Toxicity of Carbon Dioxide: A Review

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Toxicity of Carbon Dioxide: A Review

Adeline Guais,[†] Gerard Brand,[‡,§] Laurence Jacquot,[§] Mélanie Karrer,[§] Sam Dukan,[∥] Georges Grévillot,[⊥] Thierry Jo Molina,[#] Jacques Bonte,[†] Mireille Regnier,[▽] and Laurent Schwartz*[,▽,○]

[†]Biorébus, Paris, France

[‡]Centre des Sciences du Goût et de l'Alimentation (CSGA), Dijon, France

[§]Laboratoire de Neurosciences, Université de Franche-Comté, Besançon, France

[∥]Aix Marseille Université, Laboratoire de Chimie Bactérienne (UPR 9043), Institut de Microbiologie de la Méditerranée (IFR88), CNRS, Marseille, France

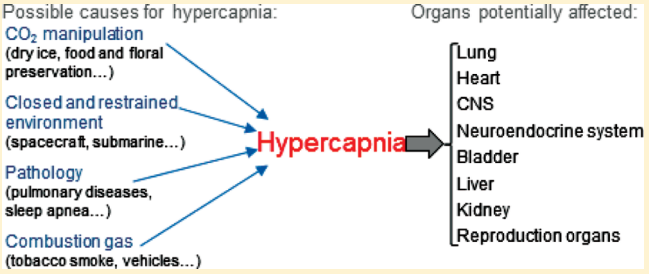
[⊥]Laboratoire des Sciences du Génie Chimique CNRS−ENSIC, Nancy, France

[#]Université Paris Descartes, AP-HP Hôtel-Dieu, Paris, France

[▽]Ecole Polytechnique, Laboratoire d'informatique, Palaiseau, France

[○]AP-HP, Hôpital Raymond Poincaré, Service d'Oncologie, Garches, France

ABSTRACT: The toxicity of carbon dioxide has been established for close to a century. A number of animal experiments have explored both acute and long-term toxicity with respect to the lungs, the cardiovascular system, and the bladder, showing inflammatory and possible carcinogenic effects. Carbon dioxide also induces multiple fetal malformations and probably reduces fertility in animals. The aim of the review is to recapitulate the physiological and metabolic mechanisms resulting from CO_2 inhalation. As smokers are exposed to a high level of carbon dioxide (13%) that is about 350 times the level in normal air, we propose the hypothesis that carbon dioxide plays a major role in the long term toxicity of tobacco smoke.



CONTENTS

1. INTRODUCTION

Carbon dioxide (CO_2) is naturally present in the atmosphere where its concentration varies from 0.03 to 0.06% (vol/vol, equivalent to 0.2 to 0.4 mmHg).[1] Its regularly increasing concentration contributes to the greenhouse effect[2] and the acceleration of global warming.[3] The average indoor concentration of CO_2 is 0.08% to 0.1%.[4] The maximal acceptable concentration has been defined between 0.5 and 3%, depending on the duration of exposure. At normal temperature and pressure, carbon dioxide is an odorless, colorless, and heavier than air gas, with a faintly pungent odor.[5] CO_2 is widely used in industries, especially in agro-productions for conserving, cooling, and medical applications. It is also known to be produced during combustion, putrefaction, and fermentation.

In air, carbon dioxide is a very stable and nonflammable compound. As CO_2 is soluble in water, it can react to form

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carbonic acid (H_2CO_3). Dissolved carbon dioxide in the water undergoes hydration according to the following reaction: $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$. This reaction can interfere with the acid−base balance: pH = pK + log [HCO_3^-/CO_2] (Henderson−Hasselbach equation).

Carbon dioxide is a normal constituent of the human body arising from cellular respiration.[6] Carbon dioxide diffuses from cells into the surrounding capillaries and is carried by the blood either bound to hemoglobin or dissolved as carbon dioxide, carbonic acid, or bicarbonate ion.[7] A minor amount of CO_2 can be bound to plasma proteins to form carbamino compounds.

Carbon dioxide is synthesized in the body, and its partial pressure under normal conditions in pulmonary capillary blood (almost 7% or 46 mmHg) is greater than that in alveolar air (6% or 40 mmHg). The gas is exchanged freely through the alveolar membrane and is thus released from the lungs by diffusion because of the concentration gradient existing between blood and air in the alveoli. Its free diffusion through the lipid cell membranes allows it to be one of the main regulators of intracellular pH acting as a stimulant or a brake in numerous cellular processes. Because of its free diffusion through tissue membranes, the toxicological effects of carbon dioxide appear very rapidly and are mainly observed in the blood pH, lungs, heart and central nervous system (see Table 1 for summary).

2. PERTURBATION OF ACID/BASE BALANCE BY CARBON DIOXIDE

An increase of the partial pressure of CO_2 (pCO_2) delivered to the lungs, i.e., hypercapnia, induces an increase of pCO_2 in the alveoli. Because carbon dioxide freely diffuses through the alveolar membrane to the blood, it results in an increase of the CO_2 tension in arterial blood ($PaCO_2$). This increase in $PaCO_2$ results in turn in an acute or chronic respiratory acidosis.

2.1. Acute Respiratory Acidosis. In acute respiratory acidosis, $PaCO_2$ is elevated above the upper limit of the reference range (i.e., >6.75% or 45 mmHg) resulting in acidosis (i.e., pH <7.35). This acute hypercapnia can be compensated for in two steps. The initial response is cellular buffering that occurs within minutes to hours.[6] Cellular buffering elevates plasma bicarbonate (HCO_3^-) only slightly, approximately 1 mEq/L for each 0.15% increase (10 mmHg) in $PaCO_2$. The second step is renal compensation that occurs over 3−5 days: renal excretion of carbonic acid and bicarbonate reabsorption are increased. In renal compensation, plasma bicarbonate increases of 3.5 mEq/L for each increase of 0.15% (10 mmHg) in $PaCO_2$.

For acute respiratory acidosis, the expected change in pH with respiratory acidosis can be estimated by the following equation: change in pH = 0.008 × (40 − $PaCO_2$).[8] This means that, from a (normal) pH of 7.4 for 6% $PaCO_2$ and 0.03% CO_2 in the atmosphere, the pH could fall to 6.65 when $PaCO_2$ is increased to 20%. In guinea pigs exposed to 15% CO_2 during 1 h (acute response), the $PaCO_2$ value was reported to be 17.8% (119 mmHg).[9]

2.2. Chronic Respiratory Acidosis. In chronic respiratory acidosis, the value of the pH is subnormal secondary to renal compensation and an elevated concentration of serum bicarbonate. In rats exposed to 10% or 15% CO_2 during 11 days (chronic acidosis), Carter et al. measured a plasma CO_2 content of 45 and 52 mEq/L, respectively, and estimated the $PaCO_2$ values at 15% (102 mmHg CO_2) and 22% (148 mmHg CO_2), respectively.[10]

In guinea pigs exposed to 15% CO_2 (about 300 time the normal air level) for 73 days,[11] the uncompensated acidosis period (first variations noticed within one day) is characterized by a decline of extracellular and urine pH, inorganic phosphorus plasma concentration, and an increase of the calcium plasma concentration and urine inorganic phosphorus. During the compensated period, the extracellular pH returns to normal, but plasma calcium is still elevated, and inorganic phosphorus low level is maintained even after 20 days of exposure. This effect on calcium and inorganic phosphorus was associated with renal calcification after 48 h of exposure. In rats exposed to 10 or 15% CO_2 for 11 days,[10] an increase in urine excretion of ammonia and acidic substances was observed. During the first two days (acute response), the ammonia and titratable acid excretion was almost twice the normal values, and the urine pH value was around 6.2 (10% CO_2). Potassium and chloride ions were significantly increased during the first days of exposure.

Body adaptation to chronic high carbon dioxide level is dependent on the concentration administered: below 3% CO_2 the compensatory mechanisms occur more slowly. Volunteers were exposed to 1.5% CO_2 over a period of 42 days, and acid−base balance and changes in electrolyte metabolism were studied.[12] During the first 23 days, a slight uncompensated respiratory acidosis was present followed by a compensated acidosis. Interestingly, arterial CO_2 tension increased by 5 mmHg (0.75%) during exposure and remained at this level during the first nine days of recovery in air. Several other studies were performed in men with low levels of increased carbon dioxide exposure (1.5 to 3%) in order to mimic living conditions in submarines or in space. Interestingly, although there were some minor modifications of the pH and serum level of the electrolytes, the experimental conditions were well tolerated.[13−15]

Guinea pigs were exposed to chronic intermittent high carbon dioxide levels (8 h per day during 7 days, 15% CO_2). While animals exposed to constant CO_2 (15% CO_2 during 7 days) displayed a 3-day-long uncompensated phase and then stabilized (pH 7.37 ± 0.035), animals exposed to intermittent CO_2 could not compensate for the respiratory acidosis, and the pH value was decreased by 0.26 (pH 7.11 ± 0.07).[16] Similarly, Schaefer et al.[17] investigated the acid−base and electrolyte responses to intermittently increased carbon dioxide concentration (concentration increasing up to 3% CO_2, 15 h/day during 5 days) in human beings. The author reported doubling of urine volume on the fourth and fifth days. This increase in urine volume was accompanied by increases in organic acids, titratable acidity, and ammonia, reflecting the elimination of the accumulated carbon dioxide by the kidneys.

Thus, chronic carbon dioxide high tension exposure causes a raise of extracellular acidity that is compensated within days (constant 10% or 15%) or weeks (constant 1.5 or 3%). However, intermittent exposure to carbon dioxide does not allow the compensation mechanisms to be active.

3. METABOLIC EFFECTS OF CARBON DIOXIDE

3.1. CO_2 Implication in Cellular Metabolism. The effects of carbon dioxide on metabolism have been poorly investigated. Warburg, Posener, and Negelein[18] performed the first work on the metabolic effects of carbon dioxide, and they demonstrated the sensitivity of anaerobic glycolysis in a tumor to the concentration of the carbon dioxide−bicarbonate buffer system.

