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FACTORS AFFECTING THE ACCUMULATION OF NITRATE IN SOIL, WATER, AND PLANTS

Agriculture Handbook No. 413

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**FACTORS AFFECTING
THE ACCUMULATION OF NITRATE
IN SOIL, WATER, AND PLANTS**

By FRANK G. VIETS, JR., and RICHARD H. HAGEMAN

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**Agricultural Research Service
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Factors Affecting The Accumulation of Nitrate in Soil, Water, and Plants

By FRANK G. VIETS, JR., *Chief Soil Scientist, Soil and Water Conservation Research Division, Agricultural Research Service*, and RICHARD H. HAGEMAN, *Professor of Agronomy, University of Illinois, Urbana*

THE NITRATE PROBLEM

Human and Animal Health Aspects

Some environmentalists and ecologists say we are faced with a nitrogen (N) crisis resulting from use of commercial nitrogen fertilizers and the release of nitrogen oxides by high temperature combustion in power plants and internal combustion engines. They point to the high nitrate content of many water sources and to high nitrate in food, particularly some canned baby foods, as causes for real alarm. Commoner¹ in his address to the American Association for the Advancement of Science called for elimination of fertilizer N use for 10 years. In his paper "The Balance of Nature," Commoner (1968)² has been one of the most vociferous critics of present technology and practices.

Nitrate upon reduction yields nitrite (NO_2^-), which is commonly regarded as 10 times as toxic as nitrate in water, food, and feeds for equal concentration. Nitrite also can be produced as a lag phase in the oxidation of ammonium to nitrate by microorganisms. Not all of the biochemical effects of highly reactive nitrite are known. Nitrite has deleterious effects on red blood cells, possibly on muscle tis-

sue, and has been implicated in cancer. Nitrite can overoxidize the ferrous (Fe^{++}) iron of hemoglobin to ferric (Fe^{+++}) iron. The resulting methemoglobin in red blood cells is incapable of carrying oxygen. Nitrite can also combine irreversibly with hemoglobin to form nitrohemoglobin which, like carbonmonoxyhemoglobin, cannot carry oxygen. Nitrite can also combine with the myosin of muscle tissue. Gruener and Shuval (1970) gave a good short review of the relation of nitrate to acute and chronic subclinical methemoglobinemia and possible effects on fetal development. Winton (1970) discussed nitrate in water in relation to methemoglobinemia of infants and the complicating factors of bacterial infection, genetic imperfections, and formula preparation involved in setting standards for water quality.

If forages like corn (*Zea mays*) are high in nitrate and are put in a silo, denitrification can lead to the production of NO (colorless), N_2O (yellow), and N_2O_4 gases that are heavier than air. The gases accumulate in silo drains, chutes, and buildings that are attached to the silo. These gases can be lethal to man and animals unless fencing and adequate ventilation are provided (Hanway et al., 1963; Crawford and Kennedy, 1960).

Nitrite reacts with secondary amines in an acidic environment to produce nitrosoamines. These compounds have received recent attention because they can be carcinogenic, teratogenic, or mutagenic (Lijinsky and Epstein, 1970). DuPlessis and coworkers (1969) reported that the high incidence of esophageal cancer in

¹ Commoner, B. Threats to the integrity of the nitrogen cycle: nitrogen compounds in soil, water and atmosphere and precipitation. Presented at the Dallas, Tex., meeting of the American Association for the Advancement of Science. December 1968. In GLOBAL EFFECTS OF ENVIRONMENTAL POLLUTION. S. F. Singer, Ed., Springer-Verlag, New York, pp. 70-95. 1970.

² The year in italics after the author's name is the key to the reference in Literature Cited, p. 55.

Transkeian Bantus is related to high nitrate levels in the maize, pumpkins, and beans that they eat and have identified dimethylnitrosoamine in the fruits of *Solanum incanum*. The juice of this fruit is used to curdle milk to produce the curds eaten by the head boys. The authors claim the plants are high in nitrate because of molybdenum deficiency in the area. However, Malins and coworkers (1970) could not detect more than 10 p.p.b. of dimethylnitrosoamine in aqueous systems of secondary amines and nitrite much more acidic than those existing when smoked chub salmon is preserved with sodium nitrite.

The reader is reminded that nitrate is reported in many different ways. Much confusion results from lack of uniformity. Units used have been NO_3^- , NO_3^- -N, KNO_3 , and NaNO_3 . Forty-five p.p.m. NO_3^- equals 10 p.p.m. NO_3^- -N. One p.p.m. equals 1 mg. per liter.

Like standards for radiation exposure, DDT in foods, and safe speed on the highway, standards for acceptable levels of nitrate in drinking water, canned foods, and animal forages are highly controversial. The U.S. Department of Health, Education, and Welfare's Public Health Service (1962) standards call for less than 10 p.p.m. of NO_3^- -N (45 p.p.m. NO_3^-) in drinking water, 50 p.p.m. nitrate (dry weight basis) in asparagus, 3,000 p.p.m. nitrate in spinach (dry weight basis), none in squash and tomatoes for baby foods, 83 p.p.m. nitrate in strained spinach for babies, and 200 p.p.m. of nitrate or nitrite in preserved meats and fish.

The California State Department of Public Health notifies public water districts and the local health officer when a source of supply exceeds 45 p.p.m. NO_3^- and urges the utility to reduce the level. When the water has more than 90 p.p.m. NO_3^- in any significant portion of the community, local health officers are requested to notify all local practicing physicians of the risks and hazards involved, and the water purveyor is required to develop a special sampling program to determine the extent and levels of nitrate in the distribution system. The Policy for Administrative Procedures further states that if the water supply continues to exceed 90 p.p.m. NO_3^- that each case will be specifically

investigated to determine an appropriate course of action (Ward, 1970).

The U.S. Department of Interior (1968) Water Quality Criteria set 10 p.p.m. NO_3^- -N plus NO_2^- -N as permissible in drinking water for humans but set the desirable level at "virtually absent." No NO_3^- standards for water for animals were established. Keller and Smith (1967) said that water with 50 p.p.m. NO_3^- -N (perhaps 10) should be avoided for livestock. Case (1970) gave a good discussion of animal health in relation to nitrate N. The permissible levels of nitrate in animal forages are far from established (Crawford and Kennedy, 1960; Wright and Davison, 1964; Hanway et al., 1963). What the standards ought to be is outside the scope of this review, but to argue that the current standards are too low is of little comfort to those interested in the hazards of nitrate accumulation in soils, waters, and foods.

However, the present standards for nitrate in potable water should not be considered closed against reevaluation on the basis of new information. Many of the cases of infant death attributed to high nitrate may be caused by a combination of nitrate, enteric infection, and debility that arose from wells with fecal contamination. Winton (1970) stated that present U.S. standards for public water supplies are under review, but he cautioned against lowering the standards simply because there are methods of preparing baby formulas with waters higher in nitrate than the limit now recommended in the standards.

Gruener and Shuval (1970) stated that vitamin C can prevent or cure methemoglobinemia, and that use of citrus juices for infants in Israel may explain the scarcity of the disease in areas with high nitrate in drinking water.

What the future permissible level of nitrate in potable water ought to be is a very important consideration in the event that a substantial buildup of nitrate in underground water supplies cannot be reversed without drastic measures. Stout and Bureau (1967) were rather pessimistic about the Grover City-Arroya Grande Basin in California they studied. They suggested that eventually many areas may have to have two water supplies—one for drinking and

one for other uses. In California, some water districts mix water from different wells and surface sources to stay under the recommended standard (Ward, 1970). Israel is trying to maintain NO_3^- -N below 10 p.p.m. in the entire National Water System. This is being attempted by mixing water from ground-water supplies with the low nitrate water from the Jordan sources.

Agricultural and Aquatic Aspects

In the management of N in the environment to prevent the undesirable accumulation of nitrate above tolerable levels, several other aspects of nitrate must not be forgotten. Growth of all organisms requires nitrogen. The photosynthetic organisms, whether they be crops on land or phytoplankton and algae in water, require nitrogen either as ammonium or nitrate, although they may use some simple soluble organic nitrogen compounds. Hence, ammonium, total soluble nitrogen, and nitrate cannot be considered independently. Some species of bacteria and blue-green algae are capable of fixing atmospheric N_2 to meet their requirements. Their inputs must be considered in both land and water environments.

All crops, grasses, and forest lands must have one of these forms of nitrogen available to the plant roots during vigorous vegetative growth. Most of the nitrogen absorbed is probably absorbed as nitrate because ammonium is so quickly oxidized to nitrate by the nitrifiers in soil. In the United States, we use about 7 million tons of nitrogen annually to maintain soil productivity. The world agricultural consumption is about 25 million tons. Too much absorbable nitrogen in soil, whether it comes from too rapid oxidation of soil organic matter, too much animal manure, or too high application rates of commercial fertilizers can cause lodging in cereals, low sugar in sugar crops, and deterioration of quality of fruit and vegetable crops. Further, excessive nitrogen can be a hazard to quality of underground water because of the potential nitrate loss by leaching. Nitrate poses no danger to the soil and its properties, but excessive accumulation is generally associated with a salinity problem.

The terrestrial situation is paralleled by that in lakes and streams. Too little nitrogen can result in poor growth of photosynthetic plankton and algae—the basis of the food chain that supports fish. The water is oligotrophic. A “biological desert” results. On the other hand, too much nitrogen, along with sufficient amounts of other nutrients, can result in overfertilized or eutrophic water that supports nuisance blooms of algae. Nitrate, as well as ammonium and simple soluble organic nitrogen compounds, can be assimilated by aquatic plant life. In studies of lake eutrophication, the standard is usually total soluble nitrogen rather than nitrate. Big mistakes can be made in surface water studies by just considering nitrate or ammonium.

In Ensilage and Food Preservation

Preservation of vegetation, fodder, or foods of high nitrate content pose potential problems. These problems are caused by nitrate which is a good oxidant and which may react with reductants in the stored material, environment, or with the container. The partly reduced products formed from the nitrate are far more toxic than the nitrate itself. The products formed from the reductant may also be undesirable.

Fodder or vegetation of high nitrate content converted to silage loses variable amounts of its nitrate content during fermentation. Kurtz and Smith (1966) estimated that, in general, half of the nitrate is lost from the silage; however, the loss is variable and depends upon several factors. For example, silage made from material of 55-percent moisture content lost only 20 percent of its nitrate, while material ensiled at 80-percent moisture lost from 61 to 98 percent (Jacobson and Wiseman, 1963). The addition of sugar to high-moisture silage or laceration of the material before storage decreases nitrate loss.

The fermentative processes that occur in the ensilage including denitrification, in general, have been assumed to result from microbial contamination. The fact that many microorganisms, such as *Escherichia coli* and *Pseudomonas aeruginosa*, have a dissimilatory nitrate reductase that uses nitrate as a substrate lends credence to this view (Hewitt and Nicholas, 1964). However, Wang and Burris (1960) found that

silage made from aseptically grown soybean seedlings produced the same kinds of nitrogen oxides as nonsterile silage.

Mulder et al. (1959) and Klepper and Hageman (1969) showed that under anaerobic conditions the endogenous nitrate reductase of leaf tissue uses carbohydrate material to reduce nitrate only as far as nitrite. Thus, nitrate reductase endogenous in the silage material could explain: (a) The production of oxides of nitrogen by aseptic material; (b) the greater loss of nitrate from high-moisture silage (based on the fact that the greener, less mature, tissue would have higher levels of enzyme); and (c) the decrease in nitrate loss resulting from laceration of the tissue before storage, as this would tend to inactivate nitrate reductase.

It is conceivable that the addition of sugar decreases loss of nitrate by enhancing the fermentative production of acids that inhibit nitrate reduction or reactions that generate energy for nitrate reduction. The intent of this section is to suggest that if nitrate reductase is present in the ensiled material, it may act alone or in conjunction with the nitrate reductases of contaminating organisms to convert nitrate to nitrite. Nitrate can be subsequently converted to nitric oxide, nitrous oxide (the toxic silo gases), and ultimately nitrogen. Although the initial conversion of nitrate to nitrite is thought to be biological, some of the subsequent interconversions are chemical.

Forages, such as oat hay, beet tops, and rape, having high-nitrate content, are also potential sources of nitrite during the postharvest curing. If forages of high-moisture, -nitrate, and -nitrate reductase are handled in a way to cause anaerobic conditions, the endogenous enzyme systems can convert nitrate to nitrite. In contrast, forages stored under aerobic conditions are more likely to convert nitrate to ammonia (nitrate assimilation). Under aerobic conditions that are conducive to drying—low humidity, or high (30° to 40° C.) temperature—the endogenous nitrate reductase will denature in most tissues within a few hours. Nitrate loss from such tissue would then be minimal. Forages containing high amounts of nitrate, adequate carbohydrate, and moisture (initial or from subsequent precipitation) can become con-

taminated with microorganisms that can utilize the nitrate as an electron acceptor (dissimilatory enzyme) and produce nitrite. While forages of excessively high-nitrate content can cause nitrate toxicity (especially with ruminants), proper postharvest management is required to prevent conversion of the nitrate to nitrite by endogenous nitrate reductase or contaminating microorganisms.

Reviews of the denitrification process, general aspects of the production of toxic "silo gases," and practical management practices to minimize nitrate toxicity in ensilage are given by Petersen et al. (1958), Wright and Davison (1964), and Kurtz and Smith (1966), respectively.

Foods preserved for human consumption that frequently contain high levels of nitrate are leafy vegetables (such as spinach), beets, and to a lesser extent, green beans, carrots, and tomatoes. Three major problems that are associated with the preservation of foods of high nitrate content are: (1) The adverse effects of nitrate per se; (2) the detinning effects of nitrate when foods are preserved in tin-lined cans; and (3) the production of highly toxic nitrite from the nitrate by contaminating microorganisms once the cans or frozen foods are opened or thawed. Nitrate content of preserved foods per se is not considered a major problem to adults on a balanced diet.

The presence of nitrate in foods preserved in tin-lined cans has been associated with the "detinning" of cans and shelf life. Since these foods are heat-treated and sterilized, the oxidation reduction reactions associated with detinning of the sealed cans are chemical in nature. The reduction products of nitrate are primarily ammonia, although nitrous oxide and nitrogen have also been identified in the headspace of canned foods.

In canned whole tomatoes or tomato juice, it has been shown that tin removal continues until either the nitrate or the tin coat is exhausted. The presence of nitrate in canned carrots, beans, and spinach has been associated with detinning. However, the initial concentration of nitrate in these products is not so reliable an index to the degree of detinning as with tomato. In beans, carrots, spinach, and tomatoes, the

detinning process is more complex and dependent upon more acidic pH and oxalate content (at least for spinach) of the pack. The use of higher levels of phosphate fertilizer in the production of green beans, carrots, and spinach appears to retard detinning. Currently it is not known whether this effect is caused by increased levels of inorganic phosphate in the pack, or metabolic alterations in pH and oxalate content of the product to be processed.

These detinning problems can be avoided by use of enamel-lined cans, glass jars, and freezing the product and processing in plastic or lined cardboard containers. However, these treatments do not reduce the nitrate content of the produce. Farrow, Charbonneau, and Lao (1969) gave a more comprehensive coverage of the detinning process and associated factors.

The problem of conversion of nitrate to nitrite by microbial contamination arises when canned or frozen food products are opened and thawed and not maintained aseptically until the food is consumed. Simon (as reported by Lee et al., 1970) showed that high levels (200 mg. $\text{NO}_2^-/\text{l.}$) can be produced from nitrate of cooked spinach juice held under nonsterile conditions at 20° C. for 24 hours. Nitrite can also be formed in vivo (up to 320 mg. $\text{NO}_2^-/100$ g. fresh tissue after 4 days of storage) in fresh spinach during transport and storage. In processing high-nitrate foods for consumption, discarding of the cooking water is recommended because both nitrate and nitrite are water soluble.

Nitrate is Not a New Problem

The deleterious effects of excess nitrate on human and animal health is not a new discovery. Nitrates were used for a long time in internal medicine as depressants, but were phased out because of their severe depression of the heart. Sea captains were claimed to have used saltpeter as an aphrodisiac.

Many western U.S. creeks were given unflattering names by the miners because of the pharmacological properties of the water. According to U.S. Public Health Service (1962), nitrate in drinking water was first proved to produce serious changes in the blood chemistry

of infants in 1945 by Comly (1945). From 1947 through 1950, 139 cases of methemoglobinemia, including 14 deaths caused by nitrate farm water supplies, were reported in Minnesota (Bosch et al., 1950). Lenain (1967) stated: "In 1949, a nine-state survey revealed 262 cases of infant cyanosis. In all but nine, nitrates were in excess of 50 p.p.m. Minnesota revealed 139 cases, including 14 deaths, associated with nitrate contaminated wells. These were rural, none connected to a public system. Cases of infant cyanosis related to high nitrate wells have been reported in many parts of the world, particularly in U.S. and the central prairies of Canada."

Schwille (1969) reported that the ground waters of the Mosel River Valley have high nitrate levels, but that even adjacent wells have big differences. He says no cases of methemoglobinemia have been reported from this area, and only two cases have been reported from the entire Rhineland area.

Keller and Smith (1967) stated: "Ground water containing nitrate (including nitrite) in toxic concentrations, occasionally high enough to be lethal to human beings and livestock when the water is used regularly, has been observed in Kansas, Minnesota, Wisconsin, Iowa, Illinois, Michigan, Montana, Pennsylvania, and elsewhere as an awareness of [nitrate] NO_3^- poisoning increases." Gruener and Shuval (1970) stated that about 2,000 cases of infant methemoglobinemia have been reported in various countries since its first clinical recognition in 1945 and cited clinical studies on it in the U.S.S.R. and Czechoslovakia.

California has never had a reported case of infant cyanosis caused by drinking water. Ward (1970) also reported that "In 1962, eighty-five community water systems under the jurisdiction of the California State Board of Public Health were serving water at some time during the year which had nitrate concentrations exceeding 45 mg./l. in the whole or in part of a distribution system." Eighteen communities now have the problem. Their populations range from 2,000 to 32,000.

In the United States, no deaths from nitrate in canned baby foods have been recorded; but, in Europe several cases have occurred.

There are many recorded instances of animal deaths resulting from ingestion of high nitrate forages, often produced under drought conditions. Mayo (1895) reported cattle deaths from pasturing drought-stricken corn in Kansas 76 years ago. Case (1970) discussed the subclinical effects of nitrate in water and forages on different kinds of livestock and the dearth of adequate statistics on deaths and harmful effects.

We have not been able to locate maps or compilations of statistics of NO_3^- concentrations in ground waters, cases of methemoglobinemia, or of the incidence of livestock ailments so that we could relate them to other factors even qualitatively, or to determine whether any longterm trends of general nitrate accumulation have occurred in ground water in the United States or Canada. The infant cyanosis problem appears to have been more acute in the 1940's and the early 1950's than it is today. "Public Health Reports" published monthly by the U.S. Public Health Service carried only one article on nitrate and methemoglobinemia in the period 1960-69, inclusive. That 1962 incident involved cyanosis of a baby on a formula prepared with a municipal water supply in Colorado. According to Winton (1970), the child's mother had not heard the warning given to physicians about use of the water for babies. When the boy developed diarrhea, the mother boiled the water for the formula longer and concentrated the nitrate. The child survived after intravenous administration of methylene blue.

The apparent lower incidence in the past 15 years may be related to a number of factors: (1) More people use water from safe public supplies (2) more adequate testing and construction of rural wells to protect against fecal contamination, and (3) more widespread use of citrus juices in babies' diets and general improvement of their health. Thus, the apparent clinical picture cannot be interpreted to mean that underground water qualities, in general, have improved. Another conclusion, which cannot be fully documented, is that the central and upper Midwestern States have had high incidences of nitrate problems in comparison with the rest of the Nation. As will be discussed later, this incidence may be related to the natural high organic N content of the soils and to the long residence time of ground waters resulting from the close balance of precipitation and evapotranspiration. Another area with high NO_3^- in ground water is the San Joaquin and other valleys in California (Ward, 1970).

In Israel, Gruener and Shuval (1970) reported that a definite upward trend in nitrate in wells has occurred in the central and southern coastal plains and the problem has now reached serious proportions. In this densely populated area, about 180 water supply wells now have NO_3^- -N concentrations equal to or in excess of 10 p.p.m. Another 50 wells, now below this limit, are increasing in this concentration at the rate of 0.44 p.p.m. or more per year.

NITRATE AND THE NITROGEN AND CARBON CYCLES

Nitrate accumulation or depletion from soil and water cannot be understood without considering the pertinent parts of both the nitrogen and carbon cycles. Water is important too. Many misinterpretations of data on nitrate are possible if concurrent changes in ammonium concentration, oxidizable carbon compounds, biological oxygen demand, and oxygen supply are not simultaneously considered. The flux and direction of water movement, and sometimes the water suction, are important in transformations. The September 1970 issue of *Scientific*

American had excellent summaries of the nitrogen, carbon, oxygen, and water cycles.

Only the most pertinent aspects are briefly stated here. Nitrate in either soil or water is formed by the oxidation of ammonium ion to nitrite by *Nitrosomonas* spp. and of the nitrite to nitrate by *Nitrobacter* spp. These bacteria derive energy from the oxidation. Oxygen and favorable moisture and temperatures are required. Nitrification cannot occur if the soil is waterlogged so that oxygen becomes depleted. It cannot occur in soil or water loaded with oxidiz-

able organic compounds, such as sewage or animal wastes, to the extent that molecular oxygen is depleted. Nitrite can accumulate in soil as a lag phase in nitrification under alkaline or other conditions unfavorable to *Nitrobacter* spp.

Ammonia either in soil or water can come from ammonium fertilization, air, or rain, but one of the main sources is the oxidizable nitrogen-containing compounds contained in animal and plant waste, sewage, and soil organic matter. Favorable temperatures and moisture are required for the host of microorganisms that accomplish ammonification, either with or without oxygen. Oxygen will be used as long as it is available. Both the ammonium released and the biodegradable organic matter are part of biological oxygen demand (BOD).

Denitrification is the reduction of nitrite or nitrate chiefly to N_2 gas, but some gaseous oxides of nitrogen may be formed. This process requires favorable temperatures and absence of molecular oxygen, the nitrate and nitrite giving up their oxygen during nitrate respiration of organisms.

Nitrate data obtained on open bodies of water are difficult to interpret because nitrate concentration is affected by supplies of oxidizable carbon and dissolved oxygen and by nitrate use by green plants of all kinds. Although the NO_3^- concentration in itself may be of significance in

water supply systems, inferences about sources of pollution, total N load and time trends are almost useless without the associated data needed for interpretation. Just as the nitrate in a soil can be depleted by a plant, so can nitrate in a lake, river, or an open agricultural drain be depleted by growth of algae and phytoplankton. Just as nitrate in a soil can be depleted by mixing crop residues with high C:N ratios, such as straw in the soil, so can nitrate in a water body be depleted by "slugging" the water with material with high BOD demand. Both in water and in soil, NO_3^- will appear again under favorable temperature conditions when the oxidizable carbon is depleted, oxygen returns to the system from the air, and ammonification and nitrification can occur. Perhaps one of the best illustrations of the fluctuations in nitrate for a lake is the data of Serruya and Berman (1970) on eutrophic, temperature and chemically stratified Lake Kinaret (Sea of Galilee). They found a range of NO_3^- -N in this constant volume lake of from zero to about 2,600 metric tons over a 4-year period with definite seasonal influences apparent. Nitrate comes from the Jordan River, mostly in the spring, and from the oxidation of ammonia released from the hypolimnion on fall turnover. Highest NO_3^- occurs in the spring. This nitrate is assimilated in the summer by photosynthetic organisms or is denitrified in the hypolimnion when the lake restratifies.

NATURAL ACCUMULATIONS OF NITRATE

The accumulations of nitrate in soil, water, and geologic strata can be divided into three time periods for convenience in assessing the impacts of modern technology. We have chosen to discuss the data in three time classifications: (1) The accumulations related to "geologic" time before there was much impact of man; (2) the period before much commercial fertilizer was used, which terminated after the end of World War II; and (3) the period of accelerating commercial fertilizer use since 1945. Only the first two periods are discussed in this section. Section on Recent Field Investigation on Nitrate Accumulation and its Sources (p. 26) covers the third period.

These are arbitrary classifications, as nitrate in the environment has multiple sources from geologic deposits, decomposition of soil organic matter and peat, animal and human waste, and fertilizers. In a chronology, each new source of input is merged into the previous sources. Imperfect and as arbitrary as it may be, this classification may give the reader some appreciation of the trends with time. An opinion about trends can only be a subjective one, as there is no continuous record of nitrate measurement in any soil or water body.

To avoid the fragmentation of a particular study by this classification, we have put it where its dominant results apply.

Accumulations in Caves, Caliches, and Playas

Nitrate deposits can accumulate under special conditions of nitrogen availability, movement by water, and concentration by evaporation of water at a surface. Mansfield and Boardman (1932) reported nitrate deposits in 23 States and classified them into cave, caliche, and playa deposits. This publication was preceded by eight earlier ones of the U.S. Geological Survey on the occurrence of nitrate deposits in the United States.

Cave deposits occur on the walls and ceilings of caves where animal excreta has the opportunity to oxidize in air and the water evaporate. Oxidation of soil organic matter and plant detritus in the overburden may also contribute nitrate to the deposit. Such deposits occurred in the caves of such States as Indiana, Kentucky, and Virginia and were used by the American Confederacy in gunpowder during the American Civil War. Smith (1967) discussed these caves in the Missouri Ozarks and their contribution to nitrate in water supplies.

The best known caliche deposit is the northern Chilean deposit on the Tarabaca Plateau in the Atacama desert that contained 250 million tons of NaNO_3 . The most probable explanation of this accumulation, like that on the offshore islands of Peru, is the oxidation of guano and subsequent accumulation of nitrate. However, the theory that the Chilean deposits resulted from electrical discharge in the cloudy, but rainless, atmosphere has had its supporters.

A corollary of the caliche-forming process was used for several centuries in the production of nitrate. Nitrieries in Germany and France in the 18th and 19th centuries produced nitrate by the outdoor oxidation of animal waste. The United States imported 2,500 tons of nitrate for munitions in 1916 from India, where the nitrate was produced by composting of human and animal waste.

The accumulation of nitrate in playas, along with other salines, was one of the interesting controversies of western U.S. agricultural development. Hilgard (1906) attributed the nitre spots in western cultivated soils to the more rapid nitrification of the organic matter in the

warm arid climate when they were irrigated. Headden (1911, 1913), and in at least seven other bulletins, and Sackett (1911) attributed the nitrate accumulation in Colorado to the stimulation of *Azotobacter* spp. by cultivation. The story is best told by quotation from Stewart and Peterson (1916) of the Utah Station:

1. Certain soils in western arid America contain spots which are rich in nitric nitrogen and contain in some instances several hundred times as much nitric nitrogen as occurs in normal, cultivated, productive irrigated soil.

2. These spots are locally known as 'nitre spots.'

3. Three theories have been presented regarding the origin of these accumulations (a) Hilgard's conception was that the accumulation was due to more rapid nitrification of the organic matter of the soil of arid climates, (b) Headden mentions that they are due to the fixation of the atmospheric nitrogen by the nonsymbiotic bacteria, (c) Stewart and Peterson believe that they are the direct result of the leaching of nitrate out of the pre-existing deposits in the country rocks and locally concentrated by seepage.

* * *

6. The nitrate deposits are not limited to the country rock of a definite geologic series. They have been found in large quantities in the Cretaceous, Tertiary, and Triassic with smaller quantities in the Jurassic.

* * *

8. The accumulation in the virgin soil possesses all the characteristics of the nitre spots in cultivated soils, i.e. (1) a high nitrate content, (2) the presence of excessive quantities of other soluble salts, (3) brownish color, (4) thin crust over surface. . .

* * *

10. The non-symbiotic bacteria are not essential to the production of the nitrate or of the color of the nitre spots.

Tables 1 and 2 give some analyses of rocks reported by Stewart and Peterson (1914, 1916). Note that the nitrate is only a very small frac-

TABLE 1.—*Sodium nitrate concentrations in western rocks*¹

Age	Maximum	Minimum	Average
	Percent	Percent	Percent
Cretaceous:			
Sandstone	0.293	0.005	0.055
Shale	1.026	.003	.104
Clay115	.002	.074
Tertiary:			
Sandstone284	0	.074
Shale750	0	.147
Clay940	0	.053
Jurassic:			
Sandstone039	0	.008
Shale019	0	.005

¹ Data from Stewart and Peterson (1914).

TABLE 2.—*Nitrate and total soluble salt content of rocks*¹

Location	Number of samples	Soluble salts	Nitrate N
		P.p.m.	P.p.m.
West of St. George, Utah	8	48,962	156.0
East of St. George, Utah	10	54,256	74.3
Northern Arizona	9	51,858	27.3
Mt. Carmel, Utah	14	17,734	67.7
Cedar City, Utah	11	36,055	28.7

¹ Data from Stewart and Peterson (1916).

tion of the total soluble salts. They stated that all Cretaceous samples contained nitrate. Rankama and Sahama (1959) gave average values for total N in igneous rocks as 46 g./ton and sedimentary rocks, 510 g./ton.

The data on a drainage well constructed near Grand Junction, Colo., to relieve the artesian pressure on an experimental area illustrate the amounts of salt and nitrate that can come from an aquifer in contact with the Mancos shale. This well was pumped continuously at a rate of about 260 gallons per minute. The water was highly saline with a dissolved solids content of 9,570 and 35 p.p.m. of nitrate-N. In 24 hours,

the well pumped about 110 pounds of nitrate-N or about 20 tons of nitrate-N per year into the Colorado River.

The occurrence of natural nitrates, particularly in profiles and geological strata of semi-arid and desert environments, is sufficient to often complicate interpretation of data obtained today to determine if fertilizer, sewage, and industrial waste disposal are enriching soils and waters with nitrate. Dyer (1965) reported nitrate-N concentrations that were in the range of 1,400 to 4,200 p.p.m. of soil solution to a depth of 50 feet for four sites located on Panoche soils, formed on alluvial mud flows on the west side of the San Joaquin Valley in California. Concentrations of nitrate were low in the upper 3 or 4 feet of the profiles caused by natural leaching. On adjacent, sprinkler-irrigated sites, the upper 20 to 30 feet of profile contained very little nitrate, but large accumulations occurred below these depths.

Smith (1967) gave an interesting account of the nitrate outflow of springs in the Missouri Ozarks: “. . . largely a forested area with little use of fertilizers and a sparse population of both humans and livestock.” Smith wrote:

. . . Big Spring in Carter County has a maximum daily flow of more than 800 million gallons of water. An average daily flow of 253 million gallons has been found over a 17-year period. Analysis of the water in 1964 showed a nitrate-nitrogen content of 2.4 to 3.0 ppm. Three parts per million in 252 million gallons of water is more than 6,000 pounds of nitrogen. The drainage area of Big Spring has been estimated as 440 square miles. Assuming that one-fourth of the 48 inches of annual rainfall percolates through the soil, an average of 250 million gallons daily would be obtained. A discharge of 6,000 pounds of nitrogen daily would amount to less than 8 pounds of nitrogen per acre per year, a realistic leaching loss even on the low-fertility forest areas of the area. Five or more pounds of nitrogen per acre is added to soils in precipitation each year. This regular addition of nitrogen in rainfall could account for most of the nitrate discharged by Big Spring. It is also of interest that the use of

nitrogen fertilizer in the drainage area of Big Spring in 1963 was only about 65% of the nitrate discharged in the flow of this single spring."

Smith further stated that about one-third of the known caves in Missouri have been reported to contain bat guano. Good correlations have been obtained between the nitrate in spring waters from these caves and the presence of guano deposits. Obviously, the amount of nitrate and the concentration in water from Big Spring hold much interest, but it is impossible to assign the sources. This example is typical of the modern problem. Nitrogen in rainfall is but one input of nitrogen into a system that can yield nitrate. Smith implied that part of the nitrate could have come from rainfall, but recent studies cloud the picture. Taylor et al. (1971) reported that the total N runoff from an Ohio wooded watershed was only 12 percent of that in the precipitation over a 3-year period, and that for farmland was 30 percent. The research by Borman and Likens (Deevey, 1970) on runoff from forest land at Hubbard Brook, N.H., showed that the runoff of nitrogen is less than that in the precipitation.

Stewart et al. (1967a, b) reported that 17 cores taken from the soil surface to the water table or drilling impediment, in native grassland of northeastern Colorado, averaged 90 pounds of NO_3^- -N in 20 feet of profile. (See table 11.) Of the 124 analyses reported in profiles taken to a depth of 12 meters, 62 samples contained no nitrate, 52 contained 1 to 3 p.p.m. (dry soil basis), nine had 4 to 10 p.p.m., and only one had more than 11 p.p.m. Water that entered the drill holes when the water table was

reached on eight of the sites had nitrate-N contents varying from 0.1 to 19 p.p.m., with an average of 11.5 p.p.m.

Agricultural Accumulations Before the Fertilizer Epoch

Some of the available data on nitrate in water and the overlying mantle collected before extensive fertilizer use or from areas with little fertilizer use before sampling are pertinent. Nitrogen fertilizer use before the end of 1945 did not approach the scale of use now.

Fertilizer N consumption of 1945 amounted to only about 650,000 tons in the United States compared with about 7 million tons in 1969. In evaluating data of this "prefertilizer era," it is impossible to separate the nitrate that may have existed in "geologic" accumulations from those related to man's occupancy of the land.

Whitehead and Moxon (1952) reported that eight wells in South Dakota, some of them causing cattle losses, had NO_3^- -N concentrations varying from 235 to 525 p.p.m. These wells were sampled in the period 1944-49. They do not give the well depths. They reported that the water in one stock tank was 240 p.p.m. higher in nitrate-N than the well due to concentration of the water by evaporation and ice formation in the tank.

The data in table 3 show appreciable amounts of nitrate-nitrogen in the water of the Arkansas River, Colo., in 1940-41, and that nitrate did not become more concentrated downstream as did the other dissolved salts. As in most irrigated areas, seepage flows into the river and the water is reused. The data indicate that either crops, plants in the stream channel, or release

TABLE 3.—Salt content of Arkansas River, water year 1940-41

Station	Flow 1000s	Dissolved solids		Ca	Mg	Na	HCO_3	SO_4	Cl	NO_3^- -N
		P.p.m.	Tons							
	<i>Acre ft.</i>			<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>
Pueblo	503	346	237,000	59	15	18	137	122	7	0.56
Nepasta	568	542	419,000	92	24	40	165	249	12	.95
La Junta	108	1,088	160,000	154	45	99	207	555	27	.56
Caddoa	310	1,376	580,000	170	61	158	195	775	36	.40
Holly	169	2,034	468,000	217	83	241	243	1,050	81	.95

Source: U.S. Bureau of Reclamation.

of untreated sewage by towns selectively removed nitrate.

Table 4 gives the water and nitrate budget for the Sunnyside Valley Irrigation District. All water diverted into irrigation canals or leaving the district via drains was measured. Appreciable concentration of nitrate occurred in the return flow, even though little nitrogen fertilizer

was used in the district in those war years. The principal sources of nitrogen were barnyard manure and alfalfa. The contribution of an acre of land in 1943 was about 8.5 pounds of NO_3^- -N; in 1944 it was 8.0. This budget does not consider organic nitrogen in the water which was not measured. Crops grown in the area were corn, alfalfa, hops, sugarbeets, wheat, barley, asparagus, and mint.

Table 5 gives the input and output of mineral salts of the Sutter Basin, Calif., in 5 different years. In two of the 5 years, there was more nitrate in the irrigation water than in the drainage water. In 1947, when data were available on total water flow, a NO_3^- -N output of about 0.5 pound per acre was computed. Much of the land of the Sutter Basin is used for rice production. Rice, which is flooded from planting until shortly before harvest, is conducive to denitrification of nitrate in the soil. This may explain the much smaller outflow of nitrate from this basin compared with the Sunnyside Valley project in Washington.

Table 6 gives the nitrate concentrations in canal waters and some wells in the Columbia Basin Project, Washington, in 1954 and 1955. This is a former semidesert area where irrigation had just been started. Nitrate in the water leaving a part of the project (sampling station 2) had less nitrate than water entering the project through sampling station 1. Of particular

TABLE 4.—*Input and output of water and nitrate N from Sunnyside (Wash.) Valley Irrigation District*

Item	Unit	1943	1944
Water:			
Input	Acre-foot	167,931	164,060
Output	do.	68,033	62,456
NO_3^--N			
Concentration:			
Input	P.p.m.	0.27	(¹)
Output	do.	2.29	1.66
Total NO_3^--N:			
Input	Pounds	123,000	² 0
Output	do.	427,000	284,000
N Release:			
NO_3^- -N	Pounds per acre	8.5	8.0

¹ Trace.

² Assuming that trace equals zero. Irrigated acreage in the Division was 37,896 acres.

Source: Unpublished data of C. S. Scofield and L. V. Wilcox, U.S. Salinity Laboratory.

TABLE 5.—*Input and output of mineral salts—Sutter Basin, Calif.*¹

Year	Input Output	Salts	Ca	Mg	Na	NCO_3	SO_4	Cl	NO_3^- -N
		P.p.m.	P.p.m.	P.p.m.	P.p.m.	P.p.m.	P.p.m.	P.p.m.	P.p.m.
1931	Input	140	17	10	11	107	8	9	0.027
1931	Output	1,008	77	59	150	268	30	364	.013
1932	Input	125	15	10	12	97	5	6	.027
1932	Output	1,294	96	78	193	283	36	518	.013
1933	Input	140	15	8	16	100	6	11	.069
1933	Output	950	67	57	152	276	31	335	.110
1946	Input	103	12	6	9	75	7	5	.069
1946	Output	603	52	41	105	277	36	184	.14
1947	Input	125	14	8	13	88	11	9	(²)
1947	Output	611	52	40	102	255	25	197	0.25

¹ Acreage irrigated: 45,028. 1947 water input—299,300 acre feet; water output—152,110 acre feet.

² Trace.

Source: U.S. Salinity Laboratory.

TABLE 6.—*Salinity of water in the Columbia Basin Project*

Sampling station	EC 25° C.	Ca	Mg	Na	HCO ₃	SO ₄	Cl	NO ₃ -N
	<i>Mmhos/cm.</i>	<i>P.p.m.</i>						
1. Lake Roosevelt ¹	150	21	5	2	76	12	1	0.17
2. Frenchman Wasteway ²	213	22	8	10	91	20	4	.11
3. Moses Lake	396	28	15	33	182	33	11	.44
4. Wasteway	204	25	8	8	101	19	4	.44
5. Potholes E. Canal Mile 0	345	24	13	28	163	27	10	.24
6. Lind Coulee	301	32	10	15	113	37	12	.55
7. Scootney Wasteway	159	21	6	4	82	13	1	.15
8. Potholes E., Canal Mile 26	335	24	13	26	159	24	11	.11
9. Potholes E., Canal Mile 38	321	24	12	24	154	25	10	.04
10. Potholes E., Canal Mile 66	318	24	12	24	146	26	10	.11
A. Well	834	92	46	20	201	214	12	15.0
B. Do.	653	44	44	37	299	203	12	.40
C. Do.	431	38	16	26	201	52	10	.69
D. Do.	955	58	45	86	530	63	24	3.02
E. Do.	495	40	25	25	173	34	16	15.0
F. Do.	517	31	37	29	263	51	3	4.4

¹ Water into project.

² Water out of project.

Source: Bureau of Reclamation—data for 1954 and 1955.

interest is the high nitrate in at least two of the wells. This nitrate could not have come from

high rates of nitrogen use in irrigation farming or from livestock.

THE SOURCES OF NITROGEN AND NITRATE

Nitrate unlike ammonia is not a constituent of the igneous rocks of the earth. Only small amounts come down in rain as the result of electrical discharge or some form of atmospheric pollution. Except for factory-produced nitrate, nitrate has to come from the oxidation of ammonia. There has to be some mechanisms of concentration for it to be of much environmental concern. Hence, the processes of accumulation of organic nitrogenous compounds that can be ammonified and nitrified are of paramount importance in consideration of nitrate accumulation. The nitrate produced is then often further concentrated by evaporation of water.

Geologic Deposits

The accumulations of nitrates on cave walls and ceilings, the deposits of caliche containing nitrates and other salts, and the accumulation of nitrates in playas and seeps are all the result

of evaporation in a dry atmosphere of nitrate-bearing solutions. In all instances, the source of the ammonia to produce the nitrate appears to have been accumulations of biological waste such as dung, animal remains, and animal and plant detritus deposited in caves, on rocks, or in the bottom of a shallow sea (marine deposits) to become consolidated into sandstones, siltstone, or shales. These processes have their modern counterparts to be discussed. Although geologic may not be the best terminology for these old deposits of nitrate, the outflow from them and their occurrence in semidesert and desert soils, and in aquifers in humid regions, is a problem in estimating the impact of modern technology. In most instances, this "background" is so variable and so little attention has been paid to measuring it that we lack knowledge about what would happen if we could eliminate current sources of nitrogen input.

Soil Organic Matter, Peat and Muck

Cultivation of some soils has increased their contents of humus and organic nitrogen. Substantial accumulations of these important soil fractions have occurred in New Zealand and Australia, where white or subterranean clovers have been used in grass-legume pastures with high rates of phosphate and sulfur fertilization (Walker et al., 1959). Western U.S. soils put under irrigation may maintain their organic matter and nitrogen contents, but only when alfalfa or heavy applications of manure are used (Viets, 1959). Information is lacking on the effects of the higher rates of commercial fertilizers now in general use on humus and organic nitrogen contents of most soils. However, the predominant effect of long-time cultivation has been depletion of organic matter and nitrogen. Few people realize the magnitude of these losses of nitrogen that have occurred from grassland and woodland soils put under cultivation in the temperate zone, where nitrate problems are of most concern to people. Also, peats and mucklands, when drained and cultivated, undergo rapid subsidence and always show losses of organic matter and organic N. These losses of soil N have been well documented at numerous locations, and estimates of their significance on a regional scale have been made.

How much organic N was accumulated in our soils before they were cultivated? A figure of 10 billion tons is frequently quoted for the 48 States. Table 7 adapted from Schreiner and Brown (1938), who adapted their data from C. F. Marbut's analysis of 250 to 300 carefully selected soil samples taken to a depth of 40 inches, shows a total of 3,494 billion tons for 720 million acres. The latter is about a third of our total land area, that not represented in table 7 being the podzol soils, organic soils, various nonzonal soils, and all land west of the Rocky Mountains.

Smith and Vandecaveye (1946) reported that soil N losses on Palouse silt loam at Pullman, Wash., were greater in wheat-fallow rotation than under a continuous wheat system for a 23-year period. In the wheat-fallow system, soil N continued to decline even when N fertilizer (as NaNO_3) or crop residues, either alone or

TABLE 7.—Average total N content of virgin soils in soil regions of the United States east of the Rocky Mountains¹

Soil region	Ap- prox. area	Surface 6 inches	In 40 inches		Regional total
			Per- cent	Tons	
	<i>Mil- lion acres</i>	<i>Percent</i>			<i>Million tons</i>
Brown forest	180	0.05—0.20	0.05	3.35	603
Red and yellow	150	.05— .15	.03	2.00	300
Prairie	113	.10— .25	.12	8.00	854
Chernozem and Cherno- zem like	123	.15— .30	.12	8.00	984
Chestnut	102	.10— .20	.08	5.35	546
Brown	52	.10— .15	.06	4.00	208
Total	720	3,495
Total of 48 States	2,266	

¹ Adapted from Schreiner and Brown (1938).

combined, were used. Bracken and Greaves (1941) found that soils in wheat-fallow systems in the Cache Valley of Utah had lost 15.9 percent of their organic N in the first foot compared with adjacent virgin grassland. Of the 2,560 pounds of N per acre lost from the upper 2 feet of soil, 735 pounds could be accounted for by crop removal, leaving an unexplained loss of 1,825 pounds, or 71.3 percent of the total loss. In the Juab Valley of Utah, a loss of 1,186 pounds of N per acre had occurred in wheat-fallow systems, 402 pounds of which could be accounted for by crop removal, leaving 784 pounds or 66.1 percent of the loss unexplained.

Haas et al. (1957) concluded from a summary of the data from 11 dryland experiment stations in the Great Plains that, in 33 years of cultivation, the total N of the surface foot decreased 24 percent under continuous small grain and 29 under an alternate fallow-small grain system. Since most of these field experiments from which the data were derived were located on relatively level sites with low water erosion hazard, the average field losses of N

may have been underestimated considerably. Viets (1968) made some highly speculative calculations about 1964 N losses in relation to the 1964 needs of wheat in seven States of the Great Plains based on the data of Haas et al. (1957). The speculation lies in the extrapolation to a Statewide basis of the annual N losses and soil N contents at the experiment station when the experiments were terminated. The calculations show that for 1964 the sum of the fertilizer N applied plus that lost from the soil in 1964 about equalled that in the 1964 wheat crop for five of the seven States for which the N balances were made for annual cropping systems. However, for a wheat-fallow system, the 2-year N loss would be about twice that removed in the wheat crop.

Thompson et al. (1954) gave a list of 20 paired soil samples obtained from the surface 6 inches of cropland and adjacent virgin grassland in Iowa. The total N contents of the cultivated soils averaged only 70 percent of that in the grassland soils. The average total N content of the grassland soils was 0.3454, for the cropped soils, 0.2422—a loss of 0.1032. Assuming an acre-6 inches of soil weighs 2 million pounds, the N loss from just the surface soil averaged 2,060 pounds per acre.

Sufficient data exist to show conclusively that cultivation has taken an enormous toll of our soil organic N accumulated over centuries. What the numbers mean on a national scale and what they mean to nitrate accumulation in surface and ground waters are speculative. Although loss figures spanning a period of time from a great number of locations are available, enough measurements were not made and *are not being made now* because of the experimental difficulties to know what fractions were lost by crop removal, erosion, nitrate leaching, and denitrification. In other words, we are far from having a balance sheet in which everything was determined even for one site.

Wadleigh (1968b) credits George Stanford with the estimate that the cultivated soils in the 48 States have lost 1.75 billion tons of organic N from the top 40 inches in the last 100 years. Lipman and Conybeare (1936) estimated annual crop removal for 1930 at 4.6 million tons, loss by erosion—5.0 million tons, and leaching of

soil N—4.0 million tons. For 1947, Mehring and Parks (cited by Stanford et al., 1970) made estimates of 6.5, 4.0, and 3.0 million tons for these categories. Stanford et al. (1970) made new estimates for 1969 as 9.5, 3.0, and 2.0 for these items (table 8). Note the question marks on estimates of leaching of fertilizer N and denitrification.

All of the estimates in table 8, except those for fertilizer nitrogen, must be regarded as highly speculative. We really know little about the input and output accounts for nitrogen, even for a selected farming system and soil association.

The effect of slow organic matter mineralization on the nitrate concentration of percolating water can be computed if the amount of leachate is known or assumed. The assumption is made that nitrate is uniformly distributed in the percolate, that is, no negative or positive adsorption. Stout and Bureau (1967) in their discussion of organic matter loss in relation to nitrate in percolating water, gave a number of cases, one of which is summarized here: If a rich prairie soil contained 0.1 percent of organic N initially in the top foot (a very conservative assumption), one-fourth of this N was lost in 100 years of cultivation, and one-hundredth of the loss leached as nitrate in 12.6 inches of percolate each year, then the percolate would contain 28.6 p.p.m. of nitrate N.

Questions posed by such calculations using the known facts on organic matter loss are many. How have the known losses of soil N been distributed among erosion, deep percolation of nitrate, crop removal and denitrification? Is their active denitrification below the root zone? If the assumptions are correct, why have all of the ground waters in the North Central Region not become loaded with nitrate long ago? Are the rates of recharge and outflow from the aquifers sufficient to keep the nitrate from accumulating? Does denitrification take place in the water table? Historically, there has been a high incidence of nitrate phenomena, such as livestock deaths and methemoglobinemia, where people depended on wells for water supply, deaths in silos, silo explosions, and toxic nitrate levels in animal forages in the Midwestern States.

TABLE 8.—*Balance sheet of nitrogen (N) in the United States: Estimated changes for 1930, 1947, and 1969 on harvested cropland*¹

Item	Amount of nitrogen in—		
	1930 ²	1947 ²	1969
	Million tons	Million tons	Million tons
Inputs of nitrogen from:			
Fertilizer N	0.3	0.7	6.8
N fixed by legumes	1.7	1.7	2.0
N fixed (nonsymbiotic)	1.0	1.0	1.0
Barnyard manure	1.9	1.3	1.0
Roots of unharvested portions of crops	1.1	1.5	2.5
Rainfall8	1.0	1.5
Total	6.8	7.2	14.8
Removals of nitrogen by:			
Harvested crops	4.6	6.5	9.5
Erosion	5.0	4.0	3.0
Leaching of soil N	4.0	3.0	2.0
Leaching of fertilizer N	0	0	(?)
Denitrification	(?)	(?)	(?)
Total	13.6	13.5	14.5

¹ Data of Mehring and Parks cited from Stanford, England, and Taylor (1970).

² Lipman, J. G., and A. B. Conybeare. Preliminary note on the inventory and balance sheet of plant nutrients in the United States. N. J. Agr. Expt. Sta. Bul. 607. 1936.

³ Mehring, A. L., and R. Q. Parks. Agricultural Chemicals, v., No. 10, pp. 36-50; No. 11, pp. 33-39 and 77-78. (See Stanford, England, and Parks 1970).

Peats and mucks represent great monuments to a past or current eutrophication process that have accumulated vast amounts of organic N. When these peats and mucks are drained and cropped, large amounts of nitrate can be released by mineralization. Schreiner and Brown (1938) indicated that there are 197,500 pounds of organic N in an acre of peat 40 inches deep. Wadleigh (1969) wrote that there are 7,500,000 acres of peat in Minnesota containing 6.4 billion tons of peat. Assuming an average N content of 1.5 percent, he calculated this peat contained 102 million tons of stored N.

Many studies have been conducted on nitrogen mineralization in peat, but we cite here only a few to indicate their potential as a nitrate source. Neller (1944) reported the nitrate content with depth of Everglades peat in fallow fields that had water tables at depths of 12 and 24 inches. The data shown in table 9 have been recalculated to show the nitrate content in the soil water of the samples assuming uniform dis-

tribution of nitrate in the water. Obviously, sawgrass peat liberates high concentrations of nitrate that can pollute drainage water unless crop demands are sufficient to remove it. Deep drainage to 24 inches allowed more nitrogen to mineralize than shallow drainage.

TABLE 9.—*Nitrate-nitrogen concentration on March 31 in soil solution of fallow Everglades peat having drainage to two depths*¹

Soil depth (inches)	Drainage depth 12 in.	Drainage depth 24 in.
	P.p.m.	P.p.m.
0 to 6	84	182
6 to 12	58	100
12 to 18	34	59
18 to 24	15	54

¹ After Neller, (1944).

The problem Israel is having with nitrate coming from overdrained peat in the Hula Valley above the Sea of Galilee (now Lake Kinaret) illustrates the significance of peat as a nitrate source. In 1958, the Hula swamp and lake that had been previously drained were prepared for growing crops, mostly cotton. Soil of the dried swamp is a low moor peat containing from 30 to 80 percent organic matter. Soil of the dried lake contains 60 to 80 percent lime equivalent and 5 to 10 percent organic matter. Water is supplied by subirrigation. Rainfall is about 30 cm. and comes in the winter months. Crops grow very poorly on patches comprising about 10 percent of the total area (Levin, 1970).

Shoham and Levin (1968) showed that the subsidence in the former swamp was about 10 cm. per year for 1958 to 1964 and about 9 cm. for 1964-65. Table 10 shows the salt content as measured by electrical conductivity and the nitrate concentrations in a lake bed area with good crops (unaffected area) compared with one with poor crops. The soluble salts are almost entirely gypsum and calcium nitrate. Soil in the affected area had two to three times as much nitrate as the unaffected area depending upon the depth. Nitrate-N as high as 2,000 p.p.m. was measured in some affected areas. This situation resembles that of the playa accumulation of nitrates noted in the Western United States, except nitrate is the dominant salt, and the source of the nitrate is not shales and other rocks, but peat.

TABLE 10.—*Electrical conductivity and nitrate concentration in lake bed soil of Hula Valley, Israel*¹

Depth, cm.	Electrical conductivity		NO ₃ -N (dry soil basis)	
	Poor crops	Good crops	Poor crops	Good crops
	Mmhos/cm.	Mmhos/cm.	P.p.m.	P.p.m.
0 to 30	8.0	7.1	1,550	660
30 to 60	7.8	4.4	1,430	400
60 to 90	4.3	2.9	1,020	430
90 to 120	2.8	2.1	-----	-----

¹ Adapted from Levin, (1970).

According to Serruya and Berman (1970), largely because of the drainage of the Hula Valley peats, a large part of the total N (1,600 tons in a normal year) entering Lake Kinaret from the Jordan is nitrate. In 1969, the nitrate nitrogen inflow into Kinaret was 5,000 tons. Since Israel is trying to maintain nitrate in all water supplies at drinking standards (less than 10 p.p.m. nitrate-N), some Israelis are having second thoughts about the wisdom of draining the Hula Valley. Much of the nitrate entering the lake is metabolized or denitrified in the hypolimnion (Serruya and Berman, 1970).

Livestock and Poultry Wastes

Livestock, wild animals, and birds foraging in their natural habitats do not ordinarily constitute a concentrated source of N supply that can give rise to nitrate accumulations. The N is recycled at or near the site it came from. However, bats and some bird species that nest closely together accumulate deposits of guano that are a potent nitrate source. But livestock and poultry production has changed and is still changing. Cattle are fattened in operations in which as many as 200,000 head are crowded onto 640 acres of land. Swine are fattened in confinement. Poultry feeding is concentrated into huge operations. The urine and manure that were once scattered over the landscape are now concentrated in a small area, the nitrogen in the feeds coming from a large land area.

Wadleigh (1968a) stated that domestic animals in the United States produce over 1 billion tons of manure and 400 million tons of urine a year. He estimated that as much as 50 percent of this waste may be produced in concentrated supply. To give some idea of the significance of concentrated supply, the following calculations are given (Viets, 1970a). A typical Colorado feedyard is used continuously and has about 360 head of fattening steers and heifers per acre. In one year these cattle will produce 4,200 tons of wet manure and urine containing 24.9 tons of nitrogen. The depth of water excreted annually in the urine and manure amounts to almost 31 inches. For a feedlot with a capacity of 200,000 head, being constructed but not fully stocked now, the total N excretion will be about 14,000 tons per year.

Mature healthy monogastric animals on good diets can excrete nitrate in the urine; cows and sheep do not (Case, 1970). However, nitrifying organisms can quickly oxidize the ammonia originating from hydrolysis of urea and uric acid and from ammonification of the organic N compounds of the feces to nitrate. Animal wastes are usually high in salts so that the runoff or percolate from their oxidation on land after evaporation results in a nitrate accumulation not unlike the playa deposits noted earlier. Animal and poultry wastes volatilize ammonia which can be absorbed by water as shown by Hutchinson and Viets (1969) for lakes in the vicinity of cattle feedyards in northern Colorado.

Runoff from feedlots can contain nitrate and always contains soluble nitrogen compounds that can quickly nitrify. In the studies of runoff from beef cattle feedlots at Mead, Nebr., Gilbertson et al. (1970) reported that NO_3^- -N in winter runoff ranged from 0 to 80 p.p.m. and in rainstorm runoff from 0 to 17 p.p.m. Heavy rates of land disposal of animal wastes can be a source of nitrogen for nitrates in percolating water. Shallow wells can become polluted with nitrate. In Michigan, Deutsch (1963) reported concentrations of NO_3^- -N ranging from 54 to 216 p.p.m. in shallow dug wells in several counties. The source was human and animal excreta. Several cases of infant methemoglobinemia were observed. Near Guelph, Ontario, Canada, Gillham and Weber (1969) reported that nitrate contamination of water in shallow wells is a serious problem in the area because rural wells are frequently located close to barnyards. In a study of the contaminated zone resulting from a barnyard located on glacial till, they found that the barnyard contributed 4.4 pounds of NO_3^- -N to the water table in a 5-month period. The lowest concentration of NO_3^- in the ground water was found in the summer.

Smith (1967) contended that the major source of nitrate contamination of shallow ground water in Missouri is livestock feedlots and barnyards. Frink (1969a) estimated the N input budget for highly specialized dairy farms in the Northeastern States. He concluded that under certain conditions dairy farms could contribute significant amounts of nitrate to ground

water. He believed the loss of nutrients can be minimized by providing more cropland per cow, by selecting crops and livestock that are efficient users of N, and by applying both manure and commercial fertilizers during the growing season. For Connecticut, Frink (1969b) estimated that animal wastes contributed 10 percent of the total N input budget, whereas agricultural fertilizer N contributed 3.5 percent, and nonagricultural fertilizer N use contributed 2.2 percent. Much of the N in animal wastes comes from imported feeds.

Although some waste from hog and poultry feeding operations is being put through sewage treatment plants not unlike those used by cities, the magnitude of the physical plant required (one cow equals the sewage equivalent of 10 to 12 people), the high water requirements for transport and digestion, and the costs all make conventional sewage treatment largely out of the question. Needless to say, the animal waste problem has created problems for pollution control agencies, all of which are not solved.

Animal wastes, unlike the other sources of potential nitrates discussed in previous sections, have enough oxidizable carbon along with the nitrogen so that riddance of the nitrogen by managed denitrification is an alternative to letting nitrate accumulate where it is not wanted.

Domestic Sewage

People are not included in the section with animals and fowl because some readers might object, but people are also a concentrated source of N. Because of the high dilution of city sewage, the concentration of effluent from a sewage plant is not the problem that the runoff from a feedlot may be. The usual figure for N in city sewage is about 4.4 kg. of N per year per capita. For a city of 7 million people that amounts to about 34,000 tons of N per year. City sewage plants, depending upon their design and operation, remove part of this nitrogen in sludge and by denitrification. Although all of the organic N is not removed by treatment, most of the N in the effluent is ammonia and nitrate. The proportion between the latter depends on the aeration and holding time in the aeration process (trickling filter or activated

sludge). The ammonia in the effluent can be a significant part of the total BOD released, since the ammonia will be nitrified by the nitrifiers if molecular oxygen is available.

Stratton (1968, 1969) showed that substantial amounts of ammonia can be volatilized from the effluent of secondary sewage treatment plants when that effluent is retained in a eutrophic pond or lake. The combination of high concentration of ammonium ions and high pH resulting from CO₂ assimilation by algae are conducive to ammonia escape.

The transition from the period when we dumped raw sewage into lakes and streams to the present period of requiring at least secondary treatment greatly complicates the interpretation of water analysis for nitrate only in water bodies. Nitrate in the water body can come from agricultural areas, a sewage treatment plant, or from nitrification of sludge previously accumulated in the water body but not oxidized because of the continual pollution with wastes with high BOD.

Nitrate in rural wells can come directly from improperly located cesspools and septic tanks. This is not desirable recycling.

Commercial Fertilizers

No concentrated source of nitrogen has attracted as much attention from the ecologists and environmentalists concerned about the nitrate problem as has the spiraling increase in the use of commercial fertilizers. This is in part probably due to the availability of statistics on fertilizer use and the cases where misuse or overuse of nitrogen fertilizers have created a problem in ground water. Outright bans on N fertilizer use have been suggested and legislation has been introduced in some States to regulate N use. Viets (1970, 1971a, b) discussed this problem in its many ramifications. Suffice to say the controversy over use of commercial fertilizers is far from over. Dodge (1970) claims that the NH₄⁺ containing fire retardant chemicals, often used to fight forest and range fires, contains insufficient N to contribute to NO₃⁻ in runoff water, the amount being much less than is used in a range or pasture fertilization program.

Salt peter has been used as a nitrogen fertilizer since the time of Virgil. Up until the invention and development of the Haber process (1904-08) for fixation of atmospheric N and the first full-scale plant put on stream in 1913, the use of commercial N fertilizers grew at a very slow rate. Agriculture had to depend on guano, the Chilean salt peter deposits, which were mined from 1880, the ammonia from coke ovens, human and animal wastes, and nitrogen fixation by legumes as sources of nitrogen. Indeed, before the invention of the Haber process and some other processes, such as the electric arc and cyanamide, which turned out to be too expensive to use, Malthusian doctrine of 1798 appeared to have come true. World population would be limited by availability of N for plant protein synthesis. Crop yields in Europe had remained stationary for centuries. Yields were comparatively high in the United States because we had a rich supply of N accumulated in grasslands to exploit. But the Haber process and its commercialization into enormous N fixation plants have changed the outlook completely in all countries where N is available, and there is a market for crops in excess of family needs.

In the United States, N consumption on farms has increased from about a third of a million tons in 1930 to almost 7 million tons today. The world consumption in 1965 was about 16 million metric tons. According to "Chemical Information Services" (1969), the world synthetic ammonia capacity in 1969 was 48.7 million metric tons or 14 kg. per capita. Capacity by 1973 will be 69.7 million metric tons or 18 kg. per capita. Of course, only part of this ammonia is used for fertilizer.

The use of commercial N fertilizers is expanding at a rapid rate. For the World, the rate of increase since 1950 has been about 11 percent a year. For the United States from 1940 through 1966, it was 1,290 percent or about 40 percent a year. Ibach (1966) estimated that by 1980 U.S. consumption of N will have to be 12.2 million tons to meet our requirements for agricultural production. This estimate of need compares with the 6.8 million tons we used in 1969.

In 1968, about 90 percent of the commercial N fertilizer used on farms was ammonia, am-

monium salts, and urea, which hydrolyzes quickly to ammonium ions. Although ammonium is retained against leaching by all soils except sands, it is quickly nitrified to nitrate if soil is moist and the temperature above 45° F. Some attention is being given to the inhibition of ammonium nitrification by the use of chemicals such as azide and picolines. Applying ammonia when soils are cold or the using of nitrification inhibitors offers some control of nitrate leaching, but the long-term effect on loss of nitrate from cropland by such practices is unknown. Other developments are coating fertilizer pellets with resins and sulfur to reduce the rates of solution and nitrification.

One of the problems confronting the commercial N fertilizer enthusiast, and the organic N enthusiasts too, in these days of concern about eutrophication and possible nitrate accumulation, is to explain why the recovery of supplemental N by crops is so low. What happens to the rest of it? How can recovery be improved? The same problem is presented in explaining where all of the N went that has been lost from our former grassland and forested soils placed under cultivation.

Commoner (1968) answered the question on fertilizer N this way: ". . . we must look for some of the 6 million tons of nitrogen added to U.S. soils during the last year not in the soil, or in the harvested crops, but in the Nation's lakes and rivers, and in the rain and snow that falls on the land." He estimated that 15 percent of the fertilizer nitrogen is lost by leaching and to surface waters, and that an equal fraction is lost to the air.

Allison (1955, 1965, 1966) made three excellent reviews on the enigma of soil nitrogen balance sheets and the recovery of N from inorganic fertilizers and manures. He stated that N in harvested crops will more often be less than 50 percent instead of greater than 50 percent of the N applied or mineralized from organic matter. Recoveries slightly higher than 80 percent have been achieved in the field on soils with low N and organic matter levels, and no leaching, but such high recovery is rare. Even when leaching losses are prevented or accounted for as in lysimeters, Allison's (1955) summary for 51 uncropped lysimeters and 106 cropped to

nonlegumes showed that 15 percent of the fertilizer N added or mineralized from soil organic matter decomposition could not be found. The presumption is that this N is lost by denitrification, mostly to N₂, but this has not been proved in the field with N¹⁵ and the gaseous products identified. Allison concluded that the main mode of nongaseous loss in the field is through the leaching of nitrate in the spring or fall when there is apt to be a surplus of water, because of low evapotranspiration demands.

Agronomists and soil scientists have been interested in response of crops to various rates of N application with the objective of attaining good yields and good recovery of the fertilizer. They have not, however, had much concern for nitrogen that was lost in runoff or leached below the root zone, except that it was not available for plant use. So in spite of the perhaps hundred thousand experiments that have been conducted, we have little information on how much and how fast nitrate percolates, and whether it reaches the water table. Whether a near maximum yield of any crop can be produced with natural rainfall, or even under carefully controlled irrigation without having some nitrate percolate below the root zone, is an unanswered question. Although a plant will extract all of the NO₃⁻ from a culture solution or a soil if the plant is starved for N, can roots remove all of the NO₃⁻ from a porous media such as soil and still have sufficient N for its maximum growth?

There are many examples of overuse of N fertilizers under conditions of slope, excessive rainfall, or method of application that are not recommended. These examples often give fertilizer use a bad name and are sometimes generalized to be the condition that exists in agriculture. The reports of Moe et al. (1967, 1968) showed what can happen. They applied 200 pounds of N per acre as pelletized ammonium nitrate to fescue sod plots (clipped at 2 inches) and to bare fallow plots on Zanesville silt loam on a 13-percent slope in Ohio. The soil had a fragipan at 24 to 36 inches. They then applied 5 inches of artificial rain in 24 hours. Fallow plots with a surface crust lost 8.2 percent of the applied N in runoff. Fallow plots plowed in the spring lost only 3.1 percent of the N. Loss of or-

ganic N by erosion exceeded the loss of fertilizer N. On sod plots, loss of fertilizer N depended on the initial soil moisture content, but was 14.0 percent when the soil moisture was high (25.8 percent).

On the same plots, Moe et al. (1968) applied 400 pounds of N per acre as pelletized urea or ammonium nitrate. The fescue had been clipped at 3 to 4 inches. The second year fallow plots had been plowed in mid-May and disked twice in mid-July before the fertilizers were applied. Two and one-half inches of artificial rain were then applied. Total loss of fertilizer N from the plots ranged from 2.4 to 12 percent of the N applied. Urea was found in the runoff only from the sod plots. These losses of N are, of course, economically significant to the farmer who would apply N in this way under these conditions. Viets (1970a) pointed out that even very small losses of fertilizer N that would be of no economic significance to a farmer can contribute sufficient N to surface waters to give them concentrations frequently associated with eutrophy.

There are many examples of overuse of nitrogen fertilizers at rates that appear to be far in excess of crop needs. Ward (1970) cited a report of the California Department of Water Resources stating that the average annual application of N for strawberries was 145 pounds per acre, and for celery was 290 pounds per acre, in the Arroya-Grande—Grover City area. This area has high concentration of nitrate in the shallow water table. In some areas in California, so much residual nitrogen is left in the soil after heavy fertilization of vegetable crops that sugarbeets with high sugar content and purity cannot be produced. Bingham et al. (1971) reported that the average nitrogen fertilizer rate on a 960-acre watershed in citrus in Southern California was 130 pounds per acre per year. Nitrate in the percolate was equal to about 45 percent of the nitrogen applied.

The best estimates of how much nitrogen is used on various crops and in various subregions of the United States are the estimates of Ibach and Adams (1967) for 1964. Some examples of high average use of nitrogen fertilizers by

States expressed in pounds of N per acre are: fruits and nuts in Arizona—149, vegetables in California—136, potatoes in California—174, potatoes in Delaware—130, fruits and nuts in Florida—142, and potatoes in North Carolina—154. Since practically all growers in these States used nitrogen fertilizers, and the rates of application quoted are averages, some growers must use very high amounts of N in relation to those who use only minimal amounts. Practically no information is published on how the rate of use is distributed around the average. To put the problem another way: In a given region is 25 percent of the fertilizer N put on 10 percent of the acreage in a given crop?

The potential for pollution of ground water with high rates of nitrogen fertilizer is greater than for low rates, but it is not necessarily true that the higher rate will cause nitrate leakage into the aquifer. The kind of crop, soil, amount and distribution of rain, and many other management variables are important. Two hundred pounds of fertilizer N per acre in one circumstance may not be as hazardous as 50 in another. Therefore, statistics on fertilizer use must be interpreted with understanding.

Although a nonfertilizer use in the conventional sense, another interesting but insignificant use of nitrate salts has been their addition to water as an oxidant to decrease the biological oxygen demand (BOD) of polluted water. Sodium nitrate has been added to sewage effluent being infiltrated into sand dunes in Holland to accomplish more rapid oxidation of pollutants. Nitrate is denitrified to yield oxygen to the denitrifying bacteria while they are digesting the organic pollutants for energy.

The following are interesting remarks made by Sawyer (1970) at the Twelfth Sanitary Engineering Conference at the University of Illinois:

Now I don't want to be criticized after I'm through telling you about these things, because in the light of the gospel that is being preached today this is strict heresy. But when you get into a hole, a real hole, with regard to an odor problem, and the paint is turning black on the buildings, and

the people are up in the arms, you're going to do something about it and you turn to nitrates. This is the quickest way. Today, why sure you'd use a portable aerator and you'd do a lot of things, but in years gone by you didn't have as many facilities, and nitrates were turned to because nitrates, next to dissolved oxygen are the easiest source for organisms to utilize oxygen. As long as nitrates are present you never have an anaerobic condition, but you do have anoxic conditions. . . Now for specific examples: nitrates were used for many, many years on the Androscoggin River in Maine, because there were sulfite pulp mills on the headwaters that couldn't see their way clear to removing the B.O.D. that was necessary. Through court action they set a precedent on the Androscoggin. Dr. Lawrence of Bates College and his crews sample the water routinely, and when the dissolved oxygen would go down to a certain level at a certain point, nitrates would be added to the Androscoggin River . . .

Nonconcentrated Sources

There are many sources that contribute N to the environment and are important in consideration of input-output balances, the behaviour of ecosystems, and enter significantly into crop and water management practices, but in themselves may not be sources of hazardous concentrations of nitrate. Nitrogen content of precipitation has interested many for a long time.

Feth (1966) reviewed the recent literature for the United States and northern Europe. There are some indications that the amounts may be increasing due to air pollution with ammonia and the nitrogen oxides from high temperature combustion. There may be direct absorption of NH_3 from the air by plants, soil, and water (Hutchinson and Viets, 1969) that is not included in precipitation figures. Biggar and Cory (1969) stated: "Nitrogen derived from rainfall may range from 1 to 19 pounds per acre per year at various locations, averaging 7 or 8 pounds. This is not enough to produce marked increases in crop yield, but in runoff or

percolative waters, it becomes a steady and significant supply."

Campbell and Webber (1969) stated that precipitation contributes more N to 2,100-acre Canal Lake in Ontario, Canada, than does the 30,000 acres of unfertilized pasture land. They estimated the total stream input to be 4,845 pounds of NO_3^- -N, of which 480 pounds was from nonagricultural sources (summer cottages). The agricultural land contributed 0.15 pound per acre. Feth (1966) said: "Data on the nitrogen load added to surface water and ground water by precipitation are hard to evaluate. Precipitation might be the most important single source, for surface water at least. Inevitably, then it must be an important source of nitrogen in ground water also."

Nonsymbiotic bacteria fix nitrogen from the air, but the magnitude of fixation is generally small and low per unit of land area. In table 8, nonsymbiotic fixation was estimated at 1 million tons annually. The blue-green algae contribute significant amounts of N to rice paddies and sometimes to lakes. The symbiotic *Rhizobia* in legume root nodules and root organisms on many nonleguminous species fix large amounts of N when grown under favorable circumstances. Alfalfa can fix as much as 600 pounds of N per acre per year, but most of the N is removed in the hay. Note in table 8 that the estimates of N fixed by legumes were 1.7 million tons in 1930 and 1947, and 2.0 million tons in 1969.

Stout and Bureau (1967) suggested that nodule legumes upon decomposition can contribute nitrate to percolating water. However, Stewart et al. (1967a, b) found low concentrations of nitrate under alfalfa fields to a depth of at least 20 feet. The nitrate concentrations were about the same as those under virgin grassland. Alfalfa either absorbed the nitrate that accumulated in the deep profile, or possibly denitrified it at the root surface. Legume nodules are not efficient in fixing atmospheric N_2 if they have fixed nitrogen available. Hence, legumes are probably not an effective factor in contributing nitrate to percolating water, unless large amounts of leguminous residues are available for decomposition.

NITRATE TRANSPORT AND ACCUMULATION IN RELATION TO HYDROLOGY AND WATER MOVEMENT

Water is the solvent, the transporter, and a storage reservoir for nitrate. Microbial mineralization of soil organic nitrogen and organic wastes requires water at least in amounts sufficient to exceed the approximate wilting point of the soil. Nitrate diffusion occurs very slowly in soils or geological materials without appreciable water movement, so that significant nitrate transfer has to be in moving water. Since nitrate is not adsorbed or negatively adsorbed by soil particles, water in soil is a storage media for nitrate just as in a lake. Hence, an understanding of the physics of soil water and its movement, of hydrology, and of climatology is essential for a thorough understanding of nitrate. All we can do in this review is to point out some of the more important aspects. Some aspects of nitrate pollution in relation to hydrology have been reviewed by Goldberg (1970), Le Grand (1970), and Feth (1966).

In the Root Zone

We use the term "root zone" in a general sense since its thickness depends on the kind of plant, soil, and climate. The Monograph "Soil Nitrogen" edited by Bartholomew and Clark (1965) covered the behavior of nitrogen and nitrate in the root zone in detail. It contains a chapter by Gardner (1965) giving a mathematical treatment of nitrate movement in the soil profile. Biggar and Cory (1969) discussed the roles of diffusion and tortuosity of the migration path in porous media like soil in spreading out the advancing front resulting from a pulse of nitrate solution followed by water lacking nitrate. The further the advancing front of nitrate moves, the more diffuse or spread out it becomes. Nitrate may move faster, slower, or at the same rate as the average total water in the soil. A general rule, applicable to soluble salts too, is the depth of maximum nitrate concentration resulting from nitrate application at the surface will advance about 1 foot for each foot of water infiltrated.

If the direction of water movement is upward

resulting from absence of rain and high evaporation, then nitrate can accumulate at the soil surface as a crust along with other salts as an evaporite. As noted previously, this is probably the explanation of the playa accumulations. Malpeaux and Lefort (1912) found that sodium nitrate placed at a 40-inch depth in soil reached the surface 6 inches of soil in less than 6 weeks. This work was of significance when W. P. Headden in Colorado was trying to explain the nitre spots as accumulations resulting from nonsymbiotic N fixation by *Azotobacter*. Accumulations of nitrate in the soil surface in well-drained soils in the dry season has been measured in Uganda (Stephens, 1962), in Kenya (Robinson and Gagoka, 1962), and in northern Australia (Wetselaar, 1961), to mention only a few reports.

Lateral movement and accumulation of nitrate in ridges of crops grown under irrigation are of great significance to agriculture. Perhaps the first instance noted of such accumulation was by McBeth (1917), who reported accumulations of nitrate in nitre spots that contained as much as 1 percent N as nitrate between furrows in California citrus groves.

Harding (1954) reported that as much as 3 tons of salts containing 800 pounds of nitrate N per acre collected in the interfurrow ridges of irrigated oranges in southern California. Nielson and Banks (1960) pointed out that nitrate accumulation in ridges (where the plant grows) between irrigation furrows may be a problem in getting good N recovery from N fertilizer. Stout (1961) indicated that such nitrate accumulation in ridges in the intermountain area of the United States can be very detrimental to sugarbeet quality if fall rains wash this nitrate into the root zone and the beets resume growth. Regulation of nitrate nutrition of sugarbeets is difficult in such situations. Nelson (1953) studied nitrogen placement in relation to the position of the irrigation furrow and found that side placed fertilizer N must be placed below the water level in the furrow to

avoid nitrate concentration in the ridge and immobilization there by evaporation.

Two conditions are essential for nitrate to move below the root zone—there must be nitrate present and downward water movement. Except in regions with excessive precipitation or irrigation requiring tile drainage, there is seldom any direct estimate of the amount of water percolating below the root zone. In arid and semiarid regions, the estimates of ground water recharge are at best only estimates not based on direct measurements. Therefore, the alternative approach of measuring precipitation and subtracting estimated evapotranspiration and runoff to get the deep percolation by difference must be used.

Nelson and Uhland (1955) were perhaps the first to use this approach in relation to possible nitrate loss from the root zone. They constructed a map of the eastern half of the United States showing the hazard in four zones of applying fertilizers in the fall. The parameters were fall and winter precipitation and time when an average minimum air temperature of 40° F. was reached, the latter being significant since the nitrification of injected NH_3 and ammonium salts depends on temperature.

Allison (1965) stressed the importance of curves of precipitation and evapotranspiration plotted on a monthly basis and the soil storage capacity for water in evaluating the potential for nitrate leaching. Presumably, runoff would also have to be considered. Allison stated: "Probably the first fact that needs to be emphasized is that leaching of available nitrogen beyond the plant root zone usually does not occur to any marked extent in cultivated, medium-textured humid soils in the United States during the main growing season, unless the annual rainfall is above about 50 inches. Most of the leaching occurs in the late fall, in the winter if the soil is not frozen, and in the early spring."

In using the precipitation-evapotranspiration concept, it is important to have current data rather than long-term averages. The latter may be of value for broad generalizations spanning both area and time, but there is no way to explain the nitrate accumulation below the root zone in wheat-fallow systems observed in north-eastern Colorado by Stewart et al. (1967a, b),

where the average rainfall is only 14 to 17 inches and the root zone seldom becomes filled with water. It is possible to explain the downward movement by a sequence of several years with precipitation above normal. These do occur. A series of wet years could result in a "pulse" of nitrate escaping below the root zone.

One further point that needs consideration is the depth of nitrate in the root zone. The nitrate in the upper part of the soil is generally more available for plant root absorption than that which is deeper, simply because the root system of most plants has decreasing density with increasing depth. Gass (1969)³ placed K^{15}NO_3 at depths from 0.3 to 6 feet in an eastern Nebraska silty clay loam in plots, each of which had accumulated 364, 417, and 520 pounds of residual soluble nitrogen per acre in 6 feet of profile resulting from differential N fertilization the preceding 3 years. Corn absorbed N^{15} at progressively deeper placement depths with continued growth, but more N^{15} was present in the mature stover from the 0.3-foot placement than from deeper ones. More isotope in the grain, however, was absorbed from the 2- and 4-foot depths. Recovery of N^{15} from the 6-foot placement decreased with increase in the amount of residual nitrogen.

As noted in another section, alfalfa has the apparent capacity to remove nitrate from the profile to at least a depth of 20 feet in alluvial soils and so is a promising crop for "decontaminating" soil profiles of their nitrate.

Nitrate distribution in the root zone, particularly when a growing crop or furrow irrigation is involved, has shown so much heterogeneity that researchers interested in studying its uptake by plants or movement below the root zone have been completely frustrated by soil sampling procedures.

In irrigation projects and in irrigated fields, the soluble salts applied in the irrigation water must be balanced over a period of time by the salt draining from the root zone. The leaching requirement can be calculated and water in excess of evapotranspiration applied to remove

³ Gass, W. B. Recovery of residual nitrogen by corn plants from various soil depths as measured by ^{15}N tracer techniques. Thesis, Univ. Nebr., Lincoln, Nebr. 1969.

salts from the profile. In most situations when adequate water is available, excess water is applied in most irrigations during the season because of the operational difficulties of matching amount of irrigation to the amount of water needed to bring the root zone back to field capacity. Obviously, the best time to accomplish leaching is late in the season after the crop has removed most of the nitrate from the root zone.

Research agronomists and soil scientists are now giving increasing attention to the effect of fertilization with commercial fertilizers, animal wastes, sewage sludge, and effluents on nitrogen recovery by crops, and to subsequent availability and distribution of the nitrogen unused by the crop. A review of all the literature, most of which cannot be related directly to the question of nitrate accumulation in ground waters because of lack of direct measurement of nitrate in percolate below the root zone, is beyond the scope of this paper. However, such a review needs to be made.

Although an unsurfaced cattle feedlot or a dairy cow loafing pen does not have a root zone, it can soon have one if the livestock are removed. Weeds grow promptly and crops usually can be grown without difficulty. The soil under an animal enclosure has many forces operating that are at least more intense, if not different, than in a cropped field. All of the forces and the results are not understood, but this is an active area of research.

Viets (1971c) indicated some of the effects on nitrogen transformations derived from field and laboratory studies. In a cattle feedyard stocked at high density and continuously, the ammonia coming from hydrolysis of urea in urine makes the surface extremely alkaline so that the ammonia is volatilized rather than converted to nitrate by the nitrifiers. In lower density yards where there may be some nitrification, the nitrate may be leached to a lower depth along with soluble carbon compounds where the combination of high BOD along with high water content results in denitrification. In a feedyard that is operated only part of the year, opportunities for nitrification and leaching of the nitrate toward the water table are undoubtedly much greater than in a yard used

continuously. These differences in possible behavior of the N wastes under different conditions of management are undoubtedly the explanation for the results of Stewart et al. (1967a, b), who found that the NO_3^- -N in 20 feet of profile varied from none to over 5,000 pounds per acre under cattle feedyards in northeastern Colorado. Each profile had different distribution of nitrate with depth. These studies suggested the possibility of choosing sites for feedlots and operating them so that there will be a zone of nitrification near the surface and an anaerobic zone below where the nitrate is denitrified. Studies of a feedlot with the desired site conditions are now underway at Central City, Nebr., by the U.S. Department of Agriculture in cooperation with the Nebraska Agricultural Experiment Station and the Water Quality Office of the Environmental Protection Agency (Mielke et al., 1970).

In the Zone Below Roots and Above the Aquifer

The presumption is that nitrate below the root zone and above the water table is stable, there being an insufficient supply of oxidizable carbon for the denitrifiers. There is no significant source of ammonium for the nitrifiers, the ammonia being adsorbed and held against leaching by the clays and humus of the root zone. F. E. Clark found low populations of bacteria in this zone compared with surface soil, but there was some increase in population in the capillary fringe above the water table under irrigated fields and cattle feedlots (Stewart et al., 1967a).

One peculiarity observed by investigators of nitrate concentrations in cores is the marked variation with depth (Stout and Bureau, 1967; Stewart et al., 1967a). Nitrate expressed on the basis of dry weight of soil is expected to vary widely because the soil texture and water content may vary widely; for example, a layer of clay impedes water movement into a lower layer of loamy sand. However, large variations having a threefold or fourfold range are still apparent when the nitrate is expressed on the basis of the water in the strata as it existed in the field. The variations are too large to be caused by

negative adsorption of nitrate. Biggar and Corey (1969) discussed these variations. The best explanation may be that they are caused by "pulses" of solution of high nitrate concentration coming from the surface which are not obliterated by diffusion as water moves down through the profile. The possibility that denitrification is occurring in these zones of low concentration has not been excluded, however. At present, the presumption that nitrate below the root zone will eventually reach the water table appears to be correct, but it cannot be accepted as proven.

In Aquifers

Biggar and Corey (1969) discussed the mixing, stratification, and differences in flow velocities in aquifers in relation to nitrate accumulation and lateral movement. Stratification and incomplete mixing are common in ground water bodies. Rates of lateral movement may vary from 1 foot a year to several miles per month. The size and rates of turnover of the water in the basin or aquifer must also be considered. Carlson (1964) found that the mean residence time of recharged ground water in a Wisconsin drainage basin as 45 days and in a New Jersey basin as 30 days.

Contrast this humid region situation with one existing in the semiarid region of the Great Plains using the estimates cited by Duke (1967) for the Ogallala aquifer under Colorado's northern high plains before irrigation wells were drilled. Duke estimated that this aquifer contained 80 million acre-feet, the recharge rate is 430,000 acre-feet per year, and that 390,000 acre-feet underflow eastward into Kansas and Nebraska at the rate of about 2 miles per year. By dividing the aquifer storage (80 million acre-feet) by the recharge rate (430,000 acre-feet per annum), one gets a mean residence time of 186 years.

The Ogallala contains very "old" water. Obviously, on a time-scale nitrate pollution and depollution of the New Jersey basin can occur 2,265 times as fast (186 years/30 days) as the Ogallala formation. Admittedly, application of simple dilution chemistry to an aquifer is extremely simplistic, but it does illustrate the im-

portance of considering the hydrology of the region. We should take particular note of Carlson's (1964) statement that the water in 90 to 95 percent of United States' available ground water reserves is probably over 50 years old, which makes their tritium content useless for dating them.

Some concept of the slow movement in the Ogallala may be gained from the experiments of Scalf et al. (1967) near Amarillo, Tex., who injected water containing DDT, tritium, and nitrate into a recharge well for 10 days and then pumped the well, after 3 hours of equilibration, for another 12 days to recover the tracers. Water containing an average of 5.435 p.p.m. of NO_3^- -N was injected at a rate of 348 g.p.m. The injected nitrate came from NaNO_3 added to the recharge water that contained initially 1.72 p.p.m. of NO_3^- -N. Water from the supply well contained 72 pounds of NO_3^- -N and that from NaNO_3 was 156 pounds, for a total of 228 pounds. After 12 days of pumping at 504 g.p.m., all of the tritium and 94 percent of the nitrate were recovered, but most of the DDT remained in the aquifer. In accounting for the lower recovery of NO_3^- than of tritium, Scalf and co-workers stated that no denitrification occurred. They attributed the lower recovery to difficulties in estimating the nitrate concentration in the aquifer initially but do indicate the possibility that nitrate diffused further than the tritium. Such a possibility is questionable. They stated that nitrate pollution of the aquifer by nitrate is of more concern than DDT pollution because of the ability of nitrate to move through the aquifer. However, their own data indicate that such movement must be very slow.

Nitrate composition of well waters fluctuates seasonally and often erratically. Even adjacent wells will differ when sampled at the same time because of the differences in depth and position on the aquifer. Feth (1966) reviewed some of the data on fluctuations in well water composition. These fluctuations make it difficult to determine if trends in composition are occurring unless a near continuous record is kept of composition and flow.

Very little is known about the stability of nitrate in ground water. The fluctuations in nitrate content of well water discussed by Feth

(1966) are probably mostly dilution and concentration phenomena related to the source, either from infiltration from above or lateral displacement. Stratification also occurs, and so changing composition of well flow can be related to depth of drawdown. However, reduced water tables do occur containing Fe^{++} , SO_3^- , H_2S , NH_3 and even methane. Under such conditions, any infiltrating nitrate should be reduced, but Stewart et al. (1967a) found nitrate in water samples with redox potentials of -300 mv. Samples from cores of soil or earth had insignificant nitrate when the E_h was below $+300$ mv.

Ammonia occurs in high concentrations in spring waters from Lake and Colusa Counties

in California (Feth, 1966). Its source is unknown. Undoubtedly, some reduction of nitrate occurs when septic tank or percolate from feedlots reaches the water table. F. E. Clark (*in* Stewart et al., 1967a) obtained much higher counts of total bacteria in the capillary fringe above the water table than in the soil above it, both under feedlots and irrigated fields. Since from 0.9 to 1.3 g. of biodegradable carbon is needed to denitrify a gram of NO_3^- -N, it appears unlikely that much microbial denitrification can occur in the water table. The safest assumption then is that once nitrate reaches the water table it will remain until flushed through or pumped out.

RECENT FIELD INVESTIGATIONS ON NITRATE ACCUMULATION AND ITS SOURCES

In Arid and Semiarid Regions

In California, Johnston et al. (1965) in a study of the N inputs in fertilizer and irrigation water, and losses in the tile effluent and runoff from four fields in the San Joaquin Valley, found that the losses as percentages of inputs varied from 9 percent for cotton to 70 percent for a field 70 percent in cotton and 30 percent in rice. Application rates of fertilizer were 195 pounds per acre for cotton and 66 for rice. The nitrate in the irrigation water applied ranged from 4.8 (cotton) to 31 (rice) pounds of N per acre for the four tiled fields. The nitrate-N concentrations in the tile effluent varied from 1.8 to 62.4 p.p.m. The mean concentration was 25.1 p.p.m. No unfertilized controls were included, and so the amount of nitrate lost assignable to fertilizer application cannot be determined. The drainage from a recently installed line under a field that had never been farmed or irrigated contained 1 p.p.m. of nitrate N. The tile effluent from a field in first-year alfalfa, planted on land that had not been N fertilized for 2 years, ranged from 2.0 to 14.3 p.p.m. Peak discharge of nitrate N was 4.7 pounds per day for 90 acres in alfalfa. Although the alfalfa field received 1,717 pounds of N as

nitrate in the irrigation water, only 282 pounds of nitrate N appeared in the drainage.

The nitrate-N losses in runoff from a natural "1,000-acre lysimeter," located on granitic soils over granite or gabbro near Riverside, Calif., were investigated by Davis et al. (1969) and compared with the 135 pounds of N as urea per acre applied annually in split applications to the citrus grove. The losses in 1966 and 1967, respectively, were 57 and 72 pounds per acre, amounting to 42 and 53 percent of the N applied. Nitrate-N concentrations of the drainage were generally above 10 p.p.m. Both the amount of N and irrigation applied were excessive according to Davis and coworkers. The experiment lacked an unfertilized control. In a subsequent paper, Bingham et al. (1971) gave additional data for 1968 and 1969. Nitrogen losses in runoff and percolate for 1967 were 45.6 pounds per acre, for 1968—55.1 pounds, and for 1969—69.6 pounds. They stated that nitrogen in drainage averages about 45 percent of the nitrogen applied. Although the irrigation water is relatively saline, significant salinization of the soil is not developing because 40 to 50 percent of the rain and irrigation water percolates. Average NO_3^- concentration of percolate for any

2-month period in the 3 years ranged from 37 to 81 p.p.m.

Doneen (1966) measured the nitrate in the tile effluents from three irrigated fields in the San Joaquin Valley and compared the results with the nitrate in the saturation extracts of two adjacent virgin land sites that had never been irrigated, and from which no drain effluent was available. One field had grown cotton, rice, barley, and lettuce in the 1962-66 period. The average annual application of N fertilizer was 167 pounds per acre. The irrigation water contained 1.7 p.p.m. of nitrate N. Water applied ranged from 7.4 to 12.1 feet per year. The nitrate N in the tile effluent averaged 44.5 p.p.m., which was two to four times the concentration of the saturation extract of the profile samples. Another field had been cropped to tomatoes, cotton, wheat, safflower, barley, and lettuce. Annual applications of fertilizer N had been about 100 pounds per acre. Water applications ranged from 2.5 to 6.1 feet per year and contained 2.1 p.p.m. of nitrate-N. The average nitrate-N concentration of the drain water was 23 p.p.m.

Another field cropped to safflower and cotton received fertilizer N applications of 75 to 131 pounds per acre per year. The average nitrate-N concentration of the drainage was 37 p.p.m. On the basis of the saturation extracts and ground water composition, one of the virgin sites contained more nitrate and one contained less than that of the cropped, fertilized, irrigated sites. The data illustrate the problem of assigning source among fertilizer, irrigation water, organic matter decomposition, lateral movement, and earlier nitrate accumulations in evaluating a nitrate problem.

Nightingale (1970) made a statistical study of 18 years' records of the nitrate composition of the Fresno-Clovis urban area well water and the surrounding irrigated, highly N-fertilized, agricultural area. The urban area now contains two sewage treatment facilities. The data were broken into three 6-year periods. Well waters were a third more saline under the agricultural area than under the urban area. The mean nitrate-N content of the well water for the 1950-67 period for the agricultural area was 2.29 and 3.20 p.p.m., or 40 percent higher for the urban zone. In the urban zone, the

nitrate-N increased from 2.31 p.p.m. for the first 6-year period to 3.64 p.p.m. for the last. Under the agricultural zone, the nitrate-N increased from 1.22 to 3.51 p.p.m. For the last 6 years nitrate increased faster under the agricultural area than under the urban area. The declining rate of nitrate increase under the urban area in the last 6 years was attributed to decreasing use of septic tanks and more connections to sanitary sewers.

Behnke and Haskell (1968) showed that the ground water contained from 5.5 to almost 8 p.p.m. of nitrate-N down gradient from the Clovis sewage plant and leaching ponds. Similar values were noted in areas of septic tank use.

Stout and Burau (1967) conducted one of the best studies to date in trying to understand the N balance of a water basin in relation to nitrate accumulation in underground water in the Grover City-Arroya Grande Basin near San Luis Obispo, Calif. They quote State of California estimates that 450,000 pounds of N are imported and 150,000 pounds are exported in farm products annually from 2,700 acres used for crop production. The net agricultural N remaining in the Basin is 74 pounds per acre per year.

On the assumption of 2.5 feet of percolate annually from over-irrigation, Stout and Burau (1967) calculated that the nitrate-N in the leachate would be increased 10.9 p.p.m. each year. They calculated that the protein intake by the 13,500 inhabitants would add 16.4 p.p.m. of N to the water pumped for domestic use, ignoring evaporation and the input of N from lawn fertilization. They could not make estimates of other N inputs such as N-fixation by legumes and denitrification losses from the system. Cores taken under different kinds of land use were analyzed for nitrate. In an irrigated strawberry field, located on a sandy soil and irrigated with water containing 26.6 p.p.m. of nitrate-N, the water in the profile from 4 to 39 feet had an average concentration of 21 p.p.m. Water in the saturated zone below 36 feet contained 16.6 p.p.m. Water percolating downward had only 79 percent the nitrate concentration of the irrigation water. The percolating water contained 90 percent of the N in the irrigation water and fertilizer applied less that removed in the crop. Under a heavily fertilized crop of

celery located on a clay soil, they found nitrate concentrations varying from 14 to 122 p.p.m. in the soil water from depths of 10 to 43 feet. Since these depths were sealed off from surface percolation by an impermeable clay layer, they concluded that the nitrate deep in the profile was from pre-irrigation sources. For a permanent pasture they found an average soil solution concentration of 2.78 p.p.m. and estimated that only 4.4 pounds of nitrate N per acre is leached out of this pasture annually.

In sewage effluent lagoons used for ground water recharge and operated on an intermittent drying and filling basis, Stout and Burau (1967) found the nitrate concentrations in the profile were high in some layers and low in others. They attributed this stratification to "slugs" of nitrate coming from the ponds. When the ponds were dried, nitrification of the sludge produced high concentrations of nitrate that was leached when the ponds were filled. After this slug of nitrate was leached, the low nitrate water of the effluent moved down. Stout and Burau emphasized that any fertile soil will produce nitrate for leaching, and that this release of nitrate is impossible to separate from that coming from fertilizers and decomposition of crop and legume residues. For a basin with high nitrate waters at shallow depths, being continually recycled by pumping, they recommend drawing water from deep wells uncontaminated by nitrate for domestic use, and recycling of the shallow nitrate-laden water for agricultural use.

Dyer (1965) showed graphs of nitrate concentration with soil depth for four nonirrigated and four adjacent sprinkler-irrigated profiles of the Panoche series in the San Joaquin Valley. The unirrigated sites had from 1,400 to 2,800 p.p.m. of nitrate N in the soil water to the depth of drilling, 50 feet on three sites, and 14 on the other. Soil material in these sites was dry throughout the profile. Irrigation and cropping had removed most of the nitrate from the upper 20 feet of the irrigated profiles, but accumulations were noted at depths of 20 to 30 feet. The NO_3^- concentration-depth curves in these zones of accumulation were sigmoidal in shape, characteristic of a wetting front. Nitrate concentrations as high as 4,200 p.p.m. of soil water in

these zones of accumulation were noted. Since soil water data are not given and these irrigated fields had been in alfalfa about half the time since inception of irrigation, no further analysis is possible. Stewart et al. (1967b) found little nitrate under an alfalfa crop to a depth of at least 20 feet and concluded that alfalfa has the ability to remove nitrate from the profile to an appreciable depth.

Meek et al. (1969) showed that in the Imperial Valley of California only 1.5 percent of the 250 pounds of N per acre applied to cotton on a silty clay loam reached the drainage tile as nitrate. They attributed the loss to denitrification in the poorly drained soil above the tile, and showed that the redox potential dropped below 300 mv. below 152 cm. of depth. The tile lines were 183 and 244 cm. in the two parts of the field. Tile lines in the Imperial Valley often become clogged with MnO_2 and Fe precipitates, indicating that there is a zone of intense reduction in the water above the tile lines.

In the Coachella Valley of California, Bower et al. (1969) found little nitrate in the drainage. Lack of nitrate in the drainage may be related to limited use of water for leaching of salts. On a chemical equivalent basis, nitrate amounted to about 0.20 percent of the salts in the irrigation water from the All American Canal. In the valley main drain, nitrates constituted about 0.77 percent of the soluble salts.

For the San Joaquin Valley of California, Glandon and Beck (1969) reported the results of an investigation on tile drain flows and the nitrate concentrations in them. In the 42 tile systems monitored, the annual discharge ranged from 0.3 to 17.0 acre-feet per acre per year. The average nitrate N concentrations varied from 2 to 400 p.p.m. Flows were highest during April through September and decreased when irrigation was stopped. Nitrate concentrations in tile drainage from several rice fields declined rapidly during flooding and increased when the fields were drained. In the northern part of the valley where an average of 42 pounds of N fertilizer had been used per acre per year during the 1957-67 period, the tiles delivered 35 pounds of nitrate N per acre per year for the period 1966-67, inclusive, and the average concentration was 9 p.p.m. Corresponding

numbers for the central San Joaquin Valley were 88 and 83 pounds per acre and 33 p.p.m., and for the southern part of the valley the values were 74, 2, and 9. They reported that many of the soils contained residual (geologic) nitrate, and that one experimental tile system located in a basin with an accumulation of salt delivered water ranging from 370 to 2,000 p.p.m. of nitrate N. No conclusions about the impact of fertilizer use on nitrate in drainage can be drawn from these studies. However, the data do show that the San Joaquin Valley had a vast accumulation of nitrate in the subsoils before the beginning of agriculture.

In his summary of the California situation, Ward (1969) pointed out that there were five areas in California where nitrates in ground waters frequently exceed the standards. For the Verdugo Basin, he stated the California Department of Public Health had concluded that the major contributions to the high nitrates in ground water being pumped for domestic purposes were from domestic sewage discharge from individual household systems. In the Arroya Grande-Grover City Basin, high nitrates were found in wells 70 feet or less in depth or in deeper wells having gravel packing, extending to shallow formations; or deep wells having some of their perforations at shallow depths. For the Delano area, Ward summarized: "Nitrates were found to have highest concentration in areas where the ground water has risen markedly during recent years, where soils of high permeability require greater use of irrigation water, or where irrigation has been practiced for longer periods of time . . . domestic sewage was excluded as being a major source of nitrate. . ."

The problem of high nitrate in the drainage waters of the San Joaquin Valley induced two Federal agencies and the California Department of Water Resources to collaborate on a research program on their sources and methods of removal so that nitrates would not contribute to the eutrophication of San Francisco Bay. Some of the recent developments are reported in "Collected Papers Regarding Nitrates in Agricultural Waste Water" (noted in the literature citations to Price, 1969; and Glendon and Beck, 1969).

In the Hawaiian Islands, Mink (1962) found that the average NO_3^- -N concentration of wells in the nonirrigated sections of southern Oahu was 1.0 ± 0.22 p.p.m., but in the irrigated regions was 8.2 ± 2.4 p.p.m. He attributed the difference to use of commercial fertilizers.

In Idaho, Carter et al. (1971) made a balance study of the N and P entering and leaving a 202,702-acre tract near Twin Falls, irrigated from the Snake River, for the water year beginning October 1, 1968. They estimated that 36 percent of the total input of water from irrigation, precipitation, and runoff onto the tract was lost by evapotranspiration, 14 percent was lost by surface runoff, and 50 percent was lost by subsurface drainage into drainage tunnels carved out of the underlying basalt and into special drainage wells. Little opportunity existed for contamination of the drains by sewage or nitrate use by photosynthetic organisms. Two hundred and ten tons of NO_3^- -N entered the tract from irrigation water, 32 tons left it in surface drainage, and 3,194 tons were in the subsurface drainage. The net output of nitrate-N was 3,016 tons, or 30 pounds per acre. Average NO_3^- -N in the irrigation water was 0.12 p.p.m. and in the subsurface drainage water was 3.24 p.p.m. Carter and coworkers estimated that the N fertilizer use for the entire area was 53 pounds per acre per year. Salt loss from the tract was high because of excessive rate of irrigation. Nitrate loss was also high, but the relative contributions of various sources are unknown.

In Washington State, Sylvester and Seabloom (1963) reported on the nitrate balance of the Yakima River Basin for 1959 and 1960. They found that the nitrate-N in the Yakima River water become concentrated from 0.25 p.p.m. at the point of diversion to 2.5 p.p.m. in the subsurface agricultural drains and to 0.8 p.p.m. in the surface drains. On a per-acre basis of agricultural land, they give the nitrate-N inflow as 4.2 pounds and the total outflow as 19.3. They reported that nitrate concentration in the open surface drains was reduced two-thirds during the growing season by surface inflow and aquatic vegetation.

Near Spokane, Wash., Crosby et al. (1971) reported that profiles under dairy cow loafing

areas with high cow density ranged in nitrate content from 10 to 85 p.p.m. of dry soil (2 to 16 p.p.m. NO_3^- -N) from the 8-foot depth to 50 or 70 feet for the two cores taken. A field control area was very low in nitrate. Since the cores taken under the loafing areas were very dry, Crosby and coworkers postulated that the profiles were now sealed against water infiltration and that the nitrate must have leached down soon after the loafing pens were established. The association of the current dry profile in which little water is moving and the low relative nitrate content indicate no hazard to water quality. A danger could arise when the land is returned to crop production, but the effects would be very localized from the lot little more than one-fourth acre in size.

In Colorado, Stewart et al. (1967*a, b*) core-drilled 129 sites from the surface to the water table, or to consolidated rock, in the South Platte Valley and analyzed the cores for nitrate. The five kinds of land use ranged from virgin grassland to cattle feedlots, some of which had been used for over 50 years. Average amounts of nitrate in the top 20 feet of profile and the nitrate in the water table that entered the drill hole are shown in table 11. The nitrate in the grassland and alfalfa land profiles was low and was uniformly distributed with depth. The land in alfalfa previously had grown fertilized crops as part of crop rotation used in the area. Alfalfa removes nitrate from the profile to its depth of rooting by some mechanism, possibly simple absorption. The dryland fields usually grow unfertilized wheat after fallow in a 2-year sequence to store moisture. These profiles were much higher in nitrate and showed definite accumulations at depths of 8 to 12 feet in most profiles. The irrigated land, which was mostly in corn and sugarbeets, had larger accumulations of nitrate but it was more diffuse in the profile. On a soil-water basis, NO_3^- -N usually ranged from 20 to 40 p.p.m. For 1964, the estimated fertilizer N use for this irrigated area was 83 pounds per acre for corn, 100 for sugarbeets, 60 for potatoes, and 40 each for wheat and barley. These rates of fertilization are regarded as essential for good production. Cattle feedlots showed highly variable amounts of nitrate ranging from none to over 5,000 pounds in

20 acre-feet of profile. Water from shallow wells located in or near feedlots often had offensive odor and the wells were not used. The nitrate concentration in the water at the top of the water table (table 11) was highly variable for each kind of land use, making it impossible to draw the conclusion that the surface-land use was affecting directly the composition of the water in the surface of the water table. Nitrate was not found in profile samples that had field-measured redox potentials less than 300 mv.

In Nebraska, Knudsen and others (1965) found that nitrate was higher in shallow water tables than in deep ones. Water samples containing more than 10 p.p.m. of nitrate-N were collected from wells where the average depth to water was 30 feet. Samples containing less than 2 p.p.m. came from wells that had an average depth of 61 feet. Herron et al. (1968) could not find appreciable leaching of nitrate below 6 feet in irrigated cornfields located on deep loess in eastern Nebraska, unless the soils were very high in N or had been highly fertilized. Under average conditions of fertilization, they found nitrate below 6 feet but "not appreciably in excess of amounts found in nonfertilized soils." Their experiments were located on Prairie and Chernozemic soils that have high natural organic N content.

In New Mexico, Bower and Wilcox (1969) analyzed the data on nitrate accumulated over the period 1943-63 on the salt balance in the upper Rio Grande. This was a period in which fertilizer N use on the irrigated crops in the valley had increased tremendously. The total nitrate load in the river decreased during the study period. They attribute this decline to decreased river flow. Comparison of 1934-43 means with 1954-63 means showed the following annual NO_3^- -N loads of the river in metric tons: Percha Dam—161 to 73; Leasburg Dam—298 to 140; American Dam—183 to 38; and El Paso-Hudspeth County line—151 to 51. They also mention that a substantial fraction of the NO_3^- in the river at the latter location comes from the El Paso sewage treatment plant. For the four sampling stations and the three 10-year periods, the average NO_3^- -N concentration in the river varied from 0.11 to 0.68 p.p.m. The latter value was for the period 1954-63 on

TABLE 11.—Average nitrate in 20-foot profiles and water at surface of water table¹

Land use	Number of profiles	NO ₃ ⁻ -N in 20 feet	Number of water tables	NO ₃ ⁻ -N	
				Mean	Range
		<i>Lb./acre</i>		<i>P.p.m.</i>	<i>P.p.m.</i>
Virgin grassland	17	90	8	11.5	0.1-19
Dryland	21	261	4	7.4	5-9.5
Irrigated land (except alfalfa)	28	506	19	11.1	0-36
Irrigated land (alfalfa)	13	79	11	9.5	1-44
Feedlots	47	1,436	33	13.4	0-41

¹ Adapted from Stewart et al. (1967a).

samples taken below the El Paso sewage plant. The agricultural drainage water contained from a trace to 2.84 p.p.m. for the three valleys and the three decades. The volume of drainage water in relation to the irrigation water diverted varied from 0.20 to 0.61 for the three valleys and the three decades.

Power (1970) at Mandan, N. Dak., found that nitrate moved below the root zone of corn on sandy loam that was fertilized annually with a high rate of N. Fertilization of continuous wheat and grassland gave little evidence of nitrate moving below the root zone, although he does admit to the possibility of some nitrate leaching below the root zone under systems with fallow or on other kinds of land use when a series of years with precipitation above normal occurs.

In Humid Regions

In Missouri, Smith (1967) and Keller and Smith (1967) reported extensive studies of nitrate distribution and its sources in rural wells. Smith concluded that most of the nitrate pollution comes from animal waste and septic tank drainage into improperly constructed, shallow wells and not from fertilizers, except for some heavy applications on the sandier soils of southern Missouri. He reported that cattle and hog numbers by counties when correlated with N use by counties gave a correlation coefficient (*r*) of 0.75. In the northwestern part of the State where the deeper waters are saline, 50 to 75 percent of the shallow wells contain sufficient nitrate to be of concern in livestock production.

He also reported that few farm ponds contained more than 0.5 to 0.75 p.p.m. of NO₃⁻-N, even when they received drainage from livestock areas. Table 12 shows the amounts of nitrate in the soil profile he found at varying distances from old cattle feedlots. Table 13 gives similar data where the source was a septic tank. He also reported that a Putnam silt loam profile that had received 120 pounds of N annually on corn for 17 years contained 360 pounds of NO₃⁻-N per acre 10 feet, but a nonfertilized cornfield contained only 148 pounds.

Keller and Smith (1967) collected more than 5,000 samples of well water and found that about 42 percent, ranging from 12 to 75 percent of the samples for individual counties, contained over 5 p.p.m. of NO₃⁻-N. The maximum concentration observed was over 300 p.p.m. Nitrite, which they stated is 10 times as toxic as nitrate, was detected in only 1 to 2 percent of the wells in winter months and 3 to 4 percent of them in warm weather. They stated that NO₃⁻-N higher than 50 p.p.m. should be avoided for livestock. Correlation of the N use by counties with the percentage of wells containing undesirable water for livestock yielded a nonsignificant *r* of -0.0295. When livestock numbers by counties were correlated with water supply nitrate data, the *r* was 0.58. It should be noted that in 1964, which was about the time these data were obtained, Missouri was not a heavy user of fertilizer N.

Ibach and Adams (1967) showed that only 38.7 percent of the total cropland in Missouri received commercial fertilizer N and that the

TABLE 12.—Nitrate nitrogen content of two soils at varying distances from old cattle feedlots¹

Distance from contaminated area, ft.	Farm A ²		Farm B ³
	Pounds N/acre, 0 to 18 ft.	NO ₃ ⁻ -N groundwater, p.p.m.	Pounds N/acre, 0 to 13 ft.
0	2,425	73	1,375
150	1,475	48	357
300	1,014	13	317
600	780	(⁴)	275
5,280	958	-----	227

¹ Adapted from Smith (1967).

² Loess soil, level topography, approximately 80 percent silt.

³ Silt loam, but with 25 to 40 percent clay below 24 in.

⁴ Trace.

rate of application was only 52.2 pounds per acre on the land fertilized.

In Illinois, Dawes et al. (1969) analyzed the NO₃⁻ data for Illinois rivers dating back to 1945. The median concentrations of some selected rivers are shown in table 14. They stated that since 1945 the NO₃⁻ concentration has almost doubled. They blame the 129-fold increase in use of commercial N fertilizers. Their data also showed, in general, that chloride, total dissolved minerals, and hardness of the water have also increased. Although they showed the sewered population for the watershed contributing water to the sampling stations, no information is given on other stream parameters such as BOD, NH₄⁺-N, or total N in solution needed to determine the extent that installation of sewage treatment facilities, during the period of study, had on nitrate concentrations or loads in the

rivers. Viets (1970b) has indicated that the increases in NO₃⁻ concentration observed in Illinois rivers "may be the first symptoms of stream recovery from organic pollution by raw sewage."

Harmeson and Larson (1970) of the Illinois State Water Survey summarized the nitrate measurements on Illinois streams and in ground waters. The following are some of their conclusions. The list of potential surface water supply sources in which the nitrate concentration exceeds the Public Health Standards during some part of the year has increased from zero to nine in the past 14 years. The highest concentration of nitrate in surface waters is most often found in the spring and early summer when the water flow is also the highest. The highest nitrate concentrations in surface waters are more consistently found in areas of intensive agricultural production on fertile, well-drained soils which are naturally rich in organic nitrogen. For the Kaskaskia watershed above Shelbyville they made these estimates of inputs of total nitrogen: Soils—61.5 percent; commercial fertilizers—26.8; animal wastes—7.6; atmospheric sources—3.8; and domestic wastes—0.3 percent. They concluded that each watershed seems to possess unique characteristics as to sources of nitrogen, making it impossible to reach generalized conclusions about the entire State. Ground water contamination by nitrate from fertilizers is not now a problem in Illinois, at least not of the magnitude of contamination of

TABLE 13.—Nitrate nitrogen content of soil and groundwater at varying distances from septic-tank drainage field, Saline County, Mo.¹

Distance from septic tank, ft.	Pounds N/acre 0 to 13 ft. soil	N groundwater, p.p.m.
60	474	22.0
86	375	8.8
112	308	7.4

¹ Adapted from Smith (1967).

surface waters according to Harmeson and Larson.

In Illinois, Walker (1969) reported that 25 percent of the wells 50 feet deep or less contained water with 10 p.p.m. or more of NO_3^- -N.

Alldrich (1970), aware of nitrate and eutrophication problems in Illinois, strongly defended the recommended use of N fertilizers in the State. He endorsed fall application of ammonium fertilizers after the soil reaches 50°F . at the 4-inch depth. Curtailing fertilizer use would require a great expansion of crop acreage onto land with high erosion and percolation hazards. He points out that a typical deep, dark Illinois soil releases about 125 pounds of N per acre per year, which is not enough for economic production. The 1969 corn crop in Illinois absorbed over a million tons of nitrogen from the soil-water system. Just for corn, the N absorbed was more than twice the fertilizer N applied to all crops, pastures, golf courses, home lawns, and all other areas in the State, according to Alldrich.

In Michigan, Erickson and Ellis (1971) measured the composition and total flow of drainage from tile lines and in creeks in an intensively farmed area in 1969. Some of the data for nitrate are shown in table 15. The Muck Farm is an experimental farm located on deep peat. They concluded that the nitrate in the drainage is only a small fraction of the ferti-

lizer N applied and discussed the difficulties of assigning sources among fertilizer, rainfall, and organic matter decomposition.

In West Germany, Kick and Kretzschmar (1968) investigated the sources of the increasing NO_3^- in the ground water on the lower terrace of the Rhine north of Bonn. They took cores to a depth of 4 meters under different kinds of land use. They found layers of accumulation of NO_3^- , Cl^- , and SO_4^- , which were especially pronounced when concentrations were expressed on the basis of the soil water present. The nitrate concentrations were higher in intensively cropped soils than in deciduous forest and wasteland soils. Differences in Cl^- and SO_4^- were smaller. Since the concentration of ions within the undisturbed profiles also varied horizontally, they stated it was difficult to trace their changes and migrations in the field. They said: "The increase of the nitrate load to ground water cannot exclusively be attributed to fertilizing, which in the area is done in relatively high applications. The sewage from urban waste waters and water courses must also be considered."

In The Netherlands, Kolenbrander (1969) found that the nitrate in drain water from cropped land and from pastures fertilized with liquid or solid manure, and liquid ammonia in the fall or winter, showed a distinct maximum in the spring (March through May) and a minimum in November. [Some of the Illinois rivers

TABLE 14.—Nitrate concentrations in Illinois rivers not exceeded 50 percent of the time¹

River	1945-51	1951-56	1956-61	1961-66	1966-69
P.p.m.					
Illinois at Peoria	9.5	10.4	18.8	16.5
Kaskaskia at—					
New Athens	5.0	4.3	5.0
Vandalia	3.8	8.2	15.5
Shelbyville	8.2	12.9	24.3
Skillet Fork at—					
Wayne City	1.8	2.5
Ohio at Cairo	3.8	4.3
Sangamon at Mahomet	8.7	32.6
Embarass at Camargo	17.7	28.0

¹ Adapted from Dawes and others (1969).

TABLE 15.—Nitrate N in the drainage of three farms and in water of two creeks in 1969 in Michigan¹

Location	Drainage composition			Fertilizer N applied Lb./acre
	Maximum P.p.m.	Minimum P.p.m.	Total Lb./acre	
Perden Farm	8.1	0.9	10.8	80
Davis Farm	7.2	1.8	7.4	35
Muck Farm	2.8	0.2	16.7	50
Dear Creek	4.4	0.4	^a 3.2	
Sloan Creek	3.7	0.3	^a 3.1	

¹ From Erickson and Ellis (1971).

^a 8 months.

also show this pattern of concentration (Dawes et al., 1969; Harmeson and Larson, 1970).] Drainage from grassland was more constant in composition. Because of the fluctuations in nitrate content, Kolenbrander stressed the need for obtaining samples throughout the year. He found an average NO₃⁻-N concentration of 1 to 2 p.p.m. from grassland and from 4 to 10 p.p.m. for cropped arable land, values being higher for sandy soils than for heavy-textured ones. From lysimeters filled with 120 to 125 cm. of a soil profile containing more than 35 percent clay, little nitrate was lost in the 35 cm. of leachate either from mineralization or fertilizer application. With sandy soils containing zero to

10 percent clay, he found that nitrate loss amounted to 60 kg. per ha., and that 20 percent of the N fertilizer was lost from the profile.

In southwestern Ontario, Canada, Bolton et al. (1970) measured the quantity and composition of tile drainage from plots in continuous corn or bluegrass and from the crops in a 4-year rotation in which each crop was represented each year in the rotation. Half of the plots were fertilized with N, P, and K, but only the annual rates of N application are shown in table 16. Both the N concentration and the total N loss, practically all nitrate, were higher from the fertilized plots than from the unfertilized ones. They stated that the nitrogen application rate for corn in the rotation with alfalfa was much higher than that usually recommended. In spite of the high N rates and the presence of alfalfa in the rotation, the N losses appear to be small in relation to the N available. Both the concentrations and losses of nitrate under the bluegrass sod were very low. Since these experiments were conducted on a Brookston clay with tile at about 70 cm., the low losses of nitrate under all systems may be caused by denitrification in the lower root zone. They found that the nitrate loss was predominantly affected by the amount of drainage. For continuous corn the drainage was 15.5 cm. per year and for the bluegrass, 6.45 cm. This difference probably accounts for most of the difference in nitrate loss from continuous corn vs. bluegrass.

TABLE 16.—Average annual nitrate-N composition of tile drain effluent from nonfertilized (NF) and fertilized (F) continuous corn, continuous bluegrass, and a 4-year rotation on Brookston clay for 1961-67, inclusive, in southwestern Ontario, Canada¹

System	N applied Kg./ha.	N loss		NO ₃ ⁻ -N	
		NF	F	NF	F
		Kg./ha.	Kg./ha.	P.p.m.	P.p.m.
Rotation:					
Corn (1)	128.8	5.6	15.1	8.5	14.0
Oats & alfalfa (2)	16.8	4.3	5.7	6.4	8.5
Alfalfa (3)	0	4.8	3.9	6.3	5.8
Alfalfa (4)	0	4.7	8.6	9.3	10.1
Continuous corn	128.8	6.6	14.0	4.4	8.9
Continuous bluegrass	16.8	0.3	0.7	3.5	1.1

¹ Adapted from Bolton and others (1970).

Nitrate Concentration by Water Passage Through Soil

Table 17 is a compilation of the nitrate concentrations in the input water, irrigation, or rain (R), and the subsurface or tile drainage water for some investigations cited in this paper. In all cases with complete data, the drainage is much higher in nitrate than the input water. In many instances nitrate exceeds the standards for potable drinking water. Table 5 for the Sutter Basin in California showed that drainage often had lower nitrate concentration than the irrigation water. Much of this drainage was surface drainage from ricefields. In all cases, these measurements were made on soils that had good to excellent fertility as judged by the areas, fertilizer programs, and kinds of

crops grown. Reduction in nitrate concentration might have been observed if the soils had had a very low state of fertility and were cropped. These increases in nitrate in the drainage were observed, even though the system as a whole was conserving nitrogen and the total outflow of nitrate was less than the total input.

This behavior of a fertile soil in enriching the drainage with nitrate is somewhat akin to the degradation of water with soluble salts as water passes through the profile and most of the water is lost by evapotranspiration. However, the resemblance in behavior of nitrate and salt is only superficial. Salt is affected less by reactions with soil and crops as it moves in water, whereas nitrate is subject to a myriad of reactions that affect its concentration.

TABLE 17.—Nitrate enrichment of subsurface drainage compared with water source

Location	Reference	NO ₃ -N in—	
		Input	Drainage
		<i>P.p.m.</i>	<i>P.p.m.</i>
California	Johnston et al. (1965)	(?)	25.1
Do.	Davis et al. (1969)	(?)	>10
Do.	Doneen (1966)	1.7	44.5
		2.1	23
Do.	Stout and Bureau (1967)	26.6	¹ 21
Do.	Bingham et al. (1971)	0.28	8.4 to 18.2
Idaho	Carter et al. (1971)	0.12	3.24
Washington	Sylvester and Seabloom (1963)	0.25	2.5
Michigan	Erickson and Ellis (1971)	rain	0.2 to 8.1
Netherlands	Kolenbrander (1969), grassland	do.	1 to 2
Do.	Kolenbrander (1969), cropped land	do.	4 to 10
Washington, 1943	Table 4, unpub. data ²	0.27	2.29
Washington, 1944	do.	trace	1.66
Ontario, Canada	Bolton et al. (1970), bluegrass	rain	1.1 to 3.5
Do.	Bolton et al. (1970), unfertilized crops	do.	4.4 to 9.3
Do.	Bolton et al. (1970), fertilized crops	do.	5.8 to 14.0

¹ Average in soil water, 4 to 39 feet.

² Unpublished data of C.S. Schofield and L. V. Wilson, U.S. Salinity Laboratory.

NITRATE ABSORPTION, REDUCTION, AND ACCUMULATION IN PLANTS

Nitrate Absorption

Although the literature on ion and salt absorption and absorption mechanisms is voluminous, a definite and detailed mechanism for ion

absorption has not been described. It is generally concluded that entry into and accumulation of inorganic ions by plant cells is via a carrier (the carrier hypothesis) and is energy dependent. The location of adenosine triphosphatase

(ATPase) on the plasmalemma (Lott and Hodges, 1970) has led these workers to postulate that this enzyme in conjunction with adenosine triphosphate (ATP) may be a part of a metabolic system involved in active transport, at least with certain ions (Fisher et al., 1970). Whether the energy (ATP) is used to synthesize or activate the carrier or in the binding of the ion is not known. In comparison with energy dependent (active) uptake, nonenergy dependent (passive) uptake by diffusion, mass flow, etc. is currently relegated to a minor role in actual ion uptake. More complete discussions of ion absorption including the many unresolved problems are detailed in various reviews (Brouwer, 1965; Laties, 1969; Leggett, 1968; Russell and Barber, 1960).

Relatively few absorption studies with nitrate are recorded in the literature in comparison with the amount of work done with other ions, especially cations. Two reasons for the limited use of nitrate in absorption studies are the lack of a convenient radioisotope, and the fact that nitrate may be reduced and assimilated once absorbed. There is enough data available to conclude that the absorption process for nitrate is not qualitatively different than for other ions.

Evidence for Active Uptake of Nitrate

Historically, the concept of active anion uptake (anion respiration) is based on the observations of Lundegardh and Burstrom (1933). Although this work shows a relationship between respiration and salt uptake, it does not provide precise evidence for the coupling of energy to anion uptake.

Active uptake is suggested by Langmuir adsorption isotherms (evidence of binding) obtained when rates of nitrate absorption by maize roots were plotted against external nitrate concentrations (van den Honert and Hooymans, 1955). The half-values of nitrate absorption were 0.023 and 0.032 meq./l. at pH 6.0 and 7.4, respectively. It was concluded that neither pH nor temperature markedly altered the uptake (binding sites). From this data, a K_m value of $2.0 \times 10^{-5}M$ was estimated for the hypothetical nitrate carrier. Similar adsorption isotherms and half-value (0.033 meq./l.) were

found with perennial ryegrass roots (Lycklama, 1963). Lycklama (1963) also noted that increases in temperature (5° to 35° C.) caused an increase in rate of nitrate absorption over the entire range; however, the greatest increase in absorption rate occurred between 15° and 25° C.

Additional evidence for an active nitrate uptake is provided by transmembrane electropotential measurements of pea and oat seedling tissue (Higinbotham et al., 1967). With anions including nitrate, an electrochemical gradient was established from the tissue to the solution; hence, an active influx system for anions was suggested.

Indirect evidence for a nitrate carrier or a requirement for energy for nitrate uptake is suggested by the work of Minotti et al. (1968). Nitrogen deficient wheat seedlings when transferred to a solution of $Ca(NO_3)_2$ exhibited a lag period (up to 240 min.) before initiation of a steady increase in nitrate absorption. Also of interest is the report of Greidanus et al. (1970) that cranberry cuttings were unable to absorb nitrate from nutrient solutions as indicated by the lack of disappearance of nitrate from the culture medium and plant growth. This would indicate the lack of a nitrate carrier in the roots of this species. This leads one to speculate that the amount of carrier could vary among varieties within a species and for a given genotype depending on physiological age and status.

It is of interest to speculate about the nature of the binding site for the nitrate ion on the proposed nitrate carrier, especially in view of the highly soluble (relatively nonbinding) characteristics of the nitrate ion. In this vein, the observation of Lycklama (1963) seems pertinent. Ryegrass seedlings absorbed more (two-fold) nitrate from a complete nutrient solution that contained 0.2 mg./l. ammonium molybdate than from a similar solution devoid of molybdenum. Lycklama (1963) concluded ". . . nitrate absorption is hampered by molybdenum deficiency," although the seedlings were grown and cultured in nonpurified solutions. Alternatively, it is conceivable that the enhanced effect of molybdenum could be the formation at the root cell surface of a molybdo-protein carrier for nitrate. This is based on the concept

that during the reduction of nitrate by the molybdocontaining nitrate reductase, nitrate is bound near or by the molybdenum site.

One of the many complicating factors in ion absorption (influx) studies is the efflux of ions from the adsorbing tissue or cells. Recently, evidence for an appreciable efflux of nitrate that occurs simultaneously with net nitrate uptake has been reported by Morgan (1970). Ryegrass grown for 28 days in $^{14}\text{NO}_3^-$ solutions lost significant amounts of $^{14}\text{NO}_3^-$ to the external $\text{Ca} (^{15}\text{NO}_3)_2$ solution over a 6-hour period. Moreover, net nitrate uptake from $\text{Ca} (^{15}\text{NO}_3)_2$ exceeded that from $\text{Ca} (^{14}\text{NO}_3)_2$ solutions. The latter observation was interpreted as evidence of recycling of endogenous $^{14}\text{NO}_3^-$ that reached the absorbing site by efflux from the roots. The results suggest that net nitrate uptake is the resultant of the influx and efflux processes. As previously mentioned, nitrate absorption studies are further complicated by the simultaneous assimilation of the absorbed nitrate.

Influence of Companion Ion on Nitrate Absorption

Wright and Davison (1964) indicated that plants absorb more nitrate from solutions made with potassium than from calcium or sodium salts. It has also been observed that increases in nitrate absorption are associated with increases in the potassium content of the nutrient media. However, increasing the amounts of potassium supplied to field-grown plants has not consistently increased nitrate accumulation. In a similar vein, it has been noted that increased levels of phosphorus have caused a decrease in nitrate absorption by soybeans grown in nutrient cultures. Again, similar treatments with field grown plants have given variable results (Wright and Davison, 1964).

In solution cultures, the presence of ammonium ions interferes with nitrate absorption. Wallace and Mueller (1957) reported the ratio of $\text{NH}_4^+ : \text{NO}_3^-$ absorbed by lemon cuttings was approximately 1.84, even though the ratio of $\text{NH}_4^+ : \text{NO}_3^-$ in the solution varied from 2:8 to 8:2. Lycklama (1963) measured simultaneously the absorption of NH_4^+ and NO_3^- by ryegrass when nitrate content of the media was held constant (1.0 meq./l.) but ammonium ion levels

were increased from 0 to 0.9 meq./l. As the NH_4^+ levels were increased there was a decrease in NO_3^- absorption from 55 to 1.8 meq./l. $\times 10^{-3}$. In the reverse experiment (NH_4^+ supplied at a constant rate of 0.2 meq./l. and NO_3^- varied from 0 to 3.0 meq./l.) NH_4^+ absorption was decreased but slightly.

While it is established that NH_4^+ interferes with the absorption of NO_3^- , the mechanism of this interference is not clear. Since direct interference at the carrier site seems unlikely, the interference is most probably indirect and attributable to assimilation. Wallace and Mueller (1957) reported that pretreatment of lemon cuttings with $^{14}\text{NO}_3^-$ subsequently resulted in increased absorption of $^{15}\text{NH}_4^+$ but had no effect on $^{15}\text{NO}_3^-$ absorption. In contrast, pretreatment with $^{14}\text{NH}_4^+$ subsequently resulted in decreased absorption of both $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$. Since pretreatment with NH_4^+ could have lowered the level of organic acids (Kirkby, 1968) and nitrate reductase (Hewitt and Nicholas, 1964), there seems to be some supporting evidence for Lycklama's conclusion (1963) that NH_4^+ interferes with NO_3^- absorption at the assimilation stage. A decrease in assimilation of NO_3^- could in turn decrease net uptake by the influx-efflux concept (Morgan, 1970).

Effects of pH on Nitrate Absorption and Conversely

Most data indicate that maximum nitrate absorption occurs when the external media are slightly acidic (pH of 4.5 to 6.0), although some workers have indicated that nitrate absorption is not markedly affected by pH. These views may be reconciled by the findings of Wallace and Mueller (1957) that, when nitrate concentrations of the medium were low, (2 meq./l.) nitrate absorption was unaffected by pH. In contrast, when nitrate was supplied at higher concentrations (8 meq./l.), nitrate absorption was decreased at higher pH values. Nutrient solutions containing nitrate as the sole source of nitrogen frequently became more alkaline because of the differential absorption (nitrate is usually absorbed at a faster rate than its accompanying cation) by plants.

To maintain electrical neutrality, it is suggested that hydrogen ions are absorbed and me-

tabolized rapidly by the plant (Dijkshoorn, 1962) or that a basic ion (bicarbonate or hydroxyl) is excreted (leaked) by the cells (Jackson and Adams, 1962; Dijkshoorn, 1962). The bicarbonate (or hydroxyl) ion could have its origin in decarboxylation reactions in the cells, as oxidation or synthesis of organic acids is the major mechanism by which plants maintain cytoplasmic pH balance. As a consequence of unequal absorption of nitrate cellular levels of organic acid should be depleted. However, in general this is not supported by experimental results (Ulrich, 1941). In fact, unequal absorption of nitrate may be associated with an increased level of cellular organic acid (Hoagland, 1944; Dijkshoorn, 1962).

These experimental observations are explainable by the assimilation of the absorbed nitrate. If nitrate remained unassimilated, the expected depletion of organic acids could be anticipated (Dijkshoorn, 1962). Since nitrate is assimilated, metabolic changes are associated with this process. The reduction of nitrate to nitrite could enhance the production of organic acids (Klepper, 1969), and the conversion of nitrite to amino-form would alter the cation-anion balance, thus leading to the requirement for synthesis of organic acids to maintain electrical neutrality and pH balance (Dijkshoorn, 1962). Consistent with this view are Kirkby's findings (1968) that nitrate grown plants had 30 percent less reducing sugars and 30 percent more organic acids than comparable plants supplied with ammonium salt.

From a practical view, the alkalization of the nutrient medium from more rapid removal of nitrate by the plants could tend to retard nitrate uptake. However, physiological iron deficiency and related problems caused by the alkaline medium (pH 7.5 to 8.5) are more likely to cause poor growth of plants cultured in nutrient media (Hewitt, 1966). These problems can be avoided or minimized by flowing cultures, large volumes of nutrient medium, or recirculation of medium with frequent adjustments of pH, the use of a mixture of ammonium and nitrate ions as the nitrogen source, e.g. Hoagland's solution No. 2 (Hoagland and Arnon, 1950), or by the use of carboxy resins in static cultures (Hageman et al., 1961b).

Nitrate Absorption as Related to Plant Families, Species, and Genotype

In their review, Wright and Davison (1964) listed *Amaranthaceae*, *Chenopodiaceae*, *Cruciferae*, *Compositae*, *Gramineae*, and *Solanaceae* as plant families that have been shown to accumulate high levels of nitrate. Because nitrate content varies with: (a) Rate of absorption, rate of assimilation, and rate of translocation, including efflux (each of these processes are dependent on metabolic status of the plant and probably vary with plant age); (b) environment, including nitrate and molybdenum supply; and (c) tissue sampled, the available data do not permit a precise ranking of plant families even with respect to nitrate accumulation let alone absorption.

Variations in nitrate accumulation have been recorded for species within a family and for genotypes within a species (Wright and Davison, 1964). In a cooperative study involving seven universities, it was noted that varietal characteristics for each of three vegetables, was one of the major factors influencing nitrate accumulation (Farrow et al., 1969). Few reports were found of experiments specifically designed to measure nitrate variation as a function of genotype. For example, Hoener and DeTurk (1938) concluded that "high protein" corn plants absorbed and assimilated more nitrate when cultured on a medium containing 100 p.p.m. nitrate than did the related "low protein" strain. This is supported by the observation that the "low protein" strain contained 49, 48, and 71 percent as much total nitrogen, nitrate, and dry weight, respectively, as the "high protein" strain. However, when the plants were supplied with 25 p.p.m. nitrate in the nutrient media, the "low protein" strain had 3, 25, and 15 percent more total nitrogen, nitrate, and dry weight, respectively, than the "high protein" strain. From this it can be argued that the "low protein" strain had a more efficient absorption system—lower K_m for nitrate by the carrier than the "high protein" strain. In this study, root weight, type of root system, and assimilation rate factors that could influence absorption were not measured. Thus, there seems to be little or no specific data concerned with differ-

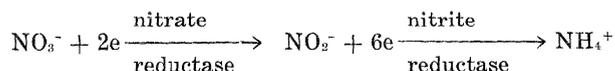
ences in nitrate absorption as a function of genotype in the literature.

Nitrate Reduction

This section will be limited to recent developments and reemphasis of certain aspects pertinent to nitrate reduction. The reader is referred to the recent review of Beevers and Hageman (1969) for a more detailed coverage

In the review article, it was estimated that on a worldwide basis, 10 billion tons of nitrogen are assimilated annually. In the United States alone, 7 million tons of nitrogen fertilizer are applied annually. Since much of the nitrogen is taken up as nitrate and the reduction of nitrate to the usable form (ammoniacal) requires eight electrons, the reduction of nitrate to ammonia utilizes a massive amount of solar energy, either directly or indirectly.

It is generally accepted that in both chlorophyllus and nonchlorophyllus tissue that the reduction of nitrate to the ammoniacal form proceeds as follows:



The first and rate limiting step is catalyzed by reduced nicotinamide adenine dinucleotide (NADH): nitrate oxidoreductase (nitrate reductase). This reaction, which occurs in the cytoplasm, is a typical transfer of two electrons from NADH. The NADH would be generated via carbohydrate oxidation and would be considered an indirect utilization of solar energy. The second step is catalyzed by nitrite reductase, presumably ferredoxin (Fd): nitrite oxido-reductase, in leaf tissue.

It has been shown *in vitro* that reduced ferredoxin can provide the electrons for the reduction of nitrite. Localization studies have indicated that both ferredoxin and nitrite reductase (but not nitrate reductase) are in the chloroplasts. Based upon these observations, but with no direct proof of *in vivo* function, the reduction of nitrite in green tissue is considered to be closely linked to solar energy. In nonchlorophyllus tissue (for example, roots and corn scutellum), the immediate electron donor for ni-

trite reduction has not been identified. It does not, however, appear to be ferredoxin. In nonchlorophyllus tissue, the source of energy for reduction of both nitrate and nitrite would be via carbohydrate oxidation and, thus, indirect utilization of solar energy. Miflin (1970) reported that part of the nitrate and nitrite reductase in barley roots is associated in a single particle. This particulate preparation when supplied with organic acids and ATP can convert nitrate to ammonia (Bourne and Miflin, 1970).

Nitrite reductase from leaf tissue behaves as a single protein that converts nitrite to ammonia without the release of free intermediates, such as hyponitrite and hydroxyl amine. The current data indicate that nitrite reductase affects the transfer of six electrons, a baffling thermodynamic and kinetic phenomenon. However, it is conceivable that there could be more than one active site on the enzyme surface. The mechanism of this complex reduction is unknown. Two hydroxylamine reductases have been isolated from leaf tissue (Hucklesby, 1968). One of these enzymes also utilized ferredoxin as an electron donor (Fd: hydroxylamine oxido-reductase). The role of these enzymes *in vivo* is not known and it should be emphasized that the reduction of nitrite to ammonia by nitrite reductase *in vitro* (illuminated chloroplasts and exogenous Fd as the electron donor system) is not dependent on either of the two hydroxylamine reductases.

Characteristics of Nitrate Reductase

Some of the more pertinent characteristics of the enzyme are: (a) Nitrate reductase is NADH-specific; (b) the enzyme is a metalloprotein and contains both iron and molybdenum; (c) maximum activities of 600 to 700 nmoles of NO_2^- produced per minute per mg. of protein have been reported for the enzyme; (d) the enzyme is adaptive, that is it is inducible by nitrate; (e) the enzyme is most unstable both *in vivo* and *in vitro* (the enzyme exhibits a 4-hour half life in detached corn leaves at 35° C.); (f) plants subjected to mild moisture and temperature stress exhibit reduced levels of enzyme activity; and (g) the enzyme exhibits both a seasonal and diurnal variation.

Because of recent developments, a few com-

ments on these characteristics are required. With respect to the electron donor for nitrate reductase, it now appears that the enzyme has a specific requirement for NADH, even for the soybean leaf enzyme. Evans and Nason (1953) originally reported that the soybean leaf enzyme could utilize either NADH or reduced nicotinamide adenine dinucleotide phosphate (NADPH) as electron donor. This finding was widely accepted and, in fact, many texts indicate NADPH to be the preferred cofactor. Beevers et al. (1964) confirmed the fact that the soybean enzyme could utilize both cofactors; however, the enzyme from other plant species had a specific requirement for NADH. Wells and Hageman (1970) have now shown that the NADPH-activity of the soybean enzyme is an artifact, and this activity is due to the presence of contaminating levels of a phosphatase that converts NADPH to NADH. The latter compound is the electron donor for nitrate reductase.

While it is true that nitrate reductase can utilize reduced flavins as well as NADH for electron donors, it does not seem likely that the reduced flavins are operative *in vivo*. This is because (a) the amounts of flavin required for optimum activity *in vitro* are in excess of the estimated *in vivo* levels; and (b) reduced flavins are auto-oxidizable, and there is no precedent for them functioning as independent (not associated with protein) electron carriers in metabolic systems. Current work would indicate that reduced nitrate reductase is composed of two tightly coupled protein subunits, (a) NADH-specific diaphorase and (b) a flavo-metallo-protein, that are not readily separated by normal purification procedures (Panaque and Losada, 1966; Schrader et al., 1968; Oji and Izawa, 1969). Thus, *in vitro* electrons can be accepted by the diaphorase from NADH and transferred to the attached flavo-protein or the flavo-protein can also accept electrons from reduced flavins.

An *in vivo* nitrate reductase assay has been used by Klepper (1969) to determine the metabolic system that generates NADH for nitrate reduction in leaf tissue. He concluded that the cytoplasmically located 3-phosphoglyceraldehyde dehydrogenase was a major source of the NADH used for nitrate reduction. This inter-

relationship between photosynthate, glycolytic metabolism, and nitrate reduction *in vivo* is shown in figure 1.

The chloroplasts when illuminated and supplied with CO₂ produce the gamut of phosphorylated glycerate, trioses, and hexoses, and sugars and starches. Certain of these intermediates termed "transport metabolites" by Heber and Willenbrincks (1964) move into the cytoplasm where they are metabolized by the glycolytic enzymes. Since the pyridine nucleotides do not move readily from the chloroplasts, the oxidation of 3-phosphoglyceraldehyde provides a source of NADH. Since nitrate reductase is also localized in the cytoplasm, it has access to this NADH and uses it as a source of energy for the reduction of nitrate. Although it can be argued that the oxidation of pyruvate (a major end product of the aerobic glycolysis) by the mitochondria could be a source of NADH for nitrate reduction, the data obtained by Klepper (1969) indicated that exogenous hexoses and trioses were much more effective in stimulating nitrate reduction than organic acids of the Krebs cycle.

This scheme (fig. 1) provides an explanation for the marked stimulation of light on nitrate reduction dependent upon CO₂ observed by Burstrom in 1943 and more recently by Kanangara and Woolhouse (1967). The data obtained are also consistent with Kirkby's (1968) observation that plants grown on ammonium salts contain 30 percent more reducing sugars and 30 percent less organic acids than comparable plants grown on a nitrate medium. In addition, figure 1 emphasizes the interrelationship of light (photosynthate), carbohydrate, and nitrogen metabolism.

It has been shown that nitrate content varies inversely with the level of nitrate reductase activity in the same tissue (Hageman et al., 1961a). Experiments with wheat (Croy and Hageman, 1970; Eilrich, 1968) reveal that additions of nitrogen fertilizer over and above that usually considered optimal for cultural practices are correlated with increases in nitrate reductase level and protein content of the plant or grain. Based on these findings, it is concluded that the level of nitrate reductase plays a major role in controlling the accumulation of nitrate.

Metabolic factors that regulate the level of nitrate reductase then should be directly related to the accumulation of nitrate. The rapid fluctuation of nitrate reductase that occurs daily (Hageman et al., 1961a), seasonably (Ziesler et al., 1963), when plants are placed in the dark (Candela et al., 1951; Hageman and Flesher, 1960), or exposed to heat or drought (Younis et al., 1965), indicate an effective *in vivo* regulatory mechanism. Evidence has been obtained that suggests that the rate of synthesis versus the rate of degradation, inactivation, or inhibition is one mechanism for controlling the enzyme level (Warner et al., 1969; Kannangara and Woolhouse, 1967).

The rate of synthesis *in vivo* is dependent upon the complex and poorly understood proc-

ess of protein synthesis. Obvious requirements are energy, the metabolic system involved such as ribosomes, messenger and transfer-nucleic acids; activating enzymes; and enzyme components, such as the amino acids, iron, and molybdenum. Travis and Key (1970) reported that synthesis of nitrate reductase by etiolated leaf tissue is light dependent. They suggest that the light requirement is related to the formation and maintenance of polyribosomes. Earlier work (Beever et al., 1965) had shown that light was also indirectly involved in induction by increasing the amount of nitrate in the inducing tissue. Although nitrate reductase is induced by substrate (nitrate), the process by which it induces synthesis of the enzyme is not well understood. It is also known that as nitrate

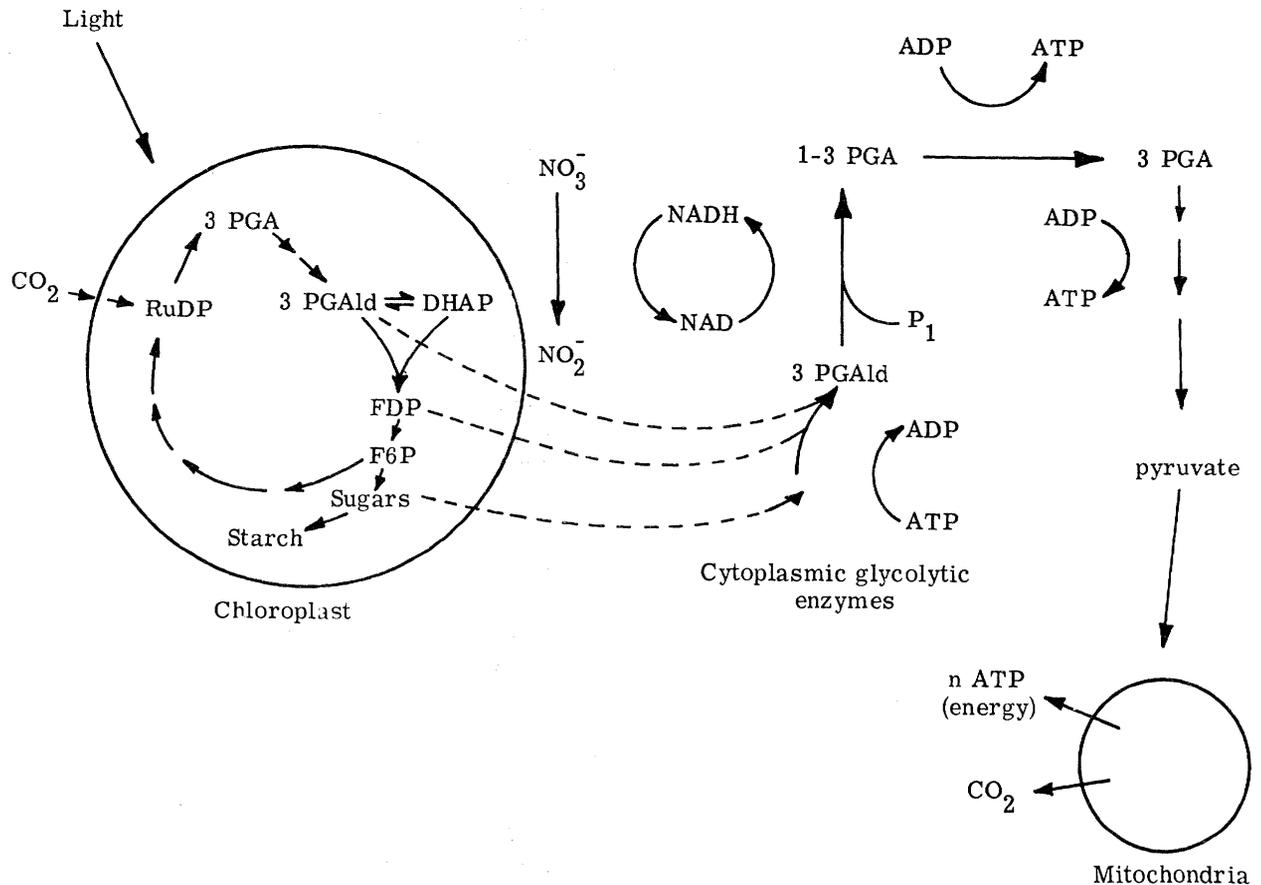


FIGURE 1.—A simplified metabolic scheme illustrating the interrelationship between photosynthesis, glycolytic metabolism, and nitrate reduction. (Abbreviations are: RuDP, ribulose diphosphate; 3PGA, 3-phosphoglyceric acid; 3 PGald, 3-phosphoglyceraldehyde, DHAP, dihydroxyacetone phosphate; FDP, fructose diphosphate; F6P, fructose-6-phosphate; NAD and NADH, oxidized and reduced nicotinamide adenine dinucleotides, respectively; and ADP and ATP, adenosine diphosphate and adenosine tri-phosphates, respectively.)

disappears or is used by the tissue, nitrate reductase disappears, thus synthesis must be maintained to balance against continual degradation.

Although Filner (1965) has reported that certain amino acids will repress nitrate reductase synthesis in cultured tobacco cells, attempts to repeat this work with corn leaf tissue were unsuccessful (Schrader and Hageman, 1967).

Coumarin, trans-cinnamic, and trans-o-hydroxy-cinnamic acids will inhibit the induction of nitrate reductase; however, it is believed that this is caused by the inhibition of protein synthesis in general and is not specific for nitrate reductase (Schrader and Hageman, 1967). With respect to rate of degradation or inactivation of nitrate reductase, it has been suggested that this may be caused by an endogenous proteinaceous inhibitor or protease. This is based on the observation of Travis et al. (1969) that the rate of disappearance of nitrate reductase activity in the dark was decreased upon the addition of protein synthesis inhibitors. In support of this view, Purvis (unpublished work at the University of Illinois) found that nitrate reductase extracted from young wheat seedlings is stable for 2 to 3 weeks at 3° C. after a single ammonium sulfate fractionation. Since the enzyme in the original extract was unstable, it is suggested that the fractionation removed an inhibitor or protease. The enzyme from young corn seedlings cannot be stabilized by similar or even more complex purification procedures.

Nitrate reductase can be inhibited *in vitro* by cyanide and cyanate, two naturally occurring metabolites (Schrader and Hageman, 1967). The effectiveness of these two compounds as *in vivo* inhibitors of nitrate reductase is not known.

High temperature and moisture stress have been associated with decreases in nitrate reductase activity *in vivo*. Younis et al. (1965) reported that increases in temperature (20° day—15° night, to 35° day—30° night) were associated with significant decreases in nitrate reductase activity of corn seedlings. Plants supplied with "optimum" levels of water had significantly higher levels of enzyme activity than

plants with "restricted" water supply at all temperatures tested. The measurement of enzyme activity indicated no significant interaction of temperature and moisture stress. Hufaker et al. (1970) demonstrated that barley seedlings subjected to 4 days of "mild" moisture stress (water potential of control was -7 atmosphere versus -11 to -12 atmosphere for the stressed plants) had 58 percent less extractable nitrate reductase than the controls. The moisture stress decreased nitrate reductase activity to a greater extent (58 percent) than three carboxylative photosynthetic enzymes (11 to 28 percent).

Genetic Variation

Corn inbreds and hybrids vary (up to fivefold) in level of nitrate reductase activity (Ziesler and Hageman, 1962). The level of nitrate reductase activity is highly heritable (Schrader et al., 1966). It has been shown for B14 and Oh43 and their progeny that the two loci control the level of activity (Warner et al., 1969). Marked differences in the level of nitrate reductase activity among wheat genotypes has also been demonstrated (Croy and Hageman, 1970; Eilrich, 1968). Higher levels of nitrate reductase activity are associated with higher rates of reduction of nitrate (Hageman et al., 1961a; Schrader and Hageman, 1965).

Intraplant Distribution of Nitrate Reductase

Because in some plant species, there is evidence that roots may play an important if not a major role in reducing nitrate (Bollard, 1960), it is worthwhile to consider the relative abundance of nitrate reductase in roots and shoots (leaves). In corn seedlings the amount of nitrate reductase in the leaves exceeds that in the roots by threefold to fivefold when expressed on a per-gram fresh weight or per-part basis (Hageman, unpublished work; and Hageman and Flesher, 1960).

Mifflin (1967) reported that the activity in roots is greater than in leaves of barley seedlings if expressed on a per unit of extractable protein; however, if expressed on a per-gram fresh weight or per-plant-part basis, the shoot has much more total activity than the root. Wallace and Pate (1965) found that the shoots had as much activity as the roots when field peas

were grown in a media that contained less than 10 p.p.m. nitrate. In contrast, when the plants were cultured on low (500 p.p.m.) levels of nitrate, the shoots had from 3 to 12 times more enzyme than the roots. In other studies with field peas, the capacity for nitrate reduction appears to be confined to the elongation zone; that is, the region of most active respiration (Carr and Pate, 1967; Pate, 1968).

Although Bollard's data (1960) conclusively showed that little or no nitrate exists in the exuded xylem sap in the many woody plant species sampled, it should be pointed out that no mention was made of the soil nitrogen status at time of sampling.

In this vein, Klepper and Hageman (1969) found that roots, stems, and petioles, and leaves from apple seedlings cultured on a medium containing 0.015 M nitrate had nitrate reductase activities of 351, 809, and 1,312 $\mu\text{moles NO}_2^-$ produced g. fresh wt.⁻¹ hr.⁻¹ and nitrate contents of 298, 21, and 45 $\mu\text{g. NO}_3^-$ -N, respectively. The higher level of enzyme activity in the leaves and petioles and stems could account for the lower levels of nitrate, at least in part. Nitrate and nitrate reductase activity were also found in leaves taken from a mature apple tree well supplied with nitrogen throughout the growing season (unpublished). Comparison under defined levels of nitrate nutrition of the nitrate reducing system of roots from plants that reduce nitrate in the roots (field pea and woody plant species) with those that do not (cocklebur), as reported by Wallace and Page (1967), would do much to clarify this problem.

Highest concentrations of nitrate reductase activity have been found in the leaf blades of the crop plants tested (corn, wheat, oats, soybeans, and sorghum). In general, maximum activity (20 to 30 $\mu\text{moles NO}_2^-$ produced g. fresh wt.⁻¹ hr.⁻¹) occurs in young (8-day-old corn and 12- to 14-day-old wheat). In older plants, maximum activity occurs in leaves before or with the attainment of maximum leaf area. Nitrate reductase activity declines in the lower (shaded) leaves of the canopy in normal field stands of corn. In contrast, all leaves maintain approximately equal and high activity, except for the extreme top and bottom leaves, when intra-plant competition is minimal (Zieserl et

al., 1963). Measurements of nitrate reductase activity made on fully expanded, unshaded, corn leaves throughout the growing season show cyclic variations with maxima in the seedling stage just before tassel emergence and during the ear filling period (Warner et al., 1969). Leaf sheathes and ear husks contain small amounts of the enzyme. Nonchlorophyllous tissue from the above ground part of the plant (stalk, ear shank, cob, and developing grain) is devoid of extractable activity.

It is interesting to note that nitrate reductase can be induced in both barley aleurone layers (Ferrari and Varner, 1970) and in corn scutellum (Eisner, 1968) by incubating the tissue in a medium containing nitrate. In intact seedlings cultured on nitrate, the corn scutellum also develops appreciable levels of nitrate reductase (Eisner, 1968).

From an agronomic standpoint and in view of the current use of high amounts of nitrogenous fertilizers, work done in our laboratory (Eilrich, 1968, Hageman and Flesher, 1960; Schrader and Hageman, 1965; Zieserl et al., 1963) and that of Wallace and Pate (1965, 1967) suggested that, except for the early seedling stages, the bulk of nitrate is reduced in the leaf blades of crop plants, excluding legumes and paddy rice.

Accumulation of Nitrate and Factors That Affect Accumulation

Nitrate is not accumulated uniformly by the various tissues and organs of plants, and even within a tissue there is variation depending on location (e.g. tip versus base of the leaf) and age. Nitrate accumulation in a tissue or organ results from the rate of absorption and translocation, the rate of reduction (assimilation) and the ability of the tissue to store the unassimilated nitrate. Because there are so many environmental and metabolic factors that influence nitrate accumulation, extreme variations are encountered in nitrate content even in the same tissue and from plant to plant and species to species.

In general terms, the stems (stalks) and petioles (mid-rib) of corn contain higher levels of nitrate than the leaf blade. Work of Deckard

(1970) indicates that the nitrate content of the basal part of the leaf mid-rib of corn is comparable with that of the stalk adjacent to its point of attachment. Although there is little data available as to the nitrate content of roots, Wright and Davison (1964) stated that the roots contain more nitrate than leaves but less than stems. Limited data suggest that very little nitrate is found in the reproductive parts. Inselberg (1956) noted that the third and non-developing corn ear initially accumulated nitrate in a linear manner (up to 420 $\mu\text{g. NO}_3^- \text{-N/g. dry wt.}$ over an 11-day period). In contrast, the first and second ear initially exhibited a loss of nitrate with development. Although these nitrate levels are relatively low in comparison with leaf tissue, the data show that some nitrate is transported to the reproductive tissue and suggest that these small amounts can be assimilated in situ.

The data of table 18 (unpublished work, G. Wells⁴) showed variation in nitrate content of leaves of several plant species throughout the 1969 growing season. Similar results were observed in 1968. These plants were grown on the

University of Illinois farm on soil of normal fertility but without preplant or supplemental fertilizer or irrigation. Although all six species exhibited a decrease in leaf nitrate content throughout the season, the two monocotyledonous species, especially corn, showed the earliest and greatest depletion of nitrate in the leaf blades.

Other works with wheat (Croy and Hageman, 1970; Eilrich, 1968) and corn (Zieserl et al., 1963) showed similar patterns [high levels of nitrate (1,000 $\mu\text{g. NO}_3^- \text{-N/g. fresh wt.}$) in young leaf tissue but rapid depletion with development]. High initial levels of nitrogen fertility and supplemental nitrogen applications will tend to increase and maintain a higher level of nitrate in the leaf blade than is shown by the corn data of table 19. However, after the early vegetative stage and with normal development, the cereal crops usually do not accumulate nitrate in the leaf blades to the extent exhibited by other plant species (table 18). One factor for the decrease in nitrate content in the leaf blades of corn may be due to its apparent inability to absorb nitrate in the later stages of development (Sayre, 1948; Deckard, 1970).

Sayre found that nitrate absorption by the

⁴ Copy on file at Urbana, Ill., Agronomy Dept.

TABLE 18.—Nitrate content of leaf tissue of several plant species throughout the 1969 growing season¹

Plant family and species	Nitrate content, ($\mu\text{g. NO}_3^- \text{-N per g. fresh wt.}$) ²								
	June 24	July 1	July 8	July 15	July 22	July 29	Aug. 5	Aug. 12	Aug. 19
<i>Amaranthus hybridus</i> (pigweed)	423	212	258	96	106	102	60	43	23
<i>Setaria Faberii</i> (foxtail)	249	81	112	51	34	257	10	11	7
<i>Abutilon theophrasti</i> (velvetleaf)	263	409	449	230	---	278	102	46	100
<i>Glycine max</i> (soybean)	386	434	280	69	---	172	40	19	13
<i>Zea mays</i> (corn) ³	322	41	44	9	---	3	9	2	4

¹ Unpublished work of G. Wells, Urbana, Ill.

² Each value is the average of three replicate samples taken from a complete random block design. Planting rates were such that there was essentially no intraplant shading.

³ The midrib was removed from the corn leaves, which in part accounts for their lower level of nitrate.

corn plant started to decrease 45 days after planting and essentially ceased 15 to 20 days later. A second factor is that corn leaf blade has a relatively active reductive and assimilatory system for nitrate. A possible third factor could be an alteration in leaf anatomy during development that precludes accumulation.

The data in table 19 show that the last supplemental application of nitrogen (applications made 44, 59, and 72 days after planting) was least effective in increasing nitrate content of stalks and leaves. It was estimated that only 10 percent of this last application was absorbed by the plant. At maturity, the amount of nitrate in the stalks (average of the six hybrids) was 33.5, 228.7, 195.1, and 145.9 mg. of NO_3^- -N per plant for the control and the three treatments (44, 59, and 72 days), respectively. These values correspond to 97.3, 99.4, 99.4, and 99.2 percent of the total nitrate content in the above-ground part of the plant. Earlier studies established that the bulk of this nitrate is confined to the lower third of the stalk (Schrader and Hageman, 1965). Since the level of nitrate reductase in the stalk is extremely low, it is assumed that the stalk has a minimal assimilatory function but does serve as a storage site for nitrate. Although there is little specific and direct evidence on this point, it is presumed that the nitrate is transported from the stalk to the leaves for reduction and assimilation as the plant develops.

Experiments with winter wheat, grown under nitrogen regimes that minimize lodging, show that the relatively high levels of nitrate (200 to 500 μg . NO_3^- -N g. fresh weight) in the leaf blade observed in March are rapidly depleted to negligible levels during the 6-week period starting in mid-March (Croy and Hageman, 1970; Eilrich, 1968). Under certain environmental conditions, the level of nitrate in the leaf blade can be depleted to nondetectible levels by the first part of April (Stage B-4 to B-5 on the Feekes scale). Although the stems (stalks) of wheat, like corn, are probably not major sites of assimilation (lacking enzymes of nitrate assimilation), they are not good storage tissue for nitrate. The level of nitrate in the stalks is

higher than that of the leaf blades and the depletion pattern parallels that of the leaf with a lag of 1 to 2 weeks. In contrast to corn, the stalks of wheat and the rest of the vegetation and grain contain negligible levels of nitrate at maturity.

Wheat also appears to differ from corn in that it can continue to absorb nitrate late into the reproductive stage of development (Barat et al., 1969; and unpublished work of Hucklesby⁵). It has been shown that wheat plants effectively absorb and rapidly reduce and assimilate nitrate applied during April and early May (Eilrich, 1968; Hucklesby et al., 1971). Eilrich applied 68 kg. N ha.⁻¹ on April 8 to Ottawa and Gage wheats at a time when the leaf nitrate was approximately 225 and 210 μg . NO_3^- -N g. fresh wt., respectively. By May 10, the leaf nitrate was approximately 30 and 10 μg . NO_3^- -N g. fresh wt. for the treated plants, and 10 to 5 μg . NO_3^- -N g. fresh wt. for the control plants of Ottