Does the nitrification of soil contribute to methemoglobinemia, one cause of infant mortality? A survey of soil and water in southern Minnesota explored the possible relationship.

Soil Nitrification and Nitrates in Waters

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VERY young infants, usually less than 2 months old, may acquire methemoglobinemia as a result of ingesting water high in nitrates. The ingested nitrate may oxidize a portion of the hemoglobin to methemoglobin with consequent loss in oxygen transport and oxygen exchange in the blood; if severe, the anoxemia may produce serious effects, and death may occur.

Cases of methemoglobinemia induced by well waters high in nitrates have been reported principally in the midwestern United States and the central Provinces of Canada (*1*, *2*). Although the high nitrate content of most of the wells studied apparently was associated with nearby sources of pollution, the origin of the nitrates that accumulate in ground waters was not always clear (*2*). Several writers have suggested that nitrate formation in normal agricultural soils may contribute significantly to nitrate accumulation in rural well waters (*1*, *3–5*). This suggestion merits further consideration, since the peculiar geographic distribution of the disease in the United States corresponds roughly to the belt of highly productive Chernozem and Prairie soils.

Nitrates are found in soils as a result of the activities of the soil microflora. Nitrogen reaches the soils principally in organic form as a part of plant and animal residues, and in this form it is subject to attack by a large and diversified group of soil micro-organisms. As the result of microbial action the nitrogen added originally in organic complexes is transformed into new organic combination as a constituent of microbial protoplasms, and the excess beyond cell needs is converted to the inorganic ammonium form. A first prerequisite of high nitrate production is the occurrence of nitrogen in the ammonium form.

Ammonium nitrogen is transformed rather rapidly under appropriate soil conditions by the activity of the specific autotrophic nitrifying bacteria of the genera *Nitrosomonas* and *Nitrobacter*. These organisms derive their energy, respectively, from the oxidation of ammonium to nitrite and the oxidation of nitrite to nitrate. Many soil factors influence the activity of the nitrifiers, but it is well established that the most fertile soils generally promote the most active nitrification.

To obtain information regarding the possible relationship between nitrate accumulation in waters and nitrate production in soils associated with those waters, a survey of soil nitrification was undertaken in the methemoglobinemia region of Minnesota in 1951. This survey was made possible by a grant from the Minnesota Department of Health.

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Organization of the Survey

The areas included in the survey were located in Renville, Nobles, Rock, and Mower Counties of southwestern and south central Minnesota. The first three of these are among the counties in the State that reported the largest number of methemoglobinemia cases in the period 1947–49, according to the report of Rosenfield and Huston (*5*). Mower County is about 100 miles east of the principal methemoglobinemia region described in that report.

Starting points for studies in each county were selected on the basis of records on file at the Minnesota Department of Health. Each initial site was a farm that, according to the health department's field studies of infant methemoglobinemia, had a high nitrate water supply. Following the survey of the initial site, additional sites in the same section and in adjoining sections were examined.

The survey of a given site centered about the water supplies of that site. In addition to farm wells, field tile drains that were carrying water, rural school wells, springs, and streams in the vicinity were included in the survey. A qualitative test for nitrate, with diphenylamine solution, was made at the site. Information was sought concerning such features as location, depth, construction, and use of farm wells; topography and surface drainage; the location of waste organic nitrogen accumulations; and land use practices. A sample from each water supply was collected for subsequent laboratory analysis. (Laboratory analysis of water samples was done by the Minnesota Department of Health.)

Soils in the vicinity of the water supplies were observed for topography and land use. Sites for sampling were selected so as to reflect the variations in soils. The usual practice was to obtain samples within a 50-foot radius of the water supply and to supplement these with samples collected from the various soils 100 to 500 feet from the water supply. Composite samples of surface soil, made up of from 4 to 6 subsamples, were taken from the 0- to 6-inch soil layer, and corresponding subsoil samples were taken at a depth of 30 to 36 inches with a soil auger.

The nitrate content of the soil and water samples returned to the laboratory was determined quantitatively by standard methods using phenoldisulfonic acid (*6*, *7*). (Nitrate is measured and reported as parts per million nitrate nitrogen, or NO_3^-N, which denotes the amount of nitrate in terms of its nitrogen.) The nitrifying capacity of each surface soil sample was determined by conventional methods of soil microbiology (*8*). Each soil was rated low, medium, high, or very high in nitrifying capacity on the basis of the quantity of nitrate produced by 100 gm. of soil incubated in the presence and in the absence of added ammonium nitrogen during a 6-week period. Solution culture studies using each surface soil as an inoculum provided additional data in rating the nitrifying capacity of each soil. Soils rated low produced less than 3 p.p.m. nitrate nitrogen during incubation for 6 weeks in the absence of added ammonium nitrogen and less than 35 p.p.m. nitrate nitrogen when 30 mg. ammonium nitrogen had been added. Soils rated very high produced more than 100 p.p.m. nitrate nitrogen without added nitrogen and more than 700 p.p.m. nitrate nitrogen in the presence of added nitrogen.

Results and Discussion

Almost all of the wells examined were of dug or bored construction and were relatively shallow, generally 15 to 50 feet deep. Exceptions to these characteristics are noted in the tables.

The concentrations of nitrate found in the waters sampled and in soils in the vicinity of the water supply are listed in tables 1, 2, and 3. (Data for soil samples taken beyond 50 feet from the water supply are not given, since these samples numbered only 7 for surface soil and 6 for subsoil.) The nitrate content of the soils varied greatly within a short distance, and the same soil varied considerably with the time of sampling. Nitrates in the surface soil maintained no constant relation to the nitrates in the parent material (subsoil samples) of that soil. There was no relation between the nitrate content of the water and that of the soils at the time of sampling. The variations in soil nitrate content are to be expected, since the nitrates that are produced in the surface soil are subject to leaching by rainfall and to utilization by microorganisms and higher plants.

The data on nitrate concentrations must be considered in connection with certain conditions at the time of sampling. The first sampling in Renville County, in early July, followed a period of about 3 weeks of abnormally heavy rainfall. The excess rainfall had caused standing water in some fields, drowned-out areas in corn, alfalfa, and bean fields, and a water table within 30 inches of the surface in many places. At the time of the second sampling, late in August, more normal conditions of rainfall prevailed. Both rainfall and crop development conditions were altered markedly between sampling periods. The nitrate content of the water supplies was lower and that of the surface soils was higher at the time of the second sampling. It is likely that the high rainfall prior to the initial sampling leached large quantities of nitrates from the surface soil at a stage of crop development that made relatively small nutritional demands on the nitrates of the soil. Much of the nitrate washed from the surface soil might well have contributed to that of the ground water supply.

Similar variations with rainfall conditions were observed in Rock and Nobles Counties, although the nitrate concentrations fluctuated less. In these counties, initial samples were taken after several weeks of near normal weather, whereas the second samples were taken soon after a short period of heavy rainfall. The variation noted in the nitrate content of the same well water is in agreement with the observations of Siemens and Mallett (*9*).

The data concerning the relationship between the nitrifying capacity of the soils and the nitrate concentration of the water supplies near those soils are summarized in table 4. The water supplies are grouped according to their nitrate content.

Soils that were obviously contaminated with organic nitrogen by livestock had the highest nitrifying capacities. These soils represented barnyards or the feeding lots so common in southwestern Minnesota or areas that so closely adjoined them as to be influenced by the organic nitrogen present. As can be seen in table

Table 1. Nitrate content of water supplies and of nearby soils in Renville County, Minn.

Site	Water supply	Date sampled in 1951	NO_3^-N in water (p.p.m.)	NO_3^-N in soils within 50 feet of water supply (p.p.m.)	
				Surface	Subsoil
1	Farm well A	July 9	120	6	23
		Aug. 23	92	212	(2)
	Farm well B	July 9	54	7	19
		Aug. 23	29	-----	-----
	Field tile	July 9	18	12	6
		Aug. 23	4	13	5
2	Farm well	July 10	75	44	14
3	Farm well	July 10	10	8	15
	Field tile	July 10	12	10	2
		Aug. 23	6	29	3
4	Farm well	July 10	87	4	2
		Aug. 24	46	67	-----
5	Pasture well	July 10	(1)	6	2
		Aug. 24	1.0	11	58
	Farm well	July 10	35	18	4
		Aug. 24	8	69	7
6	Farm well	July 10	9	10	12
7	School well	July 10	1.0	2	2
		Aug. 24	1.0	8	5
8	Farm well A	July 11	70	16	2
		Aug. 24	16	45	7
	Farm well B	July 11	4.0	-----	-----
		Aug. 24	1.0	-----	-----
26	Farm well	Aug. 24	8	71	11
27	Farm well	Aug. 24	1.0	16	5

[1] Under construction. [2] Lost.

4, water supplies with the highest concentrations of nitrate were located near soils of very high nitrifying capacity. At all but one of the sites in this group, the very high nitrifying capacity of at least one soil near each water supply was due to a concentration of organic nitrogen. At that one, site 8, no effect of organic nitrogen contamination was readily apparent, although a hog-feeding area within 150 feet of the water supply existed a few years

Table 2. Nitrate content of water supplies and of nearby soils in Rock County, Minn.

Site	Water supply	Date sampled in 1951	NO_3^-N in water (p.p.m.)	NO_3^-N in soils within 50 feet of water supply (p.p.m.)	
				Surface	Subsoil
11	School well	July 31	1.7	19	2
12	Farm well A	July 31	13	247	2
		Sept. 13	22	121	--------
	Farm well B	July 31	26	30	2
	Farm well C [1]	July 31	1.0	8	2
	Farm well D	July 31	15	11	2
		Sept. 13	23	7	2
	Field tile	Sept. 13	6.2	10	--------
13	Farm well	July 31	80	20	25
		Sept. 13	100	15	80
14	Farm well [2]	Aug. 1	2.3	33	32
15	Farm well	Aug. 1	6.3	10	2
16	Pasture well	Aug. 1	4.4	14	2
28	Farm well A	Sept. 13	130	138	--------
	Farm well B	Sept. 13	62	19	42

[1] Drilled well, 260 feet deep. [2] Drilled well, 130 feet deep.

Table 3. Nitrate content of water supplies and of nearby soils in Nobles and Mower Counties, Minn.

Site	Water supply	Date sampled in 1951	NO_3^-N in water (p.p.m.)	NO_3^-N in soils within 50 feet of water supply (p.p.m.)	
				Surface	Subsoil
	Nobles County				
10	Spring	Aug. 2	6.4	14	6
		Sept. 14	8.5	19	--------
17	Farm well	Aug. 1	4.4	28	--------
	Pasture well	Aug. 1	4.8	44	2
18	School well	Aug. 1	1.0	6	2
		Sept. 14	1.5	12	2
19	Farm well	Aug. 2	40	20	2
	Field tile	Aug. 2	5.2	63	--------
	Spring	Aug. 2	4.1	20	8
		Sept. 14	4.2	6	3
	Mower County				
20	School well	Aug. 16	1.0	7	2
21	Farm well	Aug. 16	18	38	18
22	School well	Aug. 16	1.0	11	2
23	Farm well	Aug. 16	1.0	13	3
25	Farm well	Aug. 16	1.0	8	2

Table 4. Nitrate content of water supplies in relation to their location and to the land use and nitrifying capacity of nearby soils

Site	Water supply	NO_3^-N in water (p.p.m.)		Location with respect to possible pollution [1]	Soils of vicinity	
		First sampling	Second sampling		Land use	Nitrifying capacity
	Water supplies of high NO_3^-N					
1	Farm well A	120	92	Poor	Barnyard Sod Cultivated	Very high. Low. Low.
2	Farm well	75		Poor	Near barnyard	High.
4	Farm well	87	46	Poor	Barnyard	Very high.
8	Farm well A	70	16	Questionable	Cultivated Cultivated Sod	High. Medium. Very high.
13	Farm well	80	100	Poor	Barnyard	High to very high.
28	Farm well A		130	Poor	Barnyard	Very high.
	Water supplies of moderately high NO_3^-N					
1	Field farm well B	54	29	Questionable	Sod	Low.
1	Field tile	18	4	Good	Cultivated	Medium.
3	Field tile	12	6	Good	Cultivated	Medium to high.
5	Farm well	35	8	Good	Cultivated	High.
12	Farm well A	13	22	Poor	Barnyard	High.
12	Farm well D	15	23	Good	Sod	Medium to high.
19	Farm well	40		Questionable	Cultivated	Low.
21	Farm well	18		Poor	Barnyard Cultivated	Very high. Medium.
	Water supplies of low NO_3^-N					
3	Farm well	10		Questionable	Sod	High.
6	Farm well	9		Poor	Near barnyard	Very high.
10	Spring	6.4	8.5	Good	Cultivated	Low.
11	School well	1.7		Good	Sod Cultivated	High. Low.
12	Field tile		6.2	Good	Cultivated	Medium.
14	Farm well [2]	2.3		Poor	Barnyard	Medium.
15	Farm well	6.3		Good	Cultivated	Low.
16	Pasture well	4.4		Questionable	Sod	High.
17	Farm well	4.4		Poor	Barnyard	Very high.
17	Pasture well	4.8		Questionable	Sod	Low.
18	School well	1.0	1.5	Good	Sod	Low to medium.
19	Field tile	5.2		Good	Cultivated	Medium.
19	Spring	4.1	4.2	Good	Cultivated	Medium.
26	Farm well		8	Questionable	Sod	High.
	Water supplies of very low NO_3^-N					
5	Pasture well		1.0	Good	Sod	Medium.
7	School well	1.0	1.0	Good	Sod	Medium.
12	Farm well C [3]	1.0		Good	Cultivated	Low.
20	School well	1.0		Good	Sod	High.
22	School well	1.0		Good	Sod	Medium.
23	Farm well	1.0		Good	Sod	High.
25	Farm well	1.0		Good	Sod	High.
27	Farm well		1.0	Good	Sod	Medium.

[1] Location of the water supply was evaluated on the site. Sites classed as "poor" were located within a hundred feet of obvious concentrations of organic nitrogen. Those classed as "good" were sites well removed from any concentrations of organic nitrogen. Those sites classed as "questionable" usually were within several hundred feet of manure piles or barnyards. [2] Drilled well, 130 feet deep. [3] Drilled well, 260 feet deep.

ago. Sites 6 and 17 illustrate that large concentrations of organic nitrogen in the soil adjoining a well are not necessarily reflected in a high nitrate content for that well.

Of primary interest in this study was the question of whether or not the normal field soils—the agricultural soils uncontaminated by heavy additions of organic nitrogen—might contribute to the nitrates that accumulate in the well waters of the methemoglobinemia region. The data in table 4 throw some light on this question.

The nitrifying capacity of soils other than those designated as "barnyard" ranged from low to very high. Only one of these soils, at site 8, was rated very high, and, as has already been noted, it is possible that this soil was contaminated by concentrations of organic nitrogen formerly in the vicinity. The soils of the region of Minnesota surveyed were highly productive when first placed in cultivation less than a hundred years ago. The high native fertility was due in large part to the high organic content of the soils. The effect of continued cultivation involving both good and bad land-management practices would be expected to influence the nitrifying capacities of the soils by virtue of influencing the kind and quantity of organic nitrogen present. Many of the normal soils examined demonstrated a high nitrifying capacity. The laboratory measurements of soil reaction and the field observations on the organic matter content and texture of the soils further support the conclusion that many of the soils of the region are capable of producing substantial quantities of nitrates. The addition to such soils of organic nitrogen in the form of manure fertilizers or accumulated waste from livestock will result in the very high nitrifying capacity found in the barnyard soils.

