# Survey of Literature Relating to Infant Methemoglobinemia Due to Nitrate-Contaminated Water

GRAHAM WALTON, PH.D.

*Sanitary Engineer, Environmental Health Center, U. S. Public Health Service, Cincinnati, Ohio*

METHEMOGLOBINEMIA is due to the presence of methemoglobin in the blood. It may result from the reaction of any one of several oxidizing agents with the hemoglobin normally present in the blood. Unlike the oxygen in hemoglobin, that in methemoglobin is so firmly bound that the methemoglobin cannot function as an oxygen carrier by alternate oxygenation and deoxygenation. Thus, the result may be anoxemia with serious consequences or even death.

Although methemoglobinemia may result from congenital heart diseases, or from the ingestion, inhalation, or absorption, or the medicinal administration, of any one of several drugs or chemicals, an important cause of cases in infants is the ingestion of water high in nitrate. This review of the literature is concerned with the latter cause.

## HISTORICAL

According to Eusterman and Keith,[21] Gamgee [28] demonstrated as early as 1868 that the addition of amyl nitrate to blood could result in the formation of methemoglobin. These same authors noted that Binz and Geringer [4] reported in 1901 that the administration of large doses of nitrates to animals may cause the formation of methemoglobin. Medovy, Guest, and Victor [51] credit Stokvis [68] and Talma [69] with describing cases of methemoglobinemia in 1902.

In reviewing the literature, Comly [13] mentioned that van den Bergh and Gutterink [71] reported in 1906 that nitrites could be formed in the bowels from nitrates, and that the nitrite was more likely to be absorbed if the intestine was damaged. These authors noted a few instances in which a nitrite-producing bacillus was recovered from a stool or sputum of individuals having sulfhemoglobinemia. However, it remained for Zobell [81] to demonstrate *in vitro* that many organisms commonly found in the gastrointestinal tract of man are capable of reducing nitrates to nitrites.

In 1928 Barker and O'Hare [3] described a case of cyanosis in a patient who had been treated with ammonium nitrate. The following year, Eusterman and Keith [21] reported two cases of methemoglobinemia which occurred among a large number of adults administered ammonium nitrate for diuretic purposes. However, after two dogs, to which they administered intravenous injections of the drug, failed to show symptoms of methemoglobinemia, they concluded these cases were due to abnormal metabolic processes in the two patients. In 1933, Roe [60] reported the death of a month-old infant who had been treated with bismuth subnitrate. He was the first to associate nitrates with serious or even fatal cases of infant methemoglobinemia. One year later

Chen, Rose, and Clowes [12] demonstrated that methemoglobinemia could be produced by intravenous injections of sodium nitrate.

Thus, the way was paved for Comly,[13] who in reporting on two cases of infant methemoglobinemia in 1945 concluded that methemoglobinemia may occur in an infant following ingestion of water high in nitrates, especially if the infant is suffering from gastrointestinal disturbances. Maxcy,[47] writing in 1950, noted that subsequent observations have established the validity of Comly's hypothesis.

## MEDICAL ASPECTS

*Cause*—Although methemoglobinemia in infants may be caused by congenital heart diseases,[13, 20, 21] by absorption of dyes used for laundry marking,[31, 61, 78] and by drugs administered in medical treatment,[60, 76] many of the cases reported since 1945, when Comly [13] published his conclusions that infant methemoglobinemia may be due to ingestion of high nitrate water, have been diagnosed as having been due to nitrates in the water used in the feeding formula.[8, 11, 23, 24, 48, 50, 59, 66, 84, 85] Donahue [18] alone has expressed the belief that infant methemoglobinemia may result from nitrates ingested in the mother's or cow's milk. However, no positive test for nitrates could be demonstrated in the milk from two cows fed for a period of 10 days on water containing 114 p.p.m. nitrate nitrogen.[83]

*Susceptibility*—Comly's hypothesis on infant methemoglobinemia induced by ingestion of high nitrate water caused considerable speculation concerning the effect of age and other factors on susceptibility. This led to a series of experiments conducted by Cornblath and Hartmann.[15] They fed several infants on a formula containing an artificial. well water of high nitrate-nitrogen concentration, but failed to produce apparent methemoglobinemia. Further investigation revealed that nitrate-reducing organisms, which they cultured from saliva and gastric juices of infant cases, were active only at a pH level above 4.9. On checking the gastric juices of infants, who previously had developed appreciable levels of methemoglobin, they found no free acid and pH values above 4. Based on this evidence Cornblath and Hartmann concluded that the lack of acidity of the gastric juices of a newly born infant may permit nitrate-reducing organisms to grow in the upper gastrointestinal tract and, thus, to reduce nitrates to nitrites before the former can be completely absorbed. In an older infant the acidity of the gastric juices inhibits the growth of the nitrate-reducing bacteria.

The work of Marriott, Hartmann, and Senn,[45] who investigated the acidity and bacterial flora of 200 infants suffering from diarrhea, supports this hypothesis. The pH of the stomach contents of well infants varied from 2.0 to 5.0, and averaged 3.7; that for infants suffering from bacillary dysentery ranged from 2.0 to 5.0, and averaged 3.0; but that for infants with nonspecified diarrhea averaged 5.6, and varied from 4.6 to 6.5.

Robertson and Riddell [59] considered the total amount of hemoglobin present in the blood and the energy of the enzyme system which operates to reduce methemoglobin to hemoglobin as important. Several observers [13, 61, 71] have suggested that the nitrite formed in the upper gastrointestinal tract is more readily absorbed into the blood system when the mucosa is damaged.

In an analysis of 94 cases, Bosch, *et al.*,[8] noted that the period of exposure to a feeding formula containing high nitrate water varied from 1 to 30 or more days.

Apparently, some of the factors which determine whether or not an infant may have nitrate-induced methemoglobinemia are: (a) nitrate-nitrogen content and

amount of water ingested daily; (b) duration of exposure to a feeding formula containing high nitrate water; (c) presence and activity of nitrate-reducing bacteria in the upper gastrointestinal tract—which is affected by the pH of the gastric juices; and (d) the condition of the mucosa.

*Physiological Effects*—The characteristic symptoms ordinarily associated with infant methemoglobinemia are the greyish- or brownish-blue coloration, which usually is first noticeable around the lips, spreads to the fingers and toes and over the face, and may eventually cover the entire body.[8] Other symptoms which may be noticed are drowsiness and an increased rate of respiration.[48] Although not always present, methemoglobinemia is commonly accompanied by diarrhea or other gastrointestinal disturbances.[10]

The cyanosis—the greyish- or brownish-blue coloration—is attributed to the anoxemia resulting from the decrease in the oxygen-transporting capacity of the blood.[26] The nitrites, absorbed by the blood, react with the hemoglobin to form methemoglobin. Conant[14] notes that this is an oxidation reaction in which the ferrous iron in hemoglobin is oxidized to ferric iron in methemoglobin. In the latter state the oxygen is so firmly bound that methemoglobin cannot function in the manner of hemoglobin in the transfer of oxygen. Ordinarily this reaction is reversible and occurs without permanent injury to the red blood cells.

According to Robertson and Riddell,[59] methemoglobinemia is usually first noticeable in infants when 10 per cent of the hemoglobin has been transformed to methemoglobin, and severe cases may result when it increases to 20 per cent. However, recovery has occurred in cases where the methemoglobin level has been observed to be as high as 57 per cent.[64]

*Diagnosis*—Diagnosis of cases of infant methemoglobinemia as being due to the ingestion of nitrate-contaminated water may be either presumptive or absolute. According to Bosch, *et al.*,[8] a presumptive diagnosis is justified when the physical findings are typical and either the freshly removed blood has the chocolate brown color associated with methemoglobin, or spontaneous disappearance of the cyanotic condition occurs within 48 hours after changing the water used in the feeding formula, provided an analysis of the water originally used in the feeding formula shows it to have a nitrate-nitrogen content in excess of 10 or 20 p.p.m.

An absolute diagnosis requires the demonstration of appreciable amounts of methemoglobin in the blood by either a spectroscopic examination or the photoelectric colorimetric method of Evelyn and Malloy,[22] together with analytical results showing at least 10 or 20 p.p.m. of nitrate nitrogen in the water used in the feeding formula. Finch[26] cautions that the blood should be examined within 1 or 2 hours after withdrawal since on standing methemoglobin may be spontaneously reduced to hemoglobin.

*Treatment*—In general, treatment of infants having nitrate water-induced methemoglobin has consisted of the discontinuance of the use of nitrate-contaminated water and, in the more severe cases, the intravenous injection or oral administration of methylene blue. Oxygen therapy, also the administration of such drugs as ascorbic acid, thiamine, and niacin, has been suggested, but for one reason or another none of these treatments has found general use.

In cases where a water of permissible nitrate content could not be obtained, Comly[13] recommended feeding whole milk, preferably acidified and boiled. Stafford[66] suggested that the new-born infant be retained in a hospital until the safety of the water to be used in the feeding formula has been determined.

Various observers[29, 51, 66] have reported the recovery of infant methemo-

globinemia cases following the change from use of nitrate-contaminated water to one relatively free from nitrates. In several instances, an infant has again become cyanotic when returned to his original feeding formula containing high nitrate water.[13, 61, 66] Bosch, *et al.*,[8] implied that spontaneous recovery should occur in 48 hours after discontinuance of use of a feeding formula containing high nitrate water, while Halpern and Dubost [33] stated that such recovery will occur in 4 days.

In 1939, prior to Comly's hypothesis of high nitrate water-induced methemoglobinemia, Wendel [79] reported on the effectiveness of methylene blue in treating sodium-nitrite-induced methemoglobinemia in dogs. He found that an intravenous injection of 0.1 to 0.2 ml. of a 1 per cent solution (1 to 2 mg.) of methylene blue per kilogram of body weight resulted in a rapid decrease in the methemoglobin level. In studying the molecular ratio of the hemoglobin produced per unit of methylene blue added, he could not explain the reaction on the basis of the reducing action of methylene blue. Thus, he concluded that it was a catalyst which accelerated the rate with which the enzyme systems of the red cells normally reduced methemoglobin to hemoglobin.

Warburg, *et al.*,[74] according to Stafford,[66] found that an overdose of methylene blue may produce methemoglobinemia. However, Finch [26] does not consider this important, since dosages up to 10 mg. per kilogram of body weight do not produce significant amounts of methemoglobin. It is at least significant that intravenous injections of 1 to 2 mg. per kilogram of body weight have resulted in the disappearance of cyanotic conditions in numerous cases in a matter of 30 minutes or less.[11, 13, 23, 24]

In his studies, Wendel [79] also noted that oral administration was effective. Stafford [66] stated the dosage used in oral administration should be 10 to 20 mg. of methylene blue per kilogram of body weight. This is about 10 times the amount used in intravenous injections. He reported favorable results following treatment by oral administration.

*Prevention*—In general, the prevention of infant methemoglobinemia due to nitrate-contaminated water is largely one of education. If the infant's parents and the doctor in the case are informed of the problem, steps are usually taken to prevent nitrate-induced methemoglobinemia.

Water for use in preparing the infant's formula should contain not more than 10 or possibly 20 p.p.m. nitrate nitrogen.[13, 47] In areas where there is the least question concerning the nitrate content of the water to be used, a sample of that water should be taken and analyzed. If it is high in nitrates, the infant should be retained at the hospital,[66] the feeding should be whole milk without addition of any water,[13] or water having a safe nitrate concentration should be obtained and used in preparing the formula.

An intensive program has resulted in a marked decrease in the reported cases. For example, in Minnesota the reported cases were 55 in 1947, 52 in 1948, but only 12 for the first 6 months of 1949.[8]

In some cases it may be possible to secure a low nitrate water by constructing new wells, properly located and protected from surface contamination. However, this problem appears to require further investigation.

Finally, reporting of methemoglobinemia in infants should be required. Information on the extent of the cases and the regions in which they occur would prove helpful in its prevention.

### MORBIDITY AND MORTALITY

Metzler [52] noted that methemoglobinemia is not one of the reportable diseases; and that the cases voluntarily reported are a poor indication of the true incidence. The most complete informa-

tion on the morbidity and mortality among infants due to methemoglobinemia resulting from ingestion of nitrate-contaminated water is the summary of reported cases and deaths included in the report of the Committee on Water Supply, Engineering Section, American Public Health Association.[89] Table 1 reproduces in part these data. It is based on a questionnaire sent to the 48 states and Alaska and Hawaii, to which all but one state replied. Special emphasis was placed on restricting the data to those cases definitely associated with nitrate-contaminated water. Apparently, the reported cases cover a period from 1945, or even earlier, to the dates of the replies to the questionnaires.

A total of over 278 cases and of 39 deaths are noted. Geographically the states reporting cases center around Iowa, where the first case associated with nitrate-contaminated water was recognized. Except for the north central group, only 4 states—California, Georgia, New York, and Virginia—reported cases. Infant methemoglobinemia due to high nitrate waters has also been reported for Canada [50, 59] and Belgium.[24]

Probably the most intensive study of nitrate-induced infant methemoglobinemia is that for Minnesota, which was reported by Bosch, *et al.*[8] It is interesting to note that familiarity with the problem by both physicians and parents apparently has led to a marked decrease in the reported cases.

## WATER SUPPLY ASPECTS

*Permissible Nitrate-Nitrogen Concentration*—The permissible nitrate-nitrogen concentration in water which may cause infant methemoglobinemia when used in a feeding formula is dependent on the individual's susceptibility, the increase in nitrate-nitrogen concentration due to boiling the water, the quantity of boiled water consumed per day per unit weight of the infant, the duration of exposure to the high nitrate water, and possibly other factors.[8, 15, 47]

Comly [13] considered it inadvisable to use well water containing more than 10 or 20 p.p.m. nitrate nitrogen in pre-

TABLE 1

*Reported Cases of Nitrate Water-Induced Infant Methemoglobinemia Classified According to Nitrate-Nitrogen Concentration of Water Used in Feeding Formula*

| State | *Methemoglobinemia* Reported Cases | Reported Deaths | *Number of Cases Associated with* Indicated Ranges of $NO_3$–N Conc. (P.P.M.) 0–10 | 11–20 | 21–30 | 31–50 | 51–100 | 100+ | *No. of Cases for Which* Data Are Available | *Supplementary* Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| California | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | |
| Georgia | 6 | 3 | — | — | — | — | — | — | 0 | Frith [27] |
| Illinois | 75 | 6 | 0 | 1 | 2 | 2 | 12 | 11 | 28 | |
| Indiana | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | |
| Iowa | Several | 11 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | |
| Kansas | 13 | 3 | 0 | 0 | 1 | 1 | 2 | 8 | 12 | Metzler [52] |
| Michigan | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 7 | |
| Minnesota | 139 | 14 | 0 | 2 | 25 | | 53 | 49 | 129 | Bosch et al.,[8] |
| Missouri | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | |
| Nebraska | 22 | 1 | 0 | 1 | 0 | 4 | 9 | 8 | 22 | |
| New York | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | |
| North Dakota | 9 | 1 | 0 | 1 | 1 | 0 | 0 | 6 | 8 | |
| Ohio | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Oklahoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| South Dakota | Several | 0 | — | — | — | — | — | — | — | |
| Texas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Virginia | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | |
| | 278+ | 39 | 0 | 5 | 36 | | 81 | 92 | 214 | |
| Per cent of Total | | | 0.0 | 2.3 | 16.8 | | 37.8 | 43.1 | 100.0 | |

Principal Source: Progress Report of Committee on Water Supply, A.P.H.A., Water Supply: Nitrate in Potable Waters and Methemoglobinemia. *A.P.H.A. Yearbook* 40, 5:110 (May), 1949–1950.

TABLE 2

*High Nitrate Waters, Their Geographical Distribution and Total Reported Cases of Infant Methemoglobinemia*

| State | Total No. Methemo. Cases Reported [1] | Year | Type of Supply | No. of Samples Examined | Per cent of Samples Examined within Indicated $NO_3$–N (P.P.M.) Range 0–10 | 11–20 | 21–50 | 50+ | Reference |
|---|---|---|---|---|---|---|---|---|---|
| California | 1 | — | — | — | — | — | — | — | |
| Georgia | 6 | — | — | — | — | — | — | — | |
| Illinois | 75 | 1946 | | 7,000 | 80± | ← | 20± | → | (76) |
| Indiana | 1 | — | — | 146 | ← | 98.7 | → | ← 1.3 → | (52) |
| Iowa | 69 | 1939 | Dug Wells | 454 | 66.5 | 10.6 | 14.3 | 8.6 | (38) |
| | | | Drilled Wells | 647 | 95.5 | 2.5 | 1.5 | 0.4 | |
| | | | Bored Wells | 278 | 79.5 | 7.9 | 7.2 | 5.4 | |
| | | 1943 | Dug Wells | 243 | 48.5 | 11.1 | 12.4 | 28.0 | |
| | | | Private Wells | | | | | | |
| | | | < 100′ deep | 1,943 | ← | 72± | → ← 28± | → | (7) |
| | | | > 100′ deep | 370 | ← | 96.5 | → ← 3.5 | → | |
| Kansas | 13 | — | Rural Wells | — | 67± | ← | 33± | → | (52) |
| Massachusetts | 0 | 1948 | Private | — | — | ← | 47 samples | → | (89) |
| Michigan | 7 | — | — | 2,847 | 93.0 | ← | 7.0 | → | (48) |
| Minnesota | 139 | 1947–9 | Municipal | 514 | 96.9 | ← | 3.1 | → | (8) |
| | | | | 2,912 | 84.9 | ← | 15.1 | → | |
| Missouri | 2 | — | — | — | — | — | — | — | |
| Nebraska | 22 | 1948–9 | Municipal | 416 | 95.0 | ← | 5.0 | → | (83) |
| | | | Non-municipal | 2,687 | 79.8 | ← | 20.2 | → | |
| | | | School | 258 | 95.0 | ← | 5.0 | → | |
| | | | Farm | 275 | 78.0 | ← | 22.0 | → | |
| New York | 2 | 1943 | — | 2,864 | 96.1 | 3.3 | 0.6 | 0.0 | (89) |
| | | 1944 | — | 3,528 | 96.9 | 2.5 | 0.6 | 0.0 | |
| | | 1945 | — | 5,117 | 97.4 | 1.8 | 0.7 | 0.1 | |
| | | 1946 | — | 6,524 | 96.4 | 2.1 | 1.3 | 0.2 | |
| North Dakota | 9 | — | — | 509 | 84.9 | ← | 15.1 | → | (1) |
| | | — | — | 287 | 84.0 | ← | 16.0 | → | (72) |
| Ohio | 0 | | Rural Wells | 10,500 | 90.5 | 5.0 | 3.5 | 1.0 | (75) |
| | | | State Park Wells | 400 | 100.0 | 0.0 | 0.0 | 0.0 | |
| Oklahoma | 0 | — | — | 415 | Av. $NO_3$–N Conc. = 28 p.p.m. | | | | (89) |
| South Dakota | Several | — | — | — | — | — | — | — | |
| Texas | 0 | — | Rural Wells | | | | Numerous | | (89) |
| Virginia | 1 | — | Rural Wells | — | — | — | — | — | |

1. Includes all reported cases irrespective of the date of their occurrence or the type of water with which they were associated.

paring an infant's feeding formula. Data presented in Table 2 indicate that no cases were reported which were associated with water containing 10 p.p.m. nitrate nitrogen or less, and the nitrate-nitrogen content was less than 20 p.p.m. for only 2.3 per cent of the cases for which data were available. It should be noted, however, that in many of these cases clinical data were insufficient for definite diagnosis, and samples of water for the analysis were sometimes collected several months following the occurrence of the case.

Although 10 p.p.m. nitrate nitrogen has been suggested as the permissible level,[13, 47] the A.P.H.A. Committee [89] points out that most of the cases studied were associated with nitrate-nitrogen concentration in excess of 40 p.p.m. and comments that it is "impossible at this time to select any precise concentration of nitrates in potable waters fed infants which definitely will distinguish between waters which are safe or unsafe. . . ."

*Geographical Distribution*—The geographical distribution of high nitrate waters is widespread. Twenty of 37 states, which replied to this item on the A.P.H.A. Committee's questionnaire, reported wells yielding water containing in excess of 50 p.p.m. nitrate nitrogen.[89] Results of fairly extensive surveys, indicating the occurrence and concentrations of high nitrate waters, are available in the literature for Illinois,[76] Iowa,[38] Michigan,[48] Minnesota,[8] Nebraska,[83] New York,[89] and Ohio.[75] The available data are summarized in Table 2.

Although the information presented in the literature is insufficient to permit a sound analysis of the problem, it does

indicate that waters of high nitrate-nitrogen concentration occur in a sufficient number of instances to anticipate cases of nitrate water-induced methemoglobinemia in Illinois, Iowa, Michigan, Minnesota, Nebraska, Ohio, Oklahoma, and possibly New York and Texas. The absence of any reported cases of infant methemoglobinemia in Ohio, Oklahoma, and possibly Texas, does not appear to be due to the lack of occurrence of high nitrate waters.

*Types of Supply*—High nitrate waters have been found most frequently in private wells serving rural homes. However, water containing in excess of 10 p.p.m. nitrate nitrogen has been reported for wells supplying municipal water works in Illinois,[49, 76] Kansas,[52] Minnesota,[8] Nebraska,[83] and New York.[89] Available evidence indicates that surface supplies do not offer a problem except in very unusual cases.[49, 75, 89]

*Construction of Wells*—Most cases of infant methemoglobinemia have been associated with water from dug wells. Bosch, *et al.*,[8] noted that in 125 of 129 cases investigated the water came from dug wells. Data reported for Iowa [38] show nitrate-nitrogen concentrations over 10 p.p.m. in water samples from 39.7 per cent of dug wells, 21.5 per cent of the bored wells, and only 4.5 per cent of the drilled wells.

The location and physical features, also the nature of the water-bearing strata, may be important. Nearby sources of pollution were believed to be significant by 12 of 22 states reporting on this item in the A.P.H.A. Committee's questionnaire.[89] Bosch, *et al.*,[8] noted that 83 of 129 wells involved in cases of methemoglobinemia were within less than 50 feet from a barnyard, pigpen, privy, cesspool or other source of animal or human contamination. It may be significant to note the marked difference, shown in Table 2, in the percentage of high nitrate waters from school and farm wells in Nebraska, also that between state park wells and rural wells in Ohio.

In many cases farm wells, and particularly dug wells, are inadequately protected against contamination by surface water or water percolating but a short distance through the soil. Thus, as might be expected, several states have reported a high percentage of the high nitrate waters to be positive for coliform organisms.[38, 59, 75, 76] However, there appears to be no relationship between nitrate concentration and bacterial numbers.

*Sources of Nitrate Contamination*—According to Sarles, *et al.*,[63] the principal sources of nitrogenous matter in the soil are the decomposition products of plants, animals and microörganisms; the liquid and solid wastes of animal metabolism; and fertilizers added to enrich the soil. The possibility of geological formations containing appreciable amounts of nitrates must also be considered.

It may be significant that 12 of 22 states replying to this item on the A.P.H.A. Committee's questionnaire expressed the opinion that nearby sources —barnyards, pigpens, privies, cesspools, etc.—were important in causing high nitrate waters.[89] Such a belief is substantiated by the variation in nitrate-nitrogen content of waters from wells, such as noted by Borts,[7] Maxcy,[47] and in the New York studies.[89] However, Metzler [52] observed only seasonal change in the nitrate content of 4 municipal wells, and Bosch, *et al.*,[8] noted no appreciable variation in the nitrate content of samples taken bi-weekly from each of 5 wells, or in samples from 2 wells taken after more than an hour of continuous pumping. Two wells, a 30-foot dug well and a 71-foot drilled well, were reconstructed in an attempt to exclude nitrate contamination, but no significant reductions in the nitrate content of their waters were noted.

The A.P.H.A. Committee's report [89] notes that high concentration of nitrates

in waters in rural wells, which usually were poorly constructed, shallow, dug wells, did not seem to be associated with nearby sources of pollution. Weart [76] notes that the upper 40 inches of soil may contain 16,000 lbs. of organic nitrogen per acre. Sarles, Frazier, and McCarter [63] discuss the decomposition of nitrogenous matter. Proteins and nitrogenous matter are first hydrolyzed into amino acids or other water-soluble products consumed by bacteria which give off ammonia. Nitrification may occur through the action of *Nitrosomonas* or *Nitrosococcus* bacteria, which in the presence of air oxidize ammonia to nitrous acid; and by *Nitrobacter,* which oxidizes the nitrous acid to nitric acid.

Since bacteria are essential in the production of nitrates from organic nitrogen, factors influencing their activity affect the nitrate content of the soil. Sarles, *et al.,*[63] note that nitrification takes place only when the soil contains buffering substances which neutralize the nitric acid and maintain a pH around 6.5 to 8.0; when aerobic conditions, such as result from plowing, cultivating, etc., are maintained; when soil contains only about 50 per cent of its water-holding capacity; when ammonia salts and other nutrient elements are present in the soil; and at temperatures above 50° and below 100° F.

### ANALYTICAL PROCEDURES

There are several procedures by which the nitrate content of water may be determined. Most laboratories use a screening test to differentiate between low and high nitrate concentrations, and then determine the nitrate nitrogen in high nitrate waters by a more accurate procedure.

One nitrate screening test is that developed by Riehl,[75] which he adopted from "Qualitative Analysis by Spot Tests" by Fritz Feigl. Another screening procedure is that developed by Bray [9] and used by Illinois. Both of these procedures are spot plate tests which may be used in the field to prevent overburdening the laboratory with large numbers of samples of low-nitrate waters.

Metzler [52] reports that the brucine procedure is used in Kansas. Local health departments have been equipped with field test kits with which on-the-spot tests are made to determine whether the nitrate-nitrogen concentration is within the 10 p.p.m. limit usually considered the maximum permissible level.

More precise methods for use in the laboratory are the phenol-disulfonic acid and reduction methods as given in *Standard Methods.*[88] Still another method which can be used is the brucine method.[36, 55, 80] For precise work it would seem desirable to use a photoelectric colorimeter for comparing the color developed in samples with that of standard concentrations.

### FURTHER INVESTIGATIONS NEEDED

Maxcy,[47] in a report adopted by the Committee on Sanitary Engineering and Environment, Division of Medical Sciences, National Research Council, notes that there is urgent need for further study before comprehensive measures of prevention can be advocated. Such a study should consist of observations in various parts of the United States by the same unit.

Some of the factors which should be investigated are the permissible level of nitrates in waters fed to infants; the factors which predispose some infants to methemoglobinemia, whereas other infants consuming equally high nitrate waters are unaffected; the health of infants routinely consuming water containing high nitrate in regions where methemoglobinemia has not been reported; the fluctuations, if any, in the nitrate content of waters from selected wells, including factors which influence such variations.[47, 89]

## REFERENCES

The following references are listed in alphabetical order by author. Also included is a reference list arranged according to subject matter, with the individual references indicated by their number.


1. Abbott, G. A., and Voedisch, F. W. *North Dakota Geological Survey Bull. II.*
2. Barcroft, Joseph, and Muller, Franz. The Formation and Estimation of Methemoglobinemia. *J. Physiol.* 43:xx, 1911–1912.
3. Barker, M. H., and O'Hare, J. P. The Use of Salyrgan in Edema. *J.A.M.A.* 91:2060, 1928.
4. Binz, C., and Geringer, P. Die Reduktion des Natriumnitrats in Tierkorper. *Arch. internat. de pharmacodyn. et de therap.* 9:441–450, 1901.
5. Bodansky, O., and Gutmann, H. Treatment of Methemoglobinemia. *J. Pharmacol. & Exper. Therap.* 89:45–56, 1947.
6. Bohme, A. Ueber Nitrirergiftung Nach Interner Darreichung von Bismuthum Subnitriicum. *Arch. f. exper. Path. u. Pharmakol.* 57:441–453, 1907.
7. Borts, I. H. Water-borne Diseases. *A.J.P.H.* 39:974–978, (Aug.), 1949.
8. Bosch, H. M., Rosenfield, A. B., Huston, R., Shipman, H. R., and Woodward, F. L. Methemoglobinemia and Minnesota Well Supplies. *J.A.W.W.A.* 42:161 (Feb.), 1950.
9. Bray, R. H. Nitrate Tests for Soils and Plant Tissue. *Soil Sci.* 60:219, 1945.
10. Canrick, M., Polis, B. D., and Klein, T. Methemoglobinemia—Treatment with Ascorbic Acid. *Arch. Int. Med.* 78:296, 1946.
11. Chapin, Frederick J. Methemoglobinemia from Nitrates in Well Water. *J. Michigan M. Soc.* 46:938 (Aug.), 1947.
12. Chen, K. K., Rose, C. L., and Clowes, G. H. H. Comparative Values of Several Antidotes in Cyanide Poisoning. *Am. J.M. Sc.* 188:767–781 (Dec.), 1934.
13. Comly, H. H. Cyanosis in Infants Caused by Nitrates in Well Water. *J.A.M.A.* 129:112–116 (Sept. 8), 1945.
14. Conant, J. B. Oxidation of Hemoglobin and Other Respiratory Pigments. *Harvey Lect.* 159–183, 1932–1933.
15. Cornblath, M., and Hartmann, A. F. Methemoglobinemia in Young Infants. *J. Pediat.* 33:421–425 (Oct.), 1948.
16. Darling, Robert C., and Roughton, F. J. W. The Effects of Methemoglobinemia on the Equilibrium between Oxygen and Hemoglobin. *Am. J. Physiol.* 137:56, 1942.
17. Debus, Jeanette M. Nitrates and Nitrites in Nebraska Water Supplies. *Univ. Nebraska Publication,* Second Annual Waterworks Conference.
18. Donahue, W. E. Cyanosis in Infants with Nitrates in Drinking Water as a Cause. *Pedriatics* 3:308–311 (Mar.), 1949.
19. Dugan, F. C. The Effect of Nitrates in Rural Water Supplies on Infant Health. *Bull., Dept. of Health, Kentucky* 21, 3 (Oct.), 1948.
20. Elder, H. A., Finch, C., and McKee, R. W. Congenital Methemoglobinemia: A Clinical and Biochemical Study of a Case. *J. Clin. Investigation* 28:265–272 (Mar.), 1949.
21. Eusterman, G. B., and Keith, N. M. Transient Methemoglobinemia Following Administration of Ammonium Nitrate. *M. Clin. North America* 12:1489 (May), 1929.
22. Evelyn, K. A., and Malloy, H. T. Microdetermination of Oxyhemoglobin, Methemoglobin, and Sulfhemoglobin in a Single Sample of Blood. *J. Biol. Chem.* 126:655–662, 1938.
23. Faucett, R. L., and Miller, H. C. Methemoglobinemia Occurring In Infants Fed Milk Diluted with Well Water of High Nitrate Content. *J. Pediat.* 29:593–596 (Nov.), 1946.
24. Ferrant, M. Methemoglobinemia: Two Cases in Newborn Infants Caused by Nitrates in Well Water. *J. Pediat.* 29:585–592 (Nov.), 1946.
25. Finch, C. A. Treatment of Intracellular Methemoglobinemia. *Bull. New England M. Center* 9:241–245, 1947.
26. Finch, C. A. Medical Progress: Methemoglobinemia and Sulfhemoglobinemia. *New England J. Med.* 239:470–478 (Sept. 23), 1948.
27. Frith, Gilbert R. Methemoglobinemia Caused by Nitrate Pollution in Drinking Water. *J. Med. Assoc. Georgia* p. 258 (June), 1950.
28. Gamgee, Arthur. Researches on the Blood; on the Action of Nitrites on the Blood. *Phil. Tr. Roy. Soc. London* 158:589–625, 1868.
29. Gibson, Q. H. Reduction of Methemoglobin by Ascorbic Acid. *Biochem. J.* 37:615–618, 1943.
30. Goluboff, N. Methemoglobinemia in Infants. *Canad. M.A.J.* 58:601–603 (June), 1948.
31. Graubarth, J., Bloom, C. J., Coleman, F. C., and Solomon, H. N. Dye Poisoning in Nursery: Review of Seventeen Cases. *J.A.M.A.* 128:1155, 1945.
32. Greenberg, Leon A., Lester, David, and Haggard, Howard W. The Reaction of Hemoglobin with Nitrite. *J. Biol. Chem.* 151:665 (Dec.), 1943.
33. Halpern et Dubost. *Bull. Soc. chim. Biol.* 21:717, 1939.
34. Harrop, G. A., Jr., and Barron, E. S. G. Studies on Blood Cell Metabolism. 1. Effect of Methylene Blue and Other Dyes upon Oxygen Consumption of Mammalian and Avian Erythrocytes. *J. Exper. Med.* 48:207–223, 1928.
35. Hartmann, A. F., Perley, A. M., and Barrett, H. L. A Study of Some of the Physiological Effects of Sulfanilamide. II. Methemoglobin Formation and its Control. *J. Clin. Investigation* 17:699–710 (Nov.), 1938.
36. Hatfield, W. D. The Determination of Nitrates in Sewage Effluents by the Brucine Method. Unpublished paper presented before the Division of Water and Sanitation at the Meeting of the American Chemical Society, Chicago, Ill.
37. Jensen, C. W., and Anderson, H. D. Rate of Formation and Disappearance of Methemoglobin Following Oral Administration of Sodium Nitrite. *Proc. South Dakota Acad. of Sciences* 21:37, 1941.
38. Johnson, G., Kurz, A., Cerney, J., Anderson, A., and Matlack, G. Nitrate Levels in Water from Rural Iowa Wells: A Preliminary Report. *J. Iowa M. Soc.* 36:4–7 (Jan.), 1946.
39. Kolthoff, I. M., and Naponen, G. E. Diphenylamine Sulfonic Acid Method for Determining Nitrates. *J. Am. Chem. Soc.* 55:448, 1933.
40. Lange, Bruno. Lange Colormetrische Analysis. Berlin, 1941.
41. Lederer, J. A. *Arch. internat. de pharmacodyn. et de therap.* 55:357, 1937.
42. Lester, D., and Greenberg, L. A. Metabolic Fate of Acetanilid and Other Aniline Derivatives; Major Metabolites of Acetanilid (N-acetyl p-aminophenol and its hydroxy conjugates) Appearing in Blood. *J. Pharmacol. & Exper. Therap.* 90:68–75 (May), 1947.
43. Lichtenbelt, J. W. T. Intraglobular Methemoglobinemia. *Geneesk. gids.* 1:545, 1923.
44. Lundegardh, Henrik. *Environment and Plant Development.* A translation of *Klema und Boden in Ihrer Werkung auf das Pflanzenleben.* London: Edward Arnold, 1931.
45. Marriott, W. M., Hartmann, A. F., and Senn, M. J. E. Observations on the Nature and Treatment of Diarrhea and the Associated Systemic Disturbances. *J. Pediat.* 3:181, 1933.
46. Marshall, C. R., and Marshall, W. Action of Nitrites on Blood. *J. Biol. Chem.* 158:187–208 (Mar.), 1945.
47. Maxcy, Kenneth F. Report on the Relation of Nitrate Nitrogen Concentration in Well Waters to the Occurrence of Methemoglobinemia In Infants.

*Bull. San. Eng. and Envir. 264 (or Appendix D)*, National Research Council, 1950.
48. McGuire, O. E. Blue Babies and Nitrate Content of Water. *Michigan Pub. Health* 38, 2:25–26, 1950.
49. Medovy, H. Blue Babies and Well Water. *Mod. Concepts Cardiovas. Dis.* 17:23–24 (Oct.), 1948.
50. Medovy, H. Well Water Methemoglobinemia in Infants. Its Occurrence in Rural Manitoba and Ontario. *Lancet* 68:194–196 (May), 1948.
51. Medovy, H., Guest, W. C., and Victor, M. Cyanosis in Infants in Rural Areas. *Canad. M. A. J.* 56: 505–508 (May), 1947.
52. Metzler, Dwight F. High Nitrate Waters: Their Occurrence and Role in Methemoglobinemia. *Harvard Pub. Health Alumni Bull.* 7:8 (Nov.), 1950.
53. Miller, G. E. Bismuth Subnitrate Poisoning with Methemoglobinemia. *Gastroenterology* 4:430–434 (May), 1945.
54. Morrison, D. B., and Williams, E. F., Jr. Methemoglobin Reduction by Glutathionone of Cysteine. *Science* 87:15, 1938.
55. Noll, C. A. Determination of Nitrate in Boiler Water by Brucine Reagent. *Indust. & Engin. Chem., Anal. Ed.* 17:426, 1945.
56. Paul, W. D., and Kemp, C. R. Methemoglobin: A Normal Constituent of the Blood. *Proc. Soc. Exper. Biol. & Med.* 56:55 (May), 1944.
57. Rieman, William. *Quantitative Analysis.* New York: McGraw-Hill, p. 304, 1942.
58. Riggs, C. W. Nitrite Poisoning from Ingestion of Plants High in Nitrate. *Am. J. Vet. Research* 6:194–197, 1945.
59. Robertson, H. E., and Riddell, W. A. Cyanosis of Infants Produced by High Nitrate Concentration in Rural Waters of Saskatchewan. *Canad. J. Pub. Health.* 40:72 (Feb.), 1949; Abst. *J.A.W.W.A.* 42:38.
60. Roe, H. E. Methemoglobinemia Following the Administration of Bismuth Subnitrate. Report of a Fatal Case. *J.A.M.A.* 101:352–354 (July), 1933.
61. Rottinghuis, H., Fehmers, G. A., and Strengers, T. Methemoglobinemia due to Intoxication of Newborn Infants from Aniline Ink Marking of Diapers; 31 cases. *Nederl. tijdschr. v. geneesk.* 91:2125–2129 (Aug. 2), 1947.
62. Sanger, L. A., and DeFrain, O. D. Contaminated Private Water Supplies. Nebraska Dept. of Health, *Better Health* 8, 3:2 (May–June), 1949.
63. Sarles, W. B., Frazier, W. C., and McCarter, J. R. *Bacteriology* (Rev. Ed.), Madison, Wis.: Kramer Business Service, 1940.
64. Schwartz, A. S., and Rector, E. J. Methemoglobinemia of Unknown Origin in a Two Weeks Old Infant. *Am. J. Dis. Child.* 60:652–659 (Sept.), 1940.
65. Snell, Foster D., and Snell, Cornelia, T. *Colorimetric Methods of Analysis.* New York: Van Nostrand, 1936, Vol. 1, p. 629.
66. Stafford, G. E. Methemoglobinemia in Infants from Water Containing High Concentrations of Nitrates. *Nebraska M. J.* 32:392–394 (Oct.), 1947.
67. Stieglitz, E. J., and Palmer, A. E. A Colorimetric Method for the Determination of Nitrite in Blood. *J. Pharmacol. & Exper. Therap.* 51:398, 1934.
68. Stokvis, B. J. Zur Casuistik der Autotoxischen Enterogenen Cyanosen. *Internat. Beitr. z. inn. Med.* 1:597, 1902.
69. Tolma, S. Interglebulare Methemoglobinemie beim Mehschen. *Berl. klin. Wchnschr.* 37:865, 1902.
70. van den Bergh, A. A. H. Enterogene Cyanose. *Deutsches Arch. f. Klin. Med.* 83:86–106 (May 18), 1905.
71. van den Bergh, A. A. H., and Gutterink, A. Enterogene Cyanose. *Berl. klin. Wchnschr.* 43:7, 1906.
72. Van Heuvelen, W. What About Nitrates in North Dakota Drinking Water? North Dakota Water and Sewage Works Conference, *Official Bull.* 22–23 (Sept.), 1948.
73. Wallace, William M., Methemoglobinemia in an Infant as the Result of the Administration of Bismuth Subnitrate. *J.A.M.A.* 133:1280 (April 26), 1947.
74. Warburg, O., Kubowitz, E., and Christian, W. *Biochem. Ztschr.* 227:245, 1930.
75. Waring, F. H. Significance of Nitrates in Water Supplies. *J.A.W.W.A.* 41:147–50 (Feb.), 1949.
76. Weart, J. G. Effect of Nitrates in Rural Water Supplies on Infant Health. *Illinois M. J.* 93:131–133 (Mar.), 1948.
77. Weart, James G., and Klassen, C. W. High Nitrate Waters, Their Occurrence, Source and Significance. Paper presented before American Chemical Society, Chicago, April 19, 1948.
78. Weinberg, A. A. Aniline Poisoning in Newborn, with Report of 13 Cases. *Am. J. Obst. & Gynec.* 21:104–108, 1931.
79. Wendel, W. B. The Control of Methemoglobinemia with Methylene Blue. *J. Clin. Investigation* 18:179–185 (Mar.), 1939.
80. Wolf, Benjamin. Determinations of Nitrate, Nitrite and Ammonia Nitrogen. *Indust. & Engin. Chem., Anal. Ed.* 16:446, 1944.
81. Zobell, C. E. Factors Influencing the Reduction of Nitrates and Nitrites by Bacteria in Semisolid Media. *J. Bact.* 24:273–281 (Oct.), 1932.
82. Blue Babies from Nitrates in Water Supplies. Connecticut Dept. of Health *Weekly Health Bull.* 30, 22:1 (June), 1948.
83. High Nitrate Wells in Nebraska. *Better Health,* Official Bull. State of Nebraska Dept. of Health. 9, 3:1 (1940).
84. Methemoglobinemia Due to Well Water. *J. Pediat.* 33:506–507 (Oct.), 1948.
85. Nitrate Poisoning in Plattsburg Infants. *New York State Dept. of Health Bull. Weekly,* 2:31 (July 11), 1949.
86. Nitrates in Water Seen Cause of Infant Disease. *Eng. News-Record* 144:35 (April 13), 1950.
87. Report of Committee on Water Supply of the Engineering Section, A.P.H.A. *Yearbook* 39, 5:114 (May) 1949–1950.
88. *Standard Methods for the Examination of Water and Sewage* (9th Ed.). New York: American Public Health Association, 1946.
89. A.P.H.A. Committee. "Water Supply: Nitrate in Potable Waters and Methemoglobinemia." A.P.H.A. *Yearbook* 40, 5:110 (May), 1949–1950.
90. Well Water as a Source of Infant Disease. Illinois Dept. of Public Health, *Illinois Health Messenger* 20:48 (June 15), 1948.

## REFERENCES LISTED ACCORDING TO SUBJECT MATTER

(Numbers refer to numbered references in main reference list)

### HISTORICAL

3, 4, 12, 13, 21, 28, 41, 47, 51, 60, 68, 69, 71, 81.

### MEDICAL

*Cause*
8, 11, 13, 18, 20, 23, 24, 31, 48, 50, 53, 58, 59, 60, 61, 66, 73, 78, 84, 85.

*Susceptibility*
7, 8, 13, 15, 45, 46, 56, 61.

*Diagnosis*
8, 21, 26

*Treatment*
5, 8, 11, 13, 23, 24, 25, 26, 33, 34, 35, 37, 41, 51, 54, 61, 66, 74.

*Prevention*
8, 13, 47, 52, 66.

# International VD Union Meets

The 28th General Assembly of the International Union against the Venereal Diseases was held in Paris, May 21–25, 1951. It commemorated the 50th anniversary of the founding of the French Society for Moral and Sanitary Prophylaxis by Alfred Fournier. Dr. E. H. Hermans, Netherlands, president of the Union, presided, and Dr. Andre Cavaillon, secretary-general of the Union and the French Society acted as hosts. The Assembly was attended by 52 delegates from 28 countries, as well as by observers from the World Health Organization, the League of Red Cross Societies, the International Abolitionist Federation, the International Alliance of Women, and others.

Much of the Assembly's program was devoted to the discussion of sex education. Among the numerous papers presented was a report by the American Social Hygiene Association on "Progress and Trends in Sex Education in the United States." The agenda also included a joint session of Assembly delegates, doctors, educators, psychologists, and social workers on this subject.

Resolutions unanimously adopted by the Assembly propose the establishment of a Union Liasion Office in the future, possibly in Geneva; and the preparation of a new statement of Union objectives, with program and budget. These resolutions enable the Union to meet today's challenges better equipped to fulfill its responsibilities as the international voluntary agency in its field.

The United States delegation, chaired by Bailey B. Burritt of the American Social Hygiene Association's Executive Committee included: Mrs. Burritt, Dr. Robert H. Bishop, Jr. (A.S.H.A. Executive Committee and Board of Directors) and Mrs. Bishop, Dr. William A. Brumfield, Jr. (deputy health commissioner of New York State and chairman of A.S.H.A. Committee on International Activities), Lt. Col. Louis Kossuth (attached to General Eisenhower's staff in Paris), Irving J. Fasteau (Permanent Attache for Social Affairs, American Embassy, Paris), and Dr. Robert D. Wright (professor of social and environmental medicine, University of Virginia).