The Blue Baby Syndrome

Nitrate Poisoning in Humans

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Blue Baby Syndrome or Methemoglobinemia is caused by decreased ability of blood to carry oxygen, resulting in oxygen deficiency in different body parts. Infants are more susceptible than adults. The disease can be caused by intake of water and vegetables high in nitrate, exposure to chemicals containing nitrate, or can even be hereditary. Groundwater gets contaminated by leaching of nitrate generated from fertilizer used in agricultural lands and waste dumps in rural and urban areas. Prevention of water contamination, abstention from drinking contaminated water and controlling nitrate levels in drinking water by water treatment are effective preventive measures against nitrate poisoning.

Intake of drinking water containing nitrate (as NO_3^-) > 45 mg L^{-1} (or > 10 mg L^{-1} NO_3^-–N), vegetables rich in nitrate, or exposure to some drugs and chemicals may cause methemoglobinemia in infants and adults. The toxicity of nitrate in humans is an end result of the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) in the intestine. Nitrate reacts with hemoglobin to form methemoglobin (MHb), a substance that does not bind and transport oxygen to tissues, thereby causing asphyxia (lack of oxygen), resulting in cyanosis of body tissues. Methemoglobinemia may be accentuated by an inherited enzyme deficiency, structural defects in the hemoglobin molecule, or by any toxic substance, which either oxidizes hemoglobin directly, or facilitates its oxidation by oxygen (*Box* 1).

Keywords
Cyanosis, hemoglobin, infants, methemoglobinemia, nitrate.

Methemoglobinemia

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Box 1. Agents Implicated in Acquired Methemoglobinemia

Direct Oxidants

Therapeutic Agents	*Domestic & Industrial Agents*
Amyl nitrite	Well water high in nitrates
Ethyl nitrite	Food high in nitrates
Sodium nitrite	Nitrous gases
Ammonium nitrate	Corning extract
Silver nitrate	Potassium chlorate
Bismuth subnitrate	
Nitroglycerin	
Quinines	

Indirect Agents

Sulfonamides	*Miscellaneous Compounds*
Sulfamethazole	Acetanilid
Sulfanilamide	Aminobenzenes
Sulfapyridine	Aminophenol
Sulfathiazole	Benzocaine
Prontosil	Nitrobenzene
	Nitrotoluenes
Aniline Dyes	Phenacetin
Diaper marking ink	Phenazopyridine
Dyed blankets	Phenylenediamine
Laundry markings	Prilocaine
Freshly dyed shoes	Resorcin
Red wax crayons	Toluenediamine
	Trinitrotoluene

in methemoglobinemia. Much of the ingested nitrate is usually absorbed before reaching the nitrate-reducing bacteria, which reside in the intestinal tract. Most of the ingested nitrate is excreted within 24 hours mainly through urine, as well as through feces and sweat. If nitrate is introduced directly into the colon, methemoglobinemia is readily produced. Nitrite produced from nitrate enters the bloodstream mainly through the upper gastrointestinal tract. Nitrate is converted to nitrite by intestinal bacteria, and nitrite acts as the oxidizing agent to form MHb in the red blood cells.

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Oxygen is required for all human tissues to enable them to combust food materials carried to them by blood and other body fluids. Hemoglobin in red blood cells carries oxygen to tissues from lungs by forming oxyhemoglobin. Oxygen is released in the tissues and deoxyhemoglobin is left behind to return to lungs for reoxygenation. For hemoglobin to act as a carrier, iron atom in the molecule has to be in the reduced (Fe^{++}) state. When nitrite is absorbed in bloodstream, it oxidizes hemoglobin to MHb, a Fe^{+++} compound with reduced oxygen transport capacity, as this compound contains iron in its highest oxidation state which is incapable of binding oxygen. Thus, nitrate reduces the total oxygen carrying capacity of the blood. As different parts of the body get deprived of oxygen, clinical symptoms of oxygen starvation start to appear, the main being cyanosis (derived from 'cyano', meaning dark blue; from Greek, kyanos). The lips or even the skin start to take on a blue colouration, hence the common name, the blue baby syndrome/ disease. Although persons of any age can suffer from methemoglobinemia, infants are particularly susceptible during the first four months of their life, as their total fluid intake per unit body weight is approximately three times more than that of adults. The pH of the stomach fluids in infants is also higher (5-7) than in adults (<4), which allows nitrate-reducing bacteria to grow in the upper gastrointestinal tract from which nitrite is relatively easily absorbed. In adults, as the stomach fluid is more acidic, the nitrate reducing bacteria live in the lower intestine, from which absorption of nitrite to the bloodstream does not occur. Gastrointestinal illness and diarrhoea in adults may allow the bacteria responsible for conversion of nitrate to nitrite to migrate from lower intestine to upper intestine and stomach, and increase the chances of nitrite formation prior to absorption in small intestine. Another reason why this disease is not commonly seen in adults is the presence of an enzyme, MHb reductase (erythrocyte cytochrome 5*b*-reductase), in the red blood cells of adults. This enzyme reduces MHb, and thus helps avoid the danger of methemoglobinemia in adults.

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Since hemoglobin is constantly exposed to oxidative stresses, small amounts of MHb are naturally formed all the time in the human body. MHb is produced at a slow, predictable rate in vivo by the escape of an electron from heme. Following loss of an electron, binding of oxygen by the oxidized heme cannot occur unless an electron is regained by means of reducing mechanisms within the cell. Because the capacity of red cells to reduce oxidized heme exceeds the spontaneous rate of heme oxidation by several hundredfold, only 0.5-2.0% of the total hemoglobin is in MHb form at given time. In normal adults, MHb are efficiently reduced again in a reaction catalyzed by MHb reductase. The most important pathway of MHb reduction utilizes NADH-MHb reductase for the transfer of an electron from NADH to heme. The reaction appears to proceed in two steps: (i) enzymatic reduction of cytochrome *b*5, followed by (ii) nonenzymatic transfer of an electron from reduced cytochrome *b*5 to MHb (*Figure* 1a). Another pathway for MHb reduction involves the direct transfer of electrons from ascorbic acid and glutathione to heme. The NADPH-dependent MHb reductase lacks an endogenous electron acceptor and is, therefore, physiologically inert. It may be 'activated', however, by an exogenous electron acceptor, such as methylene blue. The NADPH-dependent enzyme reduces methylene blue to leukomethylene blue, which rapidly reduces MHb nonenzymatically (*Figure* 1b). Reduction of methylene blue to leukomethylene blue requires an

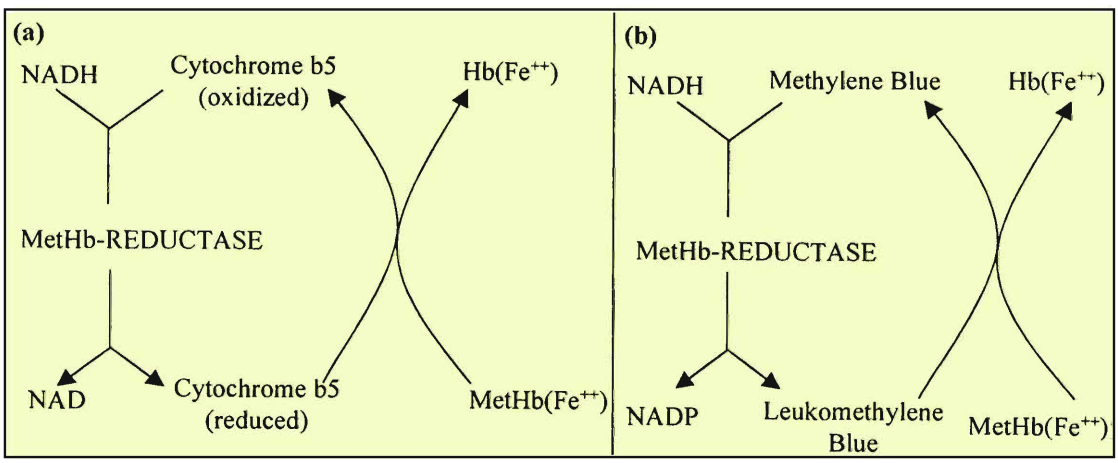

Figure 1. Metabolic pathways for the reduction of MHb to hemoglobin.
a. NADH-dependent MHb reductase pathway.
b. NADPH-dependent MHb reductase pathway, requiring an exogenous electron acceptor (methylene blue).

At approximately 10% MHb levels, the body starts to take on a slaty gray appearance of cyanosis, and the disorder becomes clinically detectable. When MHb levels reach 40% or more of the total haemoglobin, the patient may collapse and become comatose, or die.

intact pentose phosphate pathway for regeneration of NADPH. Consequently, methylene blue is without effect on MHb levels in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Unfortunately, newborn infants have a very low concentration of the enzyme MHb reductase. This concentration remains low until the age of 4 years. Apart from this, fetal hemoglobin, present in infants, is highly susceptible to oxidation to form MHb. Moreover, poor excretory power of immature kidney in infants may favour retention of nitrite ion, which may aggravate the situation.

Clinical Features

Infected infants show blueness around the mouth, hands, and feet and hence the common name 'blue baby syndrome' has come into being. At approximately 10% MHb levels, the body starts to take on a slaty gray appearance of cyanosis, and the disorder becomes clinically detectable. Diagnosis can confirm methemoglobinemia by eliminating other causes of cyanosis, and by spectrophotometric analysis of blood, which gives a characteristic absorption peak at 634 nm. Patients may show asthenia (loss of muscular strength), dizziness, headache, drowsiness, dyspnoea (lack of O_2 supply and difficult respiration), chest pain, nausea, diarrhoea and troubled breathing. Other symptoms, like renal or liver injury, and hemolysis (lysis of blood cells) may occur. In extreme cases, there is marked lethargy, stupor, an increase in the production of saliva, loss of consciousness and seizures. If and when MHb levels reach 40% or more of the total haemoglobin, the patient may collapse and become comatose, or die.

Treatment

Any patient with evidence of toxicity and/or methemoglobinemia levels > 30% should be treated. Mild cases do not require active treatment other than avoiding the contaminated source of drinking water and these patients usually recover within 24 to 72 hours, since MHb level automatically goes down to harmless

levels. A severely affected person requires a therapy of methylene blue, which is optimum at 1-2 mg kg^{-1} body wt., through a 1% solution of the same intravenously for a ten-minute period. This converts MHb to haemoglobin and gives immediate relief. Ascorbic acid, given orally in divided doses of 300 to 500 mg daily, also effects a slow, nonenzymatic reduction of MHb. Dietary intake of vitamin C has been found to help maintain lower levels of MHb by reducing nitrite to nitric oxide (NO). Vitamin E (α-tocopherol) and some unsaturated acids e.g. ferulic acid, also act as nitrite scavengers. Emergency exchange blood transfusion is indicated when levels of MHb exceed 60-70%, or if massive hemolysis occurs. Occasionally, in very severe cases, administration of high flow oxygen (100%) is done, which allows saturation of normal hemoglobin and increases dissolved oxygen.

Hereditary Methemoglobinemia

In contrast to acquired methemoglobinemia, the hereditary forms are rare. An autosomal recessive mode of transmission characterizes familial methemoglobinemia due to NADH-MHb reductase deficiency, and an autosomal dominant mode is characteristic of certain hemoglobin variants, which stabilize iron in the oxidized state.

Hereditary methemoglobinemia responsive to the administration of methylene blue or ascorbic acid was initially described in persons of European descent. Following the demonstration that these individuals were deficient in NADH-dependent MHb reductase, a near-global distribution of the disorder was recognized. Congenital deficiency of NADH-MHb reductase has been recognized as the cause of methemoglobinemia among Navajo and Alaskan Indians, Cubans, Indians, Chinese, and Japanese. Affected subjects are presumably homozygous for a rare, functionally abnormal allele of the enzyme, although double heterozygosity for two different variants has also been documented. The carrier or heterozygous state is characterized by intermediate levels of enzyme activity together with a greater

than normal susceptibility to MHb formation following exposure to oxidants, drugs, and chemicals.

The sole clinical expression of enzyme deficiency is cyanosis, often dating from birth, in the absence of associated signs or symptoms, and with no evidence of cardiopulmonary disease. The hue may be slaty gray, gray-brown or violet. It is generalized over the whole body but is particularly noticeable in the lips, the mucous membranes of the mouth, the tongue, the palate, the nose, over the cheekbones, on the ears and in the nail beds. Untreated individuals generally maintain levels of MHb between 15 and 30%. Most of the MHb is segregated in a population of older cells. Methylene blue taken orally in doses of 100 to 300 mg daily, or ascorbic acid in doses of 500 mg daily is usually sufficient to maintain the level of MHb below 10%.

Sources of Nitrate Entering the Human Body

Nitrate Contamination in Groundwater

Nitrogen accumulates in the soil naturally from rainfall, plant debris, animal residues and microbial fixation of N_2 from atmosphere. Anthropogenic nitrate (NO_3^-) comes to groundwater through nitrogenous fertilizers, organic manures, and human and industrial wastes (*Figure* 2). When nitrate-nitrogen supply exceeds plant demand, the groundwater gets contaminated by leaching which is the downward movement of NO_3^-–N with water through the soil. The potential for NO_3^-–N leaching is greater in sandy soils, but can occur on finer textured soils (e.g. clayey soils) also, albeit at a slower rate. In India, groundwater in many areas has been found to contain more than 45 mg L^{-1} NO_3, which is potentially dangerous to health, although concentration seems to vary with time, depending on season, farming practice, etc.

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Nitrate in Leafy Vegetables

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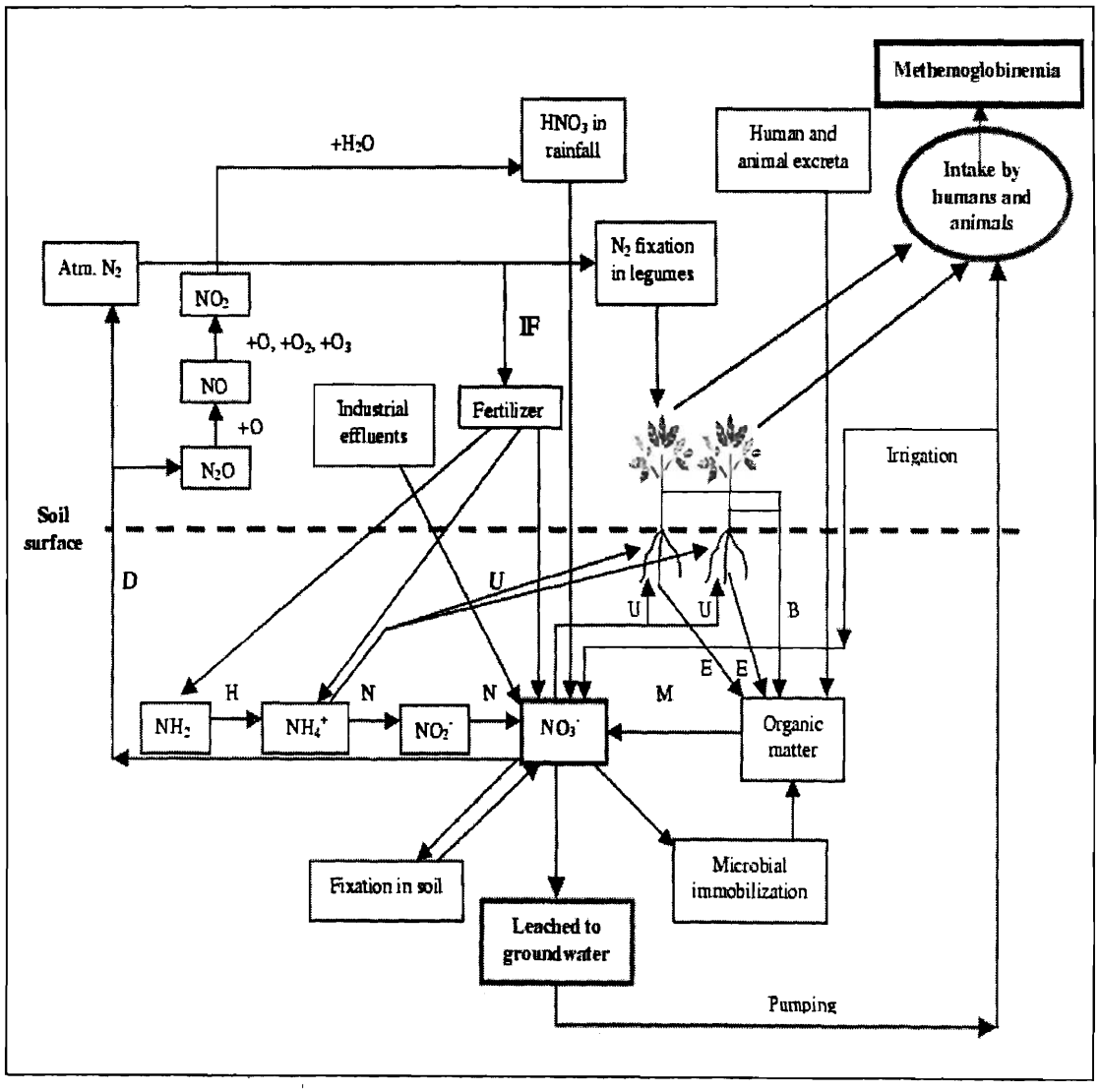