Table 1. Deleterious Effects of High CO_2 Exposure[a]

function	CO_2 level	observed effects	detailed observations	references
acid/base balance	acute hypercapnia (min to few days)	acute acidosis	low extracellular pH and inorganic phosphorus plasma concentration	6
			increase of the calcium and HCO_3^- ions plasma concentrations	
			renal compensation with excretion of NH_3 and acidic substances (low pH) and increase urine inorganic phosphorus	
			compensation within days (10 or 15%) or weeks (1.5 or 3%)	
	chronic hypercapnia (days to months)	chronic acidosis	normal extracellular pH	10−15
			elevated plasma calcium and low level of inorganic phosphorus	
	chronic intermittent hypercapnia	chronic acidosis	increase in urine volume and pH (increase in organic acids and NH_3)	16,17
			slower renal compensation than for constant exposure	
cellular metabolism	1−5%	enhanced glycolysis and respiration	in vitro	18−20
	10−20%	reduction of respiration	in vitro	20
	1.5 to 3%	body weight loss	first 25 to 35 days then partial recover, guinea pigs	25
	3% 7 days	liver function changes	depletion of glycogen vacuoles and increase of fat vacuoles	25
respiratory function	10 to 30%	loss of consciousness	observed in 1 min with 30%, 5−10 min with 10%	33
	from 1%	enhanced respiratory rate	increased respiratory minute volume	34
			increased respiratory amplitude and frequency	
		alteration of cell substructure	decrease in the airway conductance	35,36,24
	3−15% for days	hyaline membranes	elevation of $PaCO_2$	24,38
	10% for 1 h	lung inflammation	changes in the structure of type II alveolar pneumocytes	37,9
			hyperplasia and hypertrophia (increased activity?)	36
			decreased gas exchange	
cardiovascular function	from 5%	increased heart rate	cardiac frequency and arterial pressure, peripheral vasodilatation	34
	1.5 to 15%	no evidence of damage	histopathological analysis	25
CNS function	1 to 4%	low to mild effects	headaches, reduction of stereoactuity and ability to detect motion	33,40−42
	10 to 15% (min)	excitability	eye flickering, psychomotor excitation and myoclonus...	43,6
reproductive function	2.5 to 10% (hours)	testes degeneration	reversible tubular disturbance and degenerative changes in rats	44,25
			decrease in the number of mature spermatozoids	
development	6%	teratogenic	enhanced frequency of skeletal and cardiac malformations of the pups	45
	10%		retinopathy of prematurity (rats)	46,47
	10−13%		vertebral malformations (rabbits)	48
	8% chronic intermit.		body weight loss and alterations of lung maturation (rabbits)	49
cell fate and proliferation	6−30%	alteration of in vitro cell fate	retardation of cell division, alteration of the division process	51,52
	8−12%		potentialization of nicotine and NKK mitogenics effects (PNE cells)	53,54
	10%		stimulation of SCLC proliferation with MAP kinase activation	55
	0.03 to 0.1%	carcinogenic	exacerbation of H_2O_2 toxicity, aggravation of DNA damages (*E. coli*)	56
	45 to 100%		enhanced incidence of cancer and metastases (mice)	20,58−60

[a] Definitions: HCO_3^-, bicarbonates ions; NH_3, ammonia ions; $PaCO_2$, CO_2 tension in arterial blood; PNE cells, pulmonary neuroendocrine cells; SCLC, small cell lung cancer; MAP, mitogen-activated-proteins; NNK, nitrosamine 4-(methylnitrosamino)-l-(3-pyridyl)-l-butanone; *E. coli*, *Escherichia coli*.

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Table 2. Described Mechanisms of Action of Carbon Dioxide or Bicarbonate Ion[a]

target	actor	direct consequences	indirect regulation	references
acetyl-CoA carboxylase	HCO_3^-	formation of malonyl-CoA	formation of fatty acids for the cell membranes	21
pyruvate carboxylase	HCO_3^-	conversion of pyruvate to PEP during glucogenesis		21,23
carbamoyl phosphate synthetase I	HCO_3^-	synthesis of carbamoyl phosphate	urea cycle and regulation of pyrimidine biosynthesis	21,22
extracellular pH (uncompensated acidosis)	CO_2	increased plasma levels of GOT and GPT increased activities of LDH, MDH, isocitrate dehydrogenase, and cholinesterase	inhibition of lipolysis	25−27
central chemoreceptors	CO_2	regulation of cardiovascular system, lung ventilation, olfaction, cerebral circulation		21,32,61,62
PP2A/NFkB pathway	CO_2	activation of PP2A phosphatase activity causing the nuclear translocation of NFkB, not correlated with extracellular pH changes	secretion of the pro-inflammatory cytokines (TNF- α, IL- 8, IL- 6, Mip1-α) and mucin 5AC	36
MAP kinase pathway	CO_2	activation of MAP kinases, increase of the secretion of serotonin	CO_2 is a messenger molecule stimulating PNE cell proliferation	54,55

[a] Definitions: HCO_3^-, bicarbonates; CoA, coenzyme A; PEP, phosphoenolpyruvate; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; LDH, lactate dehydrogenase, MDH, malate dehydrogenase; PP2A, protein phosphatase 2A; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IL, interleukin; MAP, mitogen-activated-proteins.