The nitrate content of certain of the waters associated with the normal field soils of medium to high nitrifying capacity is of marked interest. The data in table 4 for field tile drains reveal that drainage waters directly influenced by soil nitrification contained as much as 18 p.p.m. nitrate nitrogen. At site 5, where the direct contribution of soil nitrification was more difficult to assess, a well contained 35 p.p.m. nitrate nitrogen despite its location far from any concentrations of organic nitrogen. This well served only a home, and no farm buildings were within one-half mile. The cultivated field adjoining the well had a high nitrifying capacity, and it is considered likely that soil nitrification was the important factor concerned in the nitrate content of the well water at the time of the initial sampling.

None of the samples of stream or ditch water examined showed evidence of nitrate accumulation. The streams proved to be a poor index of generalized nitrate production in the areas sampled probably because the heavy algal growth in the streams kept the supply of available nitrogen low.

Summary and Conclusions

In connection with a survey to investigate the possible relation between nitrate production in soil and nitrate concentration in waters, data on 39 water supplies from 26 sites in 4 counties of Minnesota were obtained. Samples of 59 surface soils and 50 subsurface soils associated with these waters were studied in the laboratory for nitrate content. The surface soils were also studied for capacity to produce nitrates. Laboratory data on the water and soil samples were interpreted in the light of field observations.

The 16 water supplies that were sampled a second time during the summer gave evidence that the nitrate content of the water supplies fluctuated significantly during a 5- to 6-week period. These fluctuations were considered to be due largely to soil leaching associated with the rainfall distribution. Changes in the nitrate content of the surface soils correlated well with changes in the nitrate content of the water supplies. The nitrifying capacities of the soils remained essentially constant for the two samplings.

Soils obviously contaminated with organic nitrogen by livestock had the highest nitrifying capacities. The water supplies with the highest concentrations of nitrate (75 to 130 p.p.m.) were located near such soils. However, not all water supplies near contaminated soils had unusually high nitrate content.

Normal field soils in pastures, sod, or under cultivation near the very high nitrate waters were generally much lower in nitrifying capacity, but many of the normal field soils exhibited high nitrifying capacity despite the absence of abnormal additions of organic nitrogen. Such field soils were associated with subsoil drainage waters of up to 18 p.p.m. nitrate nitrogen and well waters of up to 35 p.p.m. nitrate nitrogen.

The rich soils of southwestern Minnesota present nearly ideal conditions for soil nitrification, limited normally by the release of ammonium nitrogen from the soil organic matter. Pollution of these soils with nitrogenous wastes will provide an excess of ammonium nitrogen and result in extremely high nitrate production. It seems possible that such soils of very high nitrifying capacity may sometimes contribute to the nitrate content of water supplies ordinarily considered to be satisfactorily located. Further study of the relation of nitrification to the accumulation of nitrate in waters will be necessary before any definite conclusions can be drawn.

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Legal Note

Fluoridation of Public Water Supplies

The Supreme Court of Oregon on January 11, 1956, in the case of *Baer* v. *City of Bend*, confirmed the decree of the circuit court which had sustained a demurrer to the complaint brought to enjoin city officials from fluoridating the Bend water supply.

The plaintiff contended that fluoridation would deprive him of his liberty without due process of law secured by the 14th amendment and encroach on his freedom of religion secured against Federal intrusion by the first amendment and similarly secured against State intrusion by the 14th amendment. The court, noting the various cases and other authorities (and the express concession of the plaintiff that dental health is a proper field for the exercise of State authority), held that the fluoridation measure of the city of Bend was a reasonable law for the protection of the public health and did not violate any religious or other liberties guaranteed by the Constitution.