even higher because of fertilization practices. There have been several reports of methemoglobinemia following the consumption of spinach, but the conversion of nitrates to nitrites during storage, rather than the nitrates themselves was responsible for methemoglobinemia. Moreover, it has been suggested that other compounds, possibly ascorbic acid, present in leafy vegetables, may provide protection against in vivo reduction of nitrates to nitrites.

Figure 2. Formation and cycling of nitrate in the environment and its arrival in human and animal bodies [M: Mineralization; N: Nitrification; U: Uptake; D: Denitrification; E: Exudation; IF: Industrial fixation; B: Biomass addition to soil on death].

Nitrate Incorporation during Food Preparation

Addition of high nitrate- or nitrite-containing water to a food product during preparation will increase the nitrate/nitrite content of the final product. Heating of the water either before its addition or as part of the preparation procedure will increase the nitrate/nitrite concentration. Since methemoglobinemia is caused by nitrites, rather than nitrates, conversion of nitrates to nitrites in food preparation procedures should be avoided. Discarding the cooking liquid of high-nitrate vegetables would lower nitrate content of the diet, but some other water-soluble nutrients, including vitamin C, also would be lost. Conventional home canning and freezing practices will minimize nitrate/nitrite conversion by eliminating or limiting the microbial action responsible for the change.

Nitrate from Chemicals

Various chemical compounds used in home or industry as well as several therapeutic agents (*Box* 1) are capable of increasing the rate of heme oxidation 100 to 1000-fold, thereby overwhelming the capacity of erythrocytes to maintain hemoglobin in the reduced state. An indirect effect is postulated for certain aromatic amino and nitro compounds including acetanilid, phenacetin (Empirin, Anacin, Stanback), sulfonamides, phenazopyridine (Pyridium) and aniline dyes. Since most of these agents do not produce MHb in vitro, active intermediate compounds are presumed responsible. The ingestion of deodorant containing naphthalene and aniline or red wax crayons containing *p*-nitroaniline, contact with marking ink, dyed blankets or laundry marks on diapers and benzocaine prilocaine, resorcin, aniline dyes or other aromatic compounds absorbed orally, rectally or percutaneously have been shown to lead to methemoglobinemia.