In 1943, Craig and Beecher[19] demonstrated that the metabolism in the retina is sensitive to the concentration of the carbon dioxide−bicarbonate buffer system. Increasing the carbon dioxide from 1% to 5% at constant pH increases almost 2-fold both glycolysis and cellular respiration. Increasing the carbon dioxide at constant pH from 5% to 20% had no effect on glycolysis but depressed respiration. This was later confirmed in several studies on different normal and cancer cells (for review see [20]).

CO_2 is a product of oxidative metabolism, but CO_2 and its byproduct HCO_3^- are also substrates for important biochemical reactions occurring, for example, in the mitochondria.[21] CO_2 takes part in two types of reactions controlling respiration in animals: the formation and transport of H+ (by reversible hydration of CO_2 and by formation of carbamates from the NH_2 group of proteins) and the stimulation of metabolism.

In the cells, CO_2 is also converted into bicarbonate ion by carbonic anhydrases. HCO_3^- is required in at least three metabolic pathways in the mitochondria of the liver (Table 2).[21] Mitochondria are impermeable to HCO_3^- so that the required anion must be provided by the hydration of CO_2 which can diffuse easily across the membrane. Hydration of CO_2 is the rate limiting factor for these three metabolic pathways. HCO_3^- is involved in the formation of malonyl-CoA (enzyme: acetyl-CoA carboxylase) used for the production of fatty acid components of cell membranes. CO_2 is needed for the conversion of pyruvate to phosphoenolpyruvate during glucogenesis (enzyme: pyruvate carboxylase). Carbon dioxide is also required for the synthesis of carbamoyl phosphate (enzyme: carbamoyl phosphate synthetase I). This is known to be the entry in the urea cycle and the regulated reaction of pyrimidine biosynthesis.

It was demonstrated *in vitro* that the inhibition of a specific liver mitochondrial carbonic anhydrase isoenzyme, the catalyzer allowing a rapid conversion of CO_2 into bicarbonate, reduces the formation of glucose, urea, and fatty acids in hepatocytes.[22] Furthermore, raising the CO_2 concentration (up to approximately 8.5%) increases the carboxylation of ^{13}C labeled pyruvate independently of pH.[23]

3.2. Alteration of *In Vivo* Metabolism Caused by CO_2. Douglas et al. demonstrated that, in guinea pigs, exposure to 1% CO_2 during six weeks did not alter weight evolution as compared to that of the controls.[24] Schaefer et al.[25] studied the effect of long-term exposure of guinea pigs to higher tensions of carbon dioxide with respect to several aspects of metabolism. With exposure to 1.5% CO_2, they observed that the guinea pigs lost weight for about 25 days. The animals then start to regain weight but at a slower rate than that of the controls (2.2 g/day versus 4.75 g/day). During long-term 3% CO_2 exposure, approximately 35 days are required for the weight of the animals to start increasing above the initial level. During exposure to 15% CO_2, a 10% loss of weight occurred during the first two days. At day 20, the rodents started to gain weight for about 20 days to about 50 days.

In vivo CO_2 exposure also affects the expression or activity of certain metabolic enzymes.[25] Exposure of guinea pigs to 15% CO_2 for seven days results in a striking but transient increase in plasma levels in GOT (glutamic oxaloacetic transaminase) and GPT (glutamic pyruvic transaminase). After seven days of prolonged exposure, the concentrations of these two enzymes return to the initial values. These variations follow the pH changes corresponding to the uncompensated phase of respiratory acidosis. The activity of other serum enzymes such as lactate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, and cholinesterase

increases significantly during the first three days of exposure (uncompensated phase) and return to control level after seven days.

Histopathology analyses showed that prolonged exposure to CO_2 (3% during 7 days) causes the depletion of glycogen vacuoles and an increase in fat vacuoles in guinea pigs.[25] After three weeks of exposure to 3% CO_2 and subsequent recovery for one day breathing normal air, glycogen is again synthesized. These functional changes point to important changes in fat metabolism caused by hypercapnia. Acidosis is known to inhibit lipolysis,[26] and one could therefore expect an increase in fat since it would not be easily mobilized. It is noteworthy that both guinea pigs and rats when exposed to even low levels of CO_2 (3%) exhibit similar changes in glycogen and fat vacuolization. That would seem to suggest that modifications of fat metabolism are of special significance in hypercapnia. Lipid accumulation during chronic hypercapnia (15%) shows a specific pattern for different organs. Fat content in muscle is increased only during the first two days and that of lungs during the period from three to seven days, while the lipid content of the liver is greatly elevated throughout the exposure period.

Several *in vitro* or *in vivo* studies have demonstrated that acidosis inhibits lipolytic activity.[27,28] Adrenaline-induced lipolysis and calorigenesis is inhibited in dogs when breathing a mixture of 10% CO_2 and 25% O_2 in N_2, which results in an average pH of 7.0 and an average $PaCO_2$ of 100 mmHg.[29] A study by Longmore et al. showed increased fat synthesis in a perfused liver when the level of CO_2 was raised in the medium.[30] This suggests that another factor adds to the large increase in fat content found in the liver of guinea pigs exposed to 15% CO_2. Similar changes have been observed in both guinea pigs and rats exposed to low levels of CO_2 (3%).

