand produced *in vivo*, the free radical nitric oxide ($^{\bullet}NO$). Under virtually all circumstances, the affinity of $^{\bullet}NO$ for hemoproteins is vastly greater than that of either CO or O_2. Despite this fact, there are situations where CO will disturb the association between $^{\bullet}NO$ and hemoproteins. CO increases the steady state concentration of $^{\bullet}NO$ in, and around, both platelets and endothelial cells[24-26]. Electron paramagnetic resonance spectroscopy has provided direct evidence that exposure to CO increases the concentration of $^{\bullet}NO$ in lung and brain[27,28]. CO does not increase activity of nitric oxide synthase (NOS) in platelets or endothelial

cells, nor does CO increase NOS protein concentration in CO-exposed cells and tissues at a time when they exhibit elevated •NO levels[24-26,28-30]. In fact, CO partially inhibits NOS activity in rats exposed to 3000 ppm who have COHb levels of approximately 45 %[24]. It appears that CO increases the steady state level of unbound •NO because it competes for intracellular sites that normally would bind •NO. Toxic effects on cells occur because the liberated •NO is available to undergo reactions with superoxide anion ($O_2^{\bullet}$) which yield the potent oxidizing and nitrating agent, peroxynitrite. These events are a component to CO-mediated brain injury in some animal models[27,31-33], and they may relate to a growing body of evidence suggesting that CO functions as a cell signaling messenger analogous to •NO[34,35]. The point is that when ever CO is generated *in vivo* by heme oxygenase, if •NO is also produced in the vicinity, the CO may act to increase the effective steady state concentration of •NO.

The mechanism behind the apparent ability of CO to increase steady state concentration of •NO is under investigation. The following discussion is our current hypothesis. It is based on an assessment of potential competition between ligands using published values for the association and dissociation constants for myoglobin, which we use as a 'model' intracellular hemoprotein (Table 1)[36].

TABLE 1. RATE CONSTANTS FOR DIFFERENT LIGANDS WITH MYOGLOBIN

GAS	ASSOCIATION RATE CONSTANT ($M^{-1}sec^{-1}$)	DISSOCIATION RATE CONSTANT (sec^{-1})
O_2	14×10^6	12
•NO	17×10^6	1.2×10^{-5}
CO	0.5×10^6	1.9×10^{-2}

The calculated affinity binding constant favors •NO over CO by a factor of 10^5 when steady state concentrations of CO and •NO are equal (e.g. for •NO $1.7x10^7/1.2 \times 10^{-5} = 1.4x10^{12}$; for CO $0.5x10^6/1.9x10^{-2} = 2.6x10^7$). It is unlikely, however, for both ligands to have equal concentrations *in vivo*. Coburn (1970) demonstrated that a predictable relationship exists between the tissue concentration of CO and blood COHb up to a level of 50%. For example, at a COHb of 7 % the extravascular fluid CO concentration should be approximately 22×10^{-9} M[8,37]. Because CO is freely soluble, a similar concentration is expected to occur inside cells. The rate of •NO production by endothelial cells (which we have taken as an example because these cells are physically close to delivered CO from the blood) has been estimated to be$1.1x10^{-18}$ M /cell/min[38]. Therefore, even in a situation where there is a relatively low COHb, the CO concentration may be as much as 10^9 greater than the concentration of •NO. Therefore, competition is feasible even when considering equilibrium kinetics.

The potential for effective competition is even more favorable for CO when considering simple competition kinetics. It is more appropriate to make an assessment of competition using the association rate constants for these ligands, rather than the affinity constants. The physiological and clinical settings where this relationship may have bearing is early during a CO exposure from exogenous sources, when ever there is a change in CO production *in vivo* or when a reduction in local •NO production occurs. Since the association constant for CO and •NO differ by a factor of only 34, even a small increase in CO concentration relative to that of •NO will have an impact favoring CO competition with •NO for myoglobin. Adverse effects on endothelial cells *in vitro* that are mediated by •NO-derived oxidants can be demonstrated with exposure to a CO concentration of only $11x10^{-9}$ M[30]. There may also be physiological conditions which allow favorable competition between CO and •NO. Liver parenchyma has been estimated to generate 0.45×10^{-9} M CO/ gram liver/min[39].