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Testing recommendations

The only way to know if drinking water is contaminated with nitrates is to have it tested. It is recommended that water should be tested once every three years for nitrates, more often in an

area with a history of high nitrate levels, or if someone at home is at risk from nitrate contamination. Field tests for nitrate include diphenylamine blue (1% DPB in concentrated sulfuric acid) and nitrate dipsticks. The DPB test is more suitable to determine presence or absence of nitrate in suspected forages: a drop or two are applied on a cross-section of plant stalk material, then any dark blue color change is noted as an indicator of excessive nitrate content in the sample. Nitrate dipsticks are used primarily for testing water supplies. The dipstick method is rapid and gives indications of both nitrite and nitrate concentrations over a relatively wide range. Field tests are presumptive and should be confirmed by standard analytical methods at a recognized laboratory.

Water Treatment Methods to Produce Low-nitrate Drinking Water

1. *Distillation*

The distillation process involves heating water to boiling, resulting in evaporation, after which the resulting steam is collected and condensed using a cooled metal coil. Up to 99% of the nitrate-nitrogen can be removed by this process. Merely boiling water will increase rather than decrease the nitrate concentration. Some distillers use activated carbon filters to assist in removing any organic contaminants, whereas others any preheat the water before it enters the boiling chamber in order to help remove volatile organic contaminants.

2. *Reverse Osmosis*

In reverse osmosis, pressure is applied to the impure water forcing the more concentrated water in a reverse direction through a semi-permeable membrane. As the water passes through, the membrane filters out most of the impurities. According to manufacturers' literature, from 85 to 95% of the nitrate can be removed by this process. A disadvantage of this method is that only about 30% of the water entering the reverse osmosis unit is recovered as treated water. The remaining 70% is

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discharged as waste along with the impurities, which have been removed from the product water.

3. *Ion Exchange*

The ion exchange process removes contaminant ions in water when the water is passed through a treatment tank filled with a bead-like resin. For nitrate removal, special anion exchange resins are used that will exchange chloride ions for the nitrate and sulfate ions in the water as it passes through the resin. Most anion exchange resins have a higher selectivity for removing sulfate than nitrate. Thus, the level of sulfate in the water is an important factor in the efficiency of an ion exchange system. Another concern with nitrate ion exchange systems occurs when the resin becomes saturated with nitrate in which case the treated water may have a higher nitrate content than the untreated water. Thus, an ion exchange resin should be regenerated frequently. Ion exchange is not commonly used for household water treatment. It is more applicable for large commercial or community water system installations.

Conclusion

Although nitrate content in water may not cross the upper safe limit in many areas, people should be aware of the quality of drinking water available for them, especially in agricultural and industrial areas. Special care should be taken to monitor the water and food given to babies. In India, methemoglobinemia is not very common, which may be due to lack of awareness or lack of epidemiological studies on the disease. It seems rather surprising that in a country where agriculture is the livelihood of a major portion of population, and where general sanitation is poor in many areas, reported cases of methemoglobinemia are rare. Groundwater nitrate content in many areas of India has been found to be at dangerous levels, but in these areas little or no epidemiological studies have been carried out to find out the prevalence of the disease.

Suggested Reading

[1] T M Addiscott, A P Whitmore and D S Powlson, *Farming, Fertilizers and the Nitrate Problem*, CAB International Wallingford, Oxon.,1991.

[2] O C Bockman and T Granli, Human health aspects of nitrate intake from food and water In Chemistry, *Agriculture and the Environment* (ed M L Richardson), The Royal Society of Chemistry, Cambridge, p. 373, 1991.

[3] D D Weisenberger, Potential health consequences of groundwater contamination by nitrates in Nebraska. In *Nitrate Contamination : Exposure, consequence and control*, eds I Bogardi and RD Kuzella, NATO ASI Ser. G: Ecological Sciences 30, Springer Verlag, Berlin, pp. 309-315, 1991.

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