4. PULMONARY TOXICITY OF CARBON DIOXIDE

4.1. Respiratory Function. Most toxicological studies have been focused on respiratory damages. Under normal conditions, spontaneous breathing requires feedback controls in which detection of blood gas levels and pH are critical. CO_2 sensing depends on central chemoreceptors (CCRs) located at multiple sites.[31] They are highly sensitive to CO_2, as an evident change in ventilation occurs with an increase in $PaCO_2$ as small as 0.015% (1 mmHg). Such sensitivity is likely to be attributable to the inherent properties of CO_2/pH sensing molecules (mainly receptors and channels) and their modulation in brainstem neuronal networks. Each of these molecules covers a small range in the whole sensory spectrum. With multiple sensors arranged in parallel, both high sensitivity and broad bandwidth may be achieved.[32]

CO_2 is an asphyxiant, and loss of consciousness can occurs when exposed to 30% during 1 min or 10% during 5 to 10 min.[33] The effects of hypercapnia on the respiratory function appear immediately and at relatively low concentrations (from 1% CO_2). Following exposure to 5% carbon dioxide, there is an increased respiratory minute volume, increased respiratory amplitude and frequency, as well as a decrease in the airway conductance. In monkeys, the respiratory rate increased 2-fold until a 10% carbon dioxide concentration was reached and thereafter decreased until animals died.[34] In a human study,[35] 23 healthy men were exposed at a constant level of 1.5% CO_2 in air for 42 days in a submarine, which served as the experimental chamber. Throughout the exposure to CO_2, the respiratory minute volume and alveolar CO_2 tension were increased. During the postexposure period (9 days), the respiratory minute volume decreased event though the CO_2 tension remained elevated. The authors divided the 42-day exposure period into two parts. The first phase (days 1−23, uncompensated acidosis) was characterized by a significant increase of the alveolar carbon dioxide tension, carbon dioxide excretion, and respiratory exchange ratio. The second phase (days 24−42, compensated acidosis) was characterized by an increased excretion of carbon dioxide.

The early acute response to high CO_2 tensions (above 5%) is characterized by an enhanced respiratory volume. This is illustrated by the very rapid raise (within minutes) of respiratory distress evaluated from plethysmography-measured parameters during the exposure of mice to 5, 10, or 15%.[36]

4.2. Acute and Chronic Lung Toxicity. Douglas et al. studied the consequences of chronic exposure (up to 6 weeks) to 1% CO_2 in guinea pigs.[24] They observed an elevation of $PaCO_2$ associated with a metabolic acidosis that reached a maximum at four weeks of exposure and persisted even after two weeks of recovery in normal air. Electron microscopy analysis showed changes in cell fine structure of type II alveolar pneumocytes (granular pneumocytes) and lamellar bodies, hyperplasia (cluster of 2−4 cells), and hypertrophia of these cells as compared to those in the control, suggesting an increased activity of these cells.

Animal studies have indicated that chronic exposure to higher levels of CO_2 can cause hyaline membrane formation and atelectasis in guinea pigs and can cause edema in rat lungs. Niemoller and Schaefer[37] exposed guinea pigs and rats to different CO_2 concentrations (from 3 to 15%) during prolonged and continuous exposures (from two days to six months). Loss of surfactant (complex system of lipids, proteins, and lipoproteins which allows the alveoli to remain open throughout the normal cycle of inhalation and exhalation) was associated with hyaline membrane (fibrins, cellular debris lining, or filling the alveolar spaces) formation that led to decreased gas exchange, associated with respiratory distress syndrome. Microscopic examination indicated that guinea pigs exposed to 3 and 15% CO_2 developed hyaline membranes (respectively from fourth and first days), while those exposed to 1.5% CO_2 did not, supporting the hypothesis of a threshold for CO_2-induced lung toxicity.

In a follow-up study,[9] guinea pigs were exposed to CO_2, and data were gathered from electron microscope studies, surface tension measurements of lung tissue, and additional histochemical studies. These authors identified four phases of pulmonary changes caused by 15% carbon dioxide. The initial phase (6 h) was marked by uncompensated respiratory acidosis accompanied by pulmonary effusion (edema, congestion, atelectasis, and hemorrhage) as well as changes in the lamellar bodies (intracellular stores of surfactant) of the granular (type II) pneumocytes. This period was not associated with hyaline membrane formation. The second phase (6−24 h) was associated with hyaline membrane formation. During the third phase (days 2−7), the surface tension returned to normal, the pulmonary edema diminished, and hyaline membranes disappeared. The final phase was one of recovery despite the fact that the pCO_2 remained elevated.

Recently, Abolhassani demonstrated that inhalation of levels of carbon dioxide above 5%, for one hour, induced pulmonary inflammation. The authors showed an increase in the secretion of the pro-inflammatory cytokines TNF alpha, interleukin 8, and interleukin 6 Mip1-alpha as well as mucin 5AC, a major

pulmonary mucus glycoprotein overexpressed during inflammation. This inflammation was caused by the methylation of the C subunit of the phosphatase PP2A (protein phosphatase 2A), which in turn controls the translocation of the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells). Interestingly, complementary *in vitro* experiments do not seem to correlate pH variations to this inflammation response as IL-8 secretion was not induced in response to acidic pH imposed in the culture medium. These molecular biological findings were confirmed by microscopic examination of the lung. Extensive lung inflammation with infiltration of the parenchyma by lymphocytes and monocytes was observed.[36]

Additionally, Ryu et al. compared the consequences of the exposure of mouse neonates versus adults to 8% CO_2 for two weeks.[38] They showed that CO_2 exposure decreased lung alveolar walls thickness, reduces lung weight, and alters lung matrix protein composition (among them the decrease of interstitial collagen) in young mice. In comparison, adult lungs were not affected, which highlighted the sensitivity of young individuals to CO_2 tension variations.

5. EFFECTS OF CARBON DIOXIDE ON CARDIOVASCULAR FUNCTION

The first noticeable effects of CO_2 inhalation is an increase in heart rate. For example, exposure to levels of at least 5% CO_2 resulted in the first signs of cardiovascular and vasomotor impacts (cardiac frequency and arterial pressure, and peripheral vasodilatation) in humans. The same signs are observed in dogs and monkeys[34] at concentrations of up to 10% of the gas. In dogs with left ventricular failure (embolization of the left coronary artery), hypercapnia aggravated heart failure (increase of the left ventricular end-diastolic pressure, mean right arterial pressure, and mean right arterial pressure); however, the pump function of the heart was unchanged.[39] A reversion of the central hemodynamics changes was observed when pH was normalized during hypercapnia, meaning that pH, and not $PaCO_2$, was responsible for the observed hemodynamic deterioration.

Schaefer analyzed the heart histopathology of guinea pigs after exposure to 1.5, 3, or 15% CO_2.[25] No evidence of permanent myocardial damage was seen either in animals that expired during the period of acute acidosis or those that were sacrificed at one day or seven days following initiation of the exposure. However, a small amount of lipid (red O stain) was seen in 1 animal after 1 day, and by 7 days positive material was seen in 5 of 10 animals. To our knowledge, no similar experimentation was performed to reproduce these data.

6. EFFECTS OF CARBON DIOXIDE ON CENTRAL NERVOUS SYSTEM AND NEUROENDOCRINE FUNCTION

Carbon dioxide is a key factor in the control of respiration and cerebral circulation. It acts peripherally, both as a vasodilator and as a vasoconstrictor, and is a powerful cerebral vasodilator.

The majority of the studies reported that chronic low concentration of CO_2 induces low to mild effects: visual impairment occurred at 1% CO_2 and headaches were noticed in the first days of exposure above 2%.[32] In general, no specific neurobehavioral changes or adverse effects were reported with a level at up to 4% for the duration for up to two weeks.[40] However, more recent studies showed a decrease in stereoacuity and a decrease in the ability to detect motion with levels above 2.5%.[41,42]

At high concentrations, CO_2 exerts a stimulating effect on the central nervous system, while excessive levels exert depressant effects.[43] Exposure to 10% carbon dioxide during approximately 1.5 min causes neurologic signs including eye flickering, psychomotor excitation, and uncontrolled muscle contractions. At 15%, the same signs were recorded, as well as increased muscle tone, perspiration, flushing, restlessness, dilated pupils, and leg flexion and torsion spasms. Apart from excitability, no abnormal behavior has ever been observed after carbon dioxide exposure (psychomotor tests, resolution of problems, etc.).[6]

Carbon dioxide induces changes in the secretion of hormones. Continuous exposition (15% CO_2, 7 days)[16] stimulates the adrenal gland of guinea pigs. If animals are intermittently exposed to this same concentration of CO_2 (8 h daily for 7 days), there is an initial fall of pH but no compensation of respiratory acidosis and no changes of the sympathoadrenal responses. From these observations, the authors suggest that the stress response in chronic hypercapnia depends on extracellular and related intracellular changes and is representative of a nonspecific pH-dependent effect.

7. ALTERATION OF THE REPRODUCTIVE CAPACITY BY CARBON DIOXIDE

In rats,[44] carbon dioxide causes degenerative changes of the testes. These modifications depend on both the dose (2.5%, 5%, or 10% carbon dioxide) and the duration of exposure (1 to 8 h). Major histological effects included tubular disturbances such as sloughing as well as the loss of luminal definition (5% during 4 h) and degenerative changes such as streaking and vacuolization (10% during 4 h). These modifications are reversible, as the testes were normal 36 h after carbon dioxide exposure.

A concentration of 15% chronic CO_2 affects the spermatogenesis of guinea pigs and rats.[25] The first changes in spermatogenesis are noted after 48 h. There is a marked decrease in the number of mature spermatozoids. After 3−7 days, multinucleated giant cells are seen. However, prolonged exposure to low levels (1.5 and 3% CO_2) did not produce any spermatogenic arrest in guinea pigs and rats. Surprisingly, there are no recent data relating carbon dioxide exposure to fertility.

8. TERATOGENICITY OF CARBON DIOXIDE

Hypercapnia is teratogenic. Exposition of rats to 6% carbon dioxide (single 24-period between days 5 and 21 of pregnancy) causes some malformations in newborn pups:[45] cardiac malformations in 24% of the tested animals (7% in control), and skeletal malformations in 11% (0.6% in control). Exposure to higher CO_2 levels (10%) and consecutive acidosis of neonatal rats promote retinopathy of prematurity, a potentially blinding eye disorder that primarily affects premature infants.[46,47]

In rabbits[48] exposed to 10−13% carbon dioxide, the newborn pups had vertebral malformations. Furthermore, Nagai A. et al.[49] examined fetuses from rabbits exposed from day 21 to day 28 of gestation to 8% CO_2 for 8 h each day. These fetuses weighed less and presented numerous characteristics of increased tissue and cellular maturation of the lung (increased distended lung volumes, increased volume proportion of air spaces, decreased airspace wall, less glycogen and lamellar bodies, etc.).

Mice exposed to 20% CO_2 for 8 h on day 10 of gestation produced right-sided postaxial forelimb ectrodactyly in 23% of the offspring. Rather than metabolic acidosis, it would seem that

the primary teratogenic factor in hypercapnia is elevated CO_2 tension (low incidence of ectrodactyly associated with NH_4Cl-induced acidosis). Moreover, there is a strong correlation between maternal serum CO_2 content and the incidence of ectrodactyly.[50]

9. CARCINOGENIC POTENTIAL OF CARBON DIOXIDE

9.1. *In Vitro* Alteration of Cell Fate by CO_2 Exposure. In 1925, Bauer[51] exposed chicken tissues to carbon dioxide (concentration level not reported) *in vitro* during 6 to 8 days. The author primarily described the consequences of carbon dioxide on dividing cells, and, in particular the retardation of cell division (in prophase, anaphase, and telophase) as well as some alterations of the division process: "It was noted that the entire equatorial plate moved slowly from one pole to another without a division of the chromosomes."

In 1927, Mottram[52] described that the CO_2 tension and/or acidity applied to culture cells control cell activity, in particular cell migration. He also evoked the role that carbon dioxide could play in cancer etiology. In a follow-up experiment, Mottram cultivated kidneys cells and fibroblasts of young rats with different concentrations of carbon dioxide during 3 days; cells were then fixed and mitoses were counted (523). From these observations, he deduced that the optimum tension of CO_2 for the cell division in normal cells is the physiological CO_2 tension (6% CO_2) but that cell division occurs at concentrations above (up to 30% CO_2) and below (0 mmHg) this normal tension. Interestingly, while counting these mitoses in fibroblasts, many abnormal features were observed in the cultures grown at elevated CO_2 tensions. These features consisted of "an irregular migration of the chromatin towards the centrosomes; some chromatin remained suspended at the equator of the spindle, while other fragments had already migrated to the centrosome. This unusual arrangement was more often than not asymmetrical, a fragment of chromatin being present at one centrosome with none at the other, or more fragments at one centrosome than at the other. It was also observed that the size of the nuclei of undividing cells under high tension of CO_2 was increased, while reduced at low tensions, as compared to nuclei of cells at 40 mmHg (6%) CO_2." Thus, high carbon dioxide concentration clearly acts as a disrupter of normal cell division processes. The author also noticed that these abnormalities were similar to those observed in cells that had been subjected to X-irradiation, where, "besides fragmentation of the chromatin into fine granules, delay in its migration to the centrosomes occurs, so that whilst some chromatin has moved to the centrosomes, some remains suspended at the equator of the spindle". Similarly as after X-irradiation, an increase in size of the cell nuclei was observed under high CO_2 concentrations. These observations support the hypothesis of a role of supraphysiological concentrations of carbon dioxide in carcinogenesis via the disruption of cell division processes. Recent data partially complete these earlier findings.

Schuller et al. addressed the mechanisms of cell proliferation in response to nicotine and NNK (nitrosamine 4-(methylnitrosamino)-l-(3-pyridyl)-l-butanone) in normal pulmonary neuroendocrine (PNE) cells derived from fetal hamster lung and in two cell lines derived from human neuroendocrine lung cancers.[54] Their data demonstrated that the mitogenic effects of nicotine and NNK are potentiated by elevated levels of CO_2 (from 8 to 12%) in a concentration-dependent manner, suggesting that the two signal transduction pathways initiated by CO_2 and nicotine may converge at downstream components. In a follow-up study, Merryman demonstrated that a concentration of 10% CO_2 stimulated the proliferation of small cell lung cancer cells exposed *in vitro* with the activation of mitogen-activated-proteins (MAP) kinases, ribosomal S6 kinase, and an increase production of an autocrine growth factor, serotonin. Thus, CO_2 should be considered as an important messenger molecule in the lung.[55] Interestingly, this article also underlined that chronic non-neoplasic pulmonary diseases (chronic obstructive pulmonary disease, asthma, emphysema, chronic bronchitis) are characterized by an impaired respiration and an augmentation of carbon dioxide pulmonary tension (7 to 40%), which might promote lung cancer development.

Very recent work performed in *Escherichia coli*, demonstrated that CO_2 (from 0.004% to 0.1%) exacerbates H_2O_2 toxicity and aggravates DNA damage and mutation frequencies caused by exposure to the reactive oxygen species [56]

These articles support the hypothesis that high carbon dioxide tension might promote cancer development. These arguments are reinforced by the recent development of antitumor agents targeting carbonic anhydrase (for a review, see ref 57), a key enzyme for carbon dioxide intracellular hydration.

9.2. *In Vivo* Carcinogenicity of Carbon Dioxide. The following articles describe the carcinogenic effects of CO_2 *in vivo*. It should be noted, however, that for most of them, the concentrations of carbon dioxide used were very high.

In a transplantation experiment, skin autografts were exposed *in vitro* before transplantation during 48 h to 45 to 48% CO_2 in air (control: room air culture). Although some malignant lymphomas were observed in host animals using untreated autografts, the lymphoma incidence was highest in the recipients of the CO_2-treated grafts. Other abnormalities of the reticuloendothelial system were noted: proliferation of lymph follicles into irregular masses of pleomorphic cells, hyperplasia with concomitant atrophy of the lymphoid tissue, and replacement of the lymph follicles by malignant lymphoid cells.[20]

The long-term clinical effects of high CO_2 tensions on various normal tissues in mice have been investigated.[58] Different tissues were exposed *in vitro* to a high CO_2 proportion in air (45% CO_2) before transplantation into syngeneic or autologous hosts, or, in a second protocol, intraperitoneal tissues were exposed *in vivo* to CO_2-infusion (99.99%), thus avoiding graft−host interactions. In the autologous grafts, pretreatment by intraperitoneal CO_2-infusion induced lymphoma (60% incidence); air-infusion did not. Nonlymphoid grafts exposed *in vitro* to elevated CO_2 induced only lymphoid malignancies. But nonlymphoid tissues exposed *in vivo* to elevated CO_2 developed tumors of other tissues, such as lung tumor, in addition to lymphoid malignancies. In fact, the spontaneous pulmonary adenocarcinoma incidence doubles in the mice exposed to intraperitoneal CO_2. The same morphological lymphoid abnormalities occurred in all lymphoma-developing animals in these three experimental models: hyperplasia in the splenic T-cell areas appeared most frequently (70−75% incidence), whereas atrophy in T-cell areas of the lymph nodes and B-cell areas hyperactivity were far less frequent.

In a mouse model for multiple laparoscopies, intraperitoneal insufflations of approximately 3.5 mL of CO_2 were given daily to three groups of BALB/c mice for 11, 20, and 32 consecutive days (control: air insufflation). Proliferation of splenic T-lymphocytes (doubling of the T-cells spleen percentage) was an early, but transitory, immunologic reaction in the spleen to intraperitoneal CO_2 insufflation. This was correlated to the late occurrence of a

high incidence of malignant lymphoma (approximately 60%). The long-term survivors of CO_2 insufflation also developed a wide spectrum of malignancies that were not of lymphoid origin, specifically adenocarcinoma in various organs: lung, kidney, adrenals, ovary, gastrointestinal tract, and salivary gland.[59]

The effects of high concentrations of CO_2 on experimental murine neuroblastoma tumors have also been studied.[60] The local growth of this neuroblastoma model was not affected by concentrations of 76% and 55% of CO_2 applied for 10 and 30 min. Although, the tumor bearing animals exposed to different CO_2 concentrations tended to develop metastases more frequently than the control groups.

10. CONCLUSIONS

Below its immediately lethal concentration, carbon dioxide has long been considered as a neutral compound for the body. Although few elements are known about its mechanisms of action (Table 2), recent studies raised interest in carbon dioxide in relationship with chronic and/or intermittent long-term exposure conditions that might induce pathologic states, in particular favor DNA alterations,[56] nasal inflammation,[61,62] and pulmonary inflammation.[63,36,64]

There are various situations when pCO_2 can rise in inhaled air: first, during professional exposures such as recurrent manipulation of dry ice, food and floral preservation, wearing of masks, spacecraft, aircraft, submarine, altitude, and exposure to gas combustion;[65,33,4] second, during pathological exposures such as sleep apnea, pulmonary diseases (e.g., chronic obstructive pulmonary disease, asthma, emphysema, and chronic bronchitis).[66] In two recent studies, Schwartz et al. demonstrated the implication of CO_2 in cigarette smoke-induced lung acute inflammation, independently of hypoxia or particles content.[36,64] These results raised the hypothesis that carbon dioxide content of cigarette smoke might be implicated in smokers' pathologies. The effects of pulmonary neuroendocrine cell (PNE) exposure to carbon dioxide such as that described in part 9.1[54,55] could be linked to the observed high level of tobacco-smoke-related small cell lung cancers, proposed as likely originating from PNEs.[67] The proinflammatory action of carbon dioxide might play a role as a promoting agent in conjunction with the numerous other compounds of cigarette smoke.

The toxicity of carbon dioxide has been established for close to a century. *In vivo* carbon dioxide exposure essentially alters acid/base body balance and cellular metabolism, and also lung, heart and CNS functions are disrupted. There are elements showing a reduction of reproductive capacity and teratogenicity. The role of long-term exposure to carbon dioxide, namely, carcinogenesis, has to be investigated because it is now proven that this compound in addition to H_2O_2 can be responsible for mutagenesis in bacteria.

AUTHOR INFORMATION

Corresponding Author

*Hôpital Raymond Poincaré, Service d'Oncologie Pédiatrique, 104 Boulevard Raymond Poincaré, 92380 Garches, France. Tel: +33 681899030. E-mail: laurent.schwartz@polytechnique.edu.

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