Concomitant hypoxia and ischemia: Severe CO poisoning causes mitochondrial dysfunction and oxidative stress in the central nervous system. In experimental CO poisoning, mild manifestations occur when cerebral perfusion is maintained, whereas mortality and morbidity are profoundly enhanced when exposures to high CO concentrations occur in the presence of reduced cerebral perfusion[40]. The concomitant impairment of cerebral blood flow can be triggered by cardiac dysfunction due to CO or to fixed cerebral vascular lesions[40-43].

In the face of concomitant hypoperfusion, Coburn demonstrated that 20 to 45 % of CO present in circulating blood shifts into skeletal muscle and other tissues[44]. CO will impair mitochondrial electron transport when cells sustain a reduction in O_2 delivery, as will occur when an elevated COHb level occurs concurrent with restricted perfusion[42,43]. In the setting of hypoperfusion and high CO concentrations, CO binds to cytochrome c oxidase which will inhibit ATP synthesis and cause generation of hydroxyl-like radicals[42,45]. Energy production and mitochondrial function are restored after COHb levels decrease[42]. These observations have not explained the two historical characteristics of clinical poisoning that are correlated with a high risk for delayed morbidity: (1) A prolonged exposure to CO, called a "soaking", and (2) Syncope or temporary unconsciousness[17,46-50]. It is unclear whether the transient changes observed during CO poisoning precipitate delayed neuronal dysfunction or death[51]. Moreover, in some models with neuronal injuries, it has been difficult to document evidence of impaired mitochondrial function or a cellular hypoxic stress[43].

Large clinical surveys have reported a correlation between neurological morbidity and the occurrence of an interval of unconsciousness during CO exposure[17,47,48]. However, such an overt insult is not always necessary for neurological injuries to occur in humans[12,21-23]. Many neuroimaging techniques suggest that CO causes perivascular injuries in the CNS. Bianco[52] and Silverman[53] have hypothesized that the initial site of injury by CO may be the vasculature, based on detection of hemosiderin deposits. These deposits are thought to result from focal hemorrhages. Recently, more sophisticated neuroimaging techniques have been used to detect abnormalities in patients who, in some cases, exhibited only subtle neurological impairments. Abnormalities in resting cerebral blood flow[54,55] and abnormalities in cerebral vasoactivity to carbon dioxide[56] have been detected by single-photon emission computed tomography (SPECT). Changes have also been detected which suggest that CO causes a disturbance in coupling between neuronal O_2 demand and blood flow. DeReuck, et al.[57], examined 7 patients between 5 and 7 days after CO poisoning using positron emission tomography (PET) with $^{15}O_2$. They found a global increase in cerebral O_2 extraction along with regional areas of diminished blood flow, especially in the frontal and temporal lobes. Although these observations serve to underscore the vascular nature of CO-mediated neuropathology, they do not assist with clinical assessments of patients. To date, no objective parameters have been identified which prospectively assess the severity of poisoning. Some recent findings with state-of-the-art neuroimaging techniques have exhibited correlations with the clinical improvement in case reports, whereas others may show abnormalities when no clinical changes are noted[58-60]. In experimental studies, blood levels of glutathione, oxidized proteins and products of lipid peroxidation offer insight into CO-mediated pathology[61]. However, additional work is necessary to establish if these or some other survey may be useful to stage the severity of clinical poisonings.

Vascular injuries due to CO: The level of CO in tissues may have an equal or greater impact on the clinical status of patients and development of pathology than does the blood level of CO. This phenomenon is most clearly demonstrated with regard to CO-mediated vascular injuries. When human beings or experimental animals have been exposed to relatively low CO concentrations for extended periods of time, capillary leakage of macromolecules from the lung and systemic vasculature has been documented[28,29,62-65]. In contrast, when an hypoxic stress was established in animals by exposing them for 8 to 45 minutes to extremely high CO concentrations, sufficient to cause COHb levels of 60 to 90 %, capillary leakage was not detected[66-68]. A proposed pathological mechanism of CO which is independent of hypoxic stress has been demonstrated in experimental studies to be due to elevations in the steady state concentration of the free radical, nitric oxide ($^{\bullet}NO$)[28,29].

Cascade of pathology – vascular injury and more...: The findings outlined above for vascular injuries have provided further insight into CO-induced neuropathology because endothelial •NO-mediated changes are a prerequisite for neutrophil adherence to the cerebral microvasculature of CO poisoned animals[27,32,33]. However, •NO-mediated vascular stress is not sufficient by itself to cause neutrophil sequestration in this model of CO-mediated brain injury. During the latter portion of the exposure when rats breathe 3000 ppm CO and become unconsciousness, they invariably sustain a period of hypotension that is presumably mediated by cardiac decompensation[31,43,69]. Cerebral blood flow, which initially is approximately 150 % above normal due to •NO-mediated vasodilation, decreases to about 50 % below normal for a period of 4 to 6 minutes when rats lose consciousness[43,69]. With regard to mechanisms of brain injury, it is important to emphasize that unlike typical ischemic/hypoxic injury, there is never a time when cerebral blood flow is nil, and the interval of hypoperfusion is less than six minutes[43,69]. The sudden alteration in microvascular flow, coupled with the ·NO-mediated oxidative changes to endothelium, cause neutrophils to adhere to endothelium.

Activated neutrophils attach to the vascular wall via interactions between β_2 integrin adhesion molecules and endothelial (ICAM) counter-receptors[70]. β_2 integrin adhesion molecules are required for neutrophil adherence and progression of the oxidative stress cascade in CO poisoning[27,33,71,72]. This occurs 45 minutes after the rats are removed from CO, based on the inhibitory potential of monoclonal antibodies to β_2 integrins[33,72]. The 45 minute delay between initial adherence and β_2 integrin commitment is unusually long compared with some models of leukocyte-mediated tissue injury. The delay is due to the flux of •NO from platelets[24]. Nitric oxide will inhibit β_2 integrin function[73]. Once the animals are in fresh air and CO leaves the body, the •NO flux drops and adherent leukocytes become activated. Activated leukocytes liberate proteases and reactive O_2 species that cause conversion of endothelial xanthine dehydrogenase to xanthine oxidase, and xanthine oxidase activity is required for subsequent brain lipid peroxidation[27,31-33]. These biochemical events occur within 90 minutes after CO poisoning. Metabolic defects in the basal ganglia and hippocampus appear at 5 days, and evidence of impaired learning plateaus in 3 weeks after poisoning[74]. The cellular and biochemical events which occur during this three week period appear to be related to inflammatory processes and are under active investigation[75].

HBO_2 and experimental CO poisoning: HBO_2 has been shown to diminish cerebral edema, reduce mortality and improve neurological outcome in various models of acute CO poisoning[76-78]. The initial motivation for administering HBO_2 was to hasten removal of CO, based on the well known relationship that COHb half life is inversely related to the inspired partial pressure of O_2 [79,80]. Hyperbaric oxygen also hastens dissociation of CO from cytochrome oxidase[41,42], and vascular oxidative stress is prevented because HBO_2 inhibits $_2$ integrin-dependent leukocyte adhesion[72,81]. Neutrophils from humans exposed to HBO_2 exhibit the same diminished adherence as those in animal studies[82].

Additional studies/ more variables: Several investigations have suggested an association between CO-induced neurotoxicity and that caused by excitatory amino acids[83,84]. Although this issue is currently under investigation, at least in some studies excitotoxicity is linked to elevations of intracellular calcium, •NO and $O_2^{\bullet}$. There are three types of receptors activated by excitatory amino acids: N-methyl-D aspartic acid (NMDA), D-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid, and kainic acid. Agents that inhibit NMDA receptor activation attenuate CO-mediated delayed neuronal degeneration of pyramidal cells in the hippocampus and cochlear ganglion cells[85,86].

Monoamine neurotransmitters such as norepinephrine and dopamine are elevated after CO exposure, and enzymatic breakdown as well as auto-oxidation will generate reactive O_2 species[87,88]. These agents appear to contribute to oxidative stress after CO poisoning because free radical production in brain can be diminished by inhibiting monoamine oxidase-B, an enzyme located in microglial cell[89]. Nitric oxide will augment pre-synaptic release of monoamine neurotransmitters by activating NMDA receptors[90,91]. Activated microglia can also mediate neuronal injury by generating •NO-derived oxidants[92]. Microglia can attack oligodendroglia and

have been associated with demyelination processes[93]. Therefore, once again, •NO-mediated oxidative stress may be a common biochemical link among different pathways of CO poisoning.

Summary: Neuropathology following CO poisoning may include neuronal death in the cortex, hippocampus, substantia nigra and globus pallidus[94]. One of the most common abnormalities is demyelination of cerebral cortex, which occurs in a perivascular distribution along with evidence of a breach in the blood-brain barrier[94-96]. Blood flow and perivascular abnormalities have been shown using several neuroimaging techniques[52-57]. Acute vascular and perivascular changes also have been found in brains of experimental animals[12,27,97]. Moreover, the variability observed in lesions found in the cerebral white matter and globus pallidus of animals has been correlated with the fall in local blood flow and metabolic acidosis[16,98]. Clinical and experimental findings suggest that the effects of CO are global and variations in the clinical manifestations of poisoning arise because brain regions respond differently to the stresses. The threat to life and acute neurological compromise from CO poisoning are thought to be due to direct hypoxic stress. The syndrome of delayed neurological sequelae appears to be a consequence of a cascade of events involving oxidative stress and inflammatory responses. HBO_2 inhibits CO-mediated hypoxia and inflammation.

References:

1. National Safety Council: How people died in home accidents, 1981. In: Accident Facts, 1982 ed., National Safety Council 1982, Chicago, pp 80-84.
2. Cobb N, and Etzel RA: Unintentional carbon monoxide-related deaths in the United States, 1979 through 1988, *JAMA* 266:659-663, 1991.
3. Mathieu D, Mathieu-Nolf M, and Wattel F: Intoxication par le monoxide de carbone: aspects actuels [Carbon monoxide poisoning: present aspects], *Bull Acad Natl Med (Paris)* 180:965-973, 1996.
4. Meredith T, and Vale A: Carbon monoxide poisoning, *Br Med J* 296:77-79, 1988.
5. Cook M, Simon PA, and Hoffman RE: Unintentional carbon monoxide poisoning in Colorado, 1986 through 1991, *Am. J. Public Hlth* 85:988-990, 1995.
6. Hampson NB: Emergency department visits for carbon monoxide poisoning in the Pacific Northwest, *J Emerg Med* 16(5):695-698, 1998.
7. Douglas CG, Haldane JS, and Haldane JBS: The laws of combination of haemoglobin with carbon monoxide and oxygen, *J Physiol* (London) 44:275-304, 1912.
8. Coburn RF: The carbon monoxide body stores, *Ann NY Acad Sci* 174:11-22, 1970.
9. Garland H, and Pearce J: Neurological complications of carbon monoxide poisoning, *Q J Med* 144: 445-455, 1967.
10. Klees M, Heremans M, and Doughan S: Psychological sequelae to carbon monoxide poisoning in the child, *J Toxicol Clin Exp* 5:301-307, 1985.
11. Myers RAM, Snyder SK, Emhoff TA: Subacute sequelae of carbon monoxide poisoning, *Ann Emerg Med* 14:1163–1167, 1985.
12. Thom SR, Taber RL, Mendiguren II et al: Delayed neuropsychologic sequelae after carbon monoxide poisoning: prevention by treatment with hyperbaric oxygen, *Ann Emerg Med* 25:474–480, 1995.
13. Winter PM, and Miller JN: Carbon monoxide poisoning, *JAMA* 236:1502-1504, 1976.
14. Anderson EW, Andelman RJ, Strauch JM et al: Effects of low-level carbon monoxide exposure on onset and duration of angina pectoris, *Ann Intern Med* 79:46-50, 1973.
15. Cramlet SH, Erickson HH, and Gorman HA: Ventricular function following acute carbon monoxide exposure, *J Appl Physiol* 39:482-486, 1975.
16. Ginsberg MD: Carbon monoxide intoxication: clinical features, neuropathology and mechanisms of injury, *Clin Toxicol* 23:281-288, 1985.
17. Choi S: Delayed neurologic sequelae in carbon monoxide intoxication, *Arch Neurol* 40:433-435, 1983.
18. Gorman DF, Clayton D, Gilligan JE et al: A longitudinal study of 100 consecutive admissions for carbon monoxide poisoning to the Royal Adelaide Hospital, *Anaesth Intens Care* 20:311-316, 1992.
19. Mathieu D, Wattel F, Mathieu-Nolf M, et al: Randomized prospective study comparing the effect of HBO versus 12 hours NBO in non-comatose CO poisoned patients, *Undersea Hyperbaric Med* 23 (suppl.): 7, 1996.

20. Raphael JC, Elkharrat D, Guincestre MCJ, et al: Trial of normobaric and hyperbaric oxygen for acute carbon monoxide intoxication, *Lancet* 2:414–419, 1989.
21. Schulte JH: Effects of mild carbon monoxide intoxication, *Arch Environ Hlth* 7:524-530, 1969.
22. Remick RA and Miles JE: Carbon monoxide poisoning: neurologic and psychiatric sequelae, *Canad Med Assoc J* 117:654-657, 1977.
23. Ryan CM: Memory disturbances following chronic low level carbon monoxide exposure, *Arch Clin Neuropsychology* 5:59-67, 1990.
24. Thom SR, Ohnishi ST, Ischiropoulos H et al: Nitric oxide released by platelets inhibits neutrophil B_2 integrin function following acute carbon monoxide poisoning, *Toxicol Appl Pharmacol* 128:105-110, 1994b.
25. Thom SR, and Ischiropoulos H: Mechanism of oxidative stress from low levels of carbon monoxide, *Health Effect Institute Research Report* 80. Cambridge, MA 1997b.
26. Thom SR, Xu YA, and Ischiropoulos H: Vascular endothelial cells generate peroxynitrite in response to carbon monoxide exposure, *Chem Res Toxicol* 10:1023-1031, 1997.
27. Ischiropoulos H, Beers MF, Ohnishi ST et al: Nitric oxide and perivascular tyrosine nitration following carbon monoxide poisoning in the rat, *J Clin Invest* 97:2260-2267, 1996.
28. Thom SR, Ohnishi ST, Fisher D et al: Pulmonary vascular stress from carbon monoxide, *Toxicol Appl Pharmacol* 154:12-19,1999b.
29. Thom SR, Fisher D, Xu YA et al: Role of nitric oxide-derived oxidants in vascular injury from carbon monoxide in the rat, *Am J Physiol* 276 (*Heart and Circ. Physiol.45)*: H984-H992, 1999a.
30. Thom SR, Fisher D, and Xu YA et al: Adaptive responses and apoptosis in endothelial cells exposed to carbon monoxide, *Proc Natl Acad Sci USA* 97:1305-1310, 2000.
31. Thom SR: Carbon monoxide-mediated brain lipid peroxidation in the rat, *J Appl Physiol* 68:997-1003, 1990a.
32. Thom SR: Dehydrogenase conversion to oxidase and lipid peroxidation in brain after carbon monoxide poisoning, *J Appl Physiol* 73:1584-1589, 1992.
33. Thom SR: Leukocytes in carbon monoxide-mediated brain oxidative injury, *Toxicol Appl Pharmacol* 123:234-247, 1993b.
34. Verma A, Hirsch DJ, Glatt CE et al: Carbon monoxide: A putative neural messenger, *Science* 259:381-309, 1993.
35. Zhuo M, Small SA, Kandel ER et al: Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus, *Science* 260:1946-1950, 1993.
36. Gibson QH, Olson JS, McKinnie RE et al: A kinetic description of ligand binding to sperm whale myoglobin, *J Biol Chem* 261:10228-10239, 1986.
37. Gothert M, Lutz F, and Malorny G: Carbon monoxide partial pressure in tissue of different animals, *Environ Res* 3:303-309, 1970.
38. Schmidt HHHW, Nau H, Wittfoht W et al: Arginine is a physiological precursor of endothelium-derived nitric oxide, *Eur J Pharmacol* 154:213-216, 1988.
39. Goda N, Suzuki K, Naito M et al: Distribution of heme oxygenase isoforms in rat liver, *J Clin Invest* 101:604-612, 1998.
40. Jiang J, and Tyssebotn I: A model for acute carbon monoxide poisoning in conscious rats, *Undersea and Hyperbaric Med* 23:99-106, 1996.
41. Brown SD, and Piantadosi CA: Reversal of carbon monoxide-cytochrome C oxidase binding by hyperbaric oxygen in vivo, *Adv Exp Biol Med* 248:747–754, 1989.
42. Brown SD, and Piantadosi CA: Recovery of energy metabolism in rat brain after carbon monoxide hypoxia, *J Clin Invest* 89:666-672, 1991.
43. Mayevsky A, Meilin S, Rogatsky GG et al: Multiparametric monitoring of the awake brain exposed to carbon monoxide, *J Appl Physiol* 78:1188-1196, 1995.
44. Coburn RF, Wallace HW, and Abboud R: Redistribution of body carbon monoxide after hemorrhage, *Am. J. Physiol* 220:868-874, 1971.
45. Piantadosi CA, Zhang J, and Demchenko IT: Production of hydroxyl radical in the hippocampus after CO hypoxia ar hypoxic hypoxia in the rat, *Free Rad Biol Med* 22:725-732, 1997.

46. Bogusz M, Cholewa L, Pach J et al: A comparison of two types of carbon monoxide poisoning, *Arch Toxicol* 33:141-149, 1975.
47. Min SK: A brain syndrome associated with delayed neuropsychiatric sequelae following acute carbon monoxide intoxication, *Acta Psychiatr Scand* 73:80-86, 1986.
48. Smith JS, and Brandon S: Morbidity from acute carbon monoxide poisoning at three-year follow-up, *Br Med J* 1:318-321, 1973.
49. Wasowski J, Myslak Z, Graczyk M et al: An attempt at comparing the results of carboxyhemoglobin level in blood and gasometric determination in capillary blood in cases of carbon monoxide poisoning when treatment began at the place of accident, *Anaesth Resusc Intens Therap* 4:245-249, 1976.
50. Werner B, Back W, Akerblom H et al: Two cases of acute carbon monoxide poisoning with delayed neurological sequelae after a "free" interval, *Clin Toxicol* 23:249-265, 1985.
51. Piantadosi CA, Zhang J, Levin ED et al: Apoptosis and delayed neuronal damage after carbon monoxide poisoning in the rat, *Exp Neurol* 147:103-114, 1997.
52. Bianco F, and Floris R: Transient disappearance of bilateral low-density lesions of the globi palladi in carbon monoxide intoxication and MRI, *J Neuroradiol* 15:381-385, 1988.
53. Silverman CS, Brenner J, and Murtagh FR: Hemorrhagic necrosis and vascular injury in carbon monoxide poisoning: MR demonstration, *AJNR* 14:168-170, 1993.
54. Maeda Y, Kawasaki Y, Jibiki I et al: Effect of therapy with oxygen under high pressure on regional cerebral blood flow in the interval form of carbon monoxide poisoning: observation from subtraction of technetium-99m HMPAOSPECT brain imaging, *Eur Neurol* 31:380-383, 1991.
55. Shimosegawa E, Hatazawa J, Nagata K, et al: Cerebral blood flow and glucose metabolism measurements in a patient surviving one year after carbon monoxide intoxication, *J Nucl Med* 33:1696-1698, 1992.
56. Ducasse JL, Celsis P, and Marc-Vergnes JP: Non-comatose patients with acute carbon monoxide poisoning: hyperbaric or normobaric oxygenation? *Undersea and Hyperbaric Med* 22:9-15, 1995.
57. DeReuck J, Deccoo D, Lemahieu I. et al: A positron emmission tomography study of patients with acute carbon monoxide poisoning treated by hyperbaric oxygen, *J Neurol* 240:430-434, 1993.
58. Murata T, Itoh S, Koshino Y et al: Serial cerebral MRI with FLAIR sequences in acute carbon monoxide poisoning, *J Computer Assisted Tomography* 19(4):631-634, 1995.
59. Sakamoto K, Murata T, Omori M et al: Clinical studies on three cases of the interval form of carbon monoxide poisoning: serial proton magnetic resonance spectroscopy as a prognostic predictor, *Psych Res Neuroimaging Sect* 83:179-192, 1998.
60. Yoshii F, Kozuma R, Takahashi W et al: Magnetic resonance imaging and ^{11}C-N-methylspiperone/positron emission tomography studies in a patient with the interval form of carbon monoxide poisoning, *J Neurol Sci* 160:87-91, 1998.
61. Thom SR, Kang M, Fisher D et al: Release of glutathione from erythrocytes and other markers of oxidative stress in carbon monoxide poisoning, *J Appl Physiol* 82:1424-1432, 1997c.
62. Kjeldsen K, Astrup P and Wanstrup J: Ultrastructural intimal changes in the rabbit aorta after a moderate carbon monoxide exposure, *Atherosclerosis* 16:67-82, 1972.
63. Maurer FW: The effects of carbon monoxide anoxemia on the flow and composition of cervical lymph, *Am J Physiol* 133:170-179, 1941.
64. Parving HH, Ohlsson K, Hansen HJB, et al: Effect of carbon monoxide exposure on capillary permeability to albumin ad a_2-macroglobulin, *Scand J Clin Lab Invest* 29:381-388, 1972.
65. Siggaard-Anderson J, Bonde-Peterson F, and Hanson TI: Plasma volume and vascular permeability during hypoxia and carbon monoxide exposure, *Scand J Clin Lab Invest* 22:39-48, 1968.
66. Fisher AB, Hyde RW, Baue AE et al: Effect of carbon monoxide on function and structure of the lung, *J Appl Physiol* 26:4-12, 1969.
67. Robinson NB, Barie PS, Halebian PH et al: Distribution of ventilation and perfusion following acute carbon monoxide poisoning, *Surg Forum* 36:115-118, 1985.
68. Sugi K, Theissen JL, Traber LD et al: Impact of carbon monoxide on cardiopulmonary dysfunction after smoke inhalation injury, *Circ Res* 66:69-75, 1990.
69. Meilin S, Rogatsky GG, Thom SR et al: Effects of carbon monoxide on the brain may be mediated by nitric oxide, J Appl Physiol 81:1078-1083, 1996.

70. Von Andrian UH, Chambers JD, McEvoy LM et al: Two-step model of leukocyte-endothelial cell interaction in inflammation: Distinct roles for LECAM-1 and the leukocyte 2 integrins *in vivo, Proc Natl Acad Sci USA* 88:7538-7542, 1991.
71. Thom SR: Antagonism of CO-mediated brain lipid peroxidation by hyperbaric oxygen, *Toxicol Appl Pharmacol* 105:340-344, 1990b.
72. Thom SR: Functional inhibition of leukocyte β_2 integrins by hyperbaric oxygen in carbon monoxide-mediated brain injury in rats, *Toxicol Appl Pharmacol* 123:248–256, 1993.
73. Banick PD, Chen Q, Xu YA et al: Nitric oxide inhibits neutrophil 2 integrin function by inhibiting membrane-associated cyclic GMP synthesis, *J Cell Physiol* 172:12-24, 1997.
74. Thom SR: Learning dysfunction and metabolic defects in globus pallidus and hippocampus after CO poisoning in a rat model, *Undersea Hyperbaric Med* 23(suppl.): 20, 1997.
75. Thom SR, Xu YA, and Fisher DG: Autoimmune responses in carbon monoxide-mediated brain injury, *Undersea and Hyperbaric Med* 2000 in press.
76. Jiang J, and Tyssebotn I: Normobaric and hyperbaric oxygen treatment of acute carbon monoxide poisoning in rats, *Undersea and Hyperbaric Med* 24(2):107-116, 1997.
77. Jiang J, and Tyssebotn I: Cerebrospinal fluid pressure changes after acute carbon monoxide poisoning and therapeutic effects of normobaric and hyperbaric oxygen in conscious rats, *Undersea and Hyperbaric Med* 24(4):245-254, 1997.
78. Tomaszewski C, Rudy J, Wathen J et al: Prevention of neurologic sequelae from carbon monoxide by hyperbaric oxygen in rats, *Ann_Emerg Med* 21:1992.
79. Pace N, Strajman E, and Walker EL: Acceleration of carbon monoxide elimination in man by high pressure oxygen, *Science* 111:652–654, 1950.
80. End E, and Long CW: Oxygen under pressure in carbon monoxide poisoning, *J Ind Hyg Toxicol* 24:302–306, 1942.
81. Chen Q, Banick PD, and Thom SR: Functional inhibition of rat polymorphonuclear leukocyte B_2 integrins by hyperbaric oxygen is associated with impaired cGMP synthesis, *J Pharmacol Expt'l Therap* 276:929-933, 1996.
82. Thom SR, Mendiguren I, Hardy KR et al: Inhibition of human neutrophil β_2 integrin-dependent adherence by hyperbaric oxygen, *Am J Physiol* 272:770–771, 1997.
83. Nabeshima T, Katoh A, Ishimaru H et al: Carbon monoxide-induced delayed amnesia, delayed neuronal death and change in acetylcholine concentration in mice, *J Pharm Exp Therap* 256: 378-384, 1991.
84. Ishimaru H, Nabeshima T, Katoh A et al: Effects of successive carbon monoxide exposures on delayed neuronal death in mice under the maintenance of normal body temperature, *Biochem_Biophys Res Comm* 179:836-840, 1991.
85. Ishimaru H, Katoh A, Suzuki H et al: Effects of N-methyl-D-aspartate receptor antagonists on carbon monoxide-induced brain damage in mice, *J Pharmacol Exp Ther* 261:349-352, 1992.
86. Liu Y, and Fechter LD: MK-801 protects against carbon monoxide-induced hearing loss, *Toxicol Appl Pharmacol* 132:196-202, 1995.
87. Newby MB, Roberts RJ, and Bhatnagar RK: Carbon monoxide and hypoxia-induced effects on catecholamines in the mature and developing rat brain, *J Pharmacol Exp Ther* 206:61-68, 1978.
88. Bindoli A, Rigobello MP, and Deeble DJ: Biochemical and toxicological properties of the oxidation products of catecholamines, *Free Rad Biol Med* 13:391-405, 1992.
89. Piantadosi CA, Tatro L, and Zhang J: Hydroxyl radical production in the brain after CO hypoxia in rats, *Free Rad Biol Med* 18:603-609, 1995.
90. Hanbauer I, Wink D, Osawa Y et al: Role of nitric oxide in NMDA-evoked release of [^{3}H]-dopamine from striatal slices, *Neuroreport* 3:409-412, 1992.
91. Montague PR, Gancayco CD, Winn MJ et al: Role of NO production in NMDA receptor-mediated neurotrasmitter release in cerebral cortex, *Science* 263:973-977,1994.
92. Chao CC, Hu S, Molitor TW et al: Activated microglia mediate neuronal cell injury via a nitric oxide mechanism, *J Immunol* 149:2736-2741, 1992.
93. Merrill JE, and Zimmerman RP: Natural and induced cytotoxicity of oligodendrocytes by microglia is inhibitable by TBFβ, *Glia* 4:327-331, 1991.

94. Lapresle J, and Fardeau M: The central nervous system and carbon monoxide poisoning. In: Carbon Monoxide Poisoning edited by H. Bour and I. M. Ledingham. Amsterdam: 1967, Elsevier Publishing CO pp. 31-74.
95. Courville CB: The process of demyelination in the central nervous system, *J Nerv Ment Dis* 125:504-546, 1957.
96. Putnam TJ, McKenna JB, and Morrison LR: Studies in multiple sclerosis, *JAMA* 97:1591-1596, 1991.
97. Funata N, Okeda R, Takano T et al: Electron microscopic observations of experimental carbon monoxide encephalopathy in the acute phase, *Acta Pathol Jpn* 32:219-229, 1982.
98. Song SY, Okeda R, Funata N et al: An experimental study of the pathogenesis of the selective lesion of the globus pallidus in acute carbon monoxide poisoning in cats, Acta Neuropathol 61:232-238, 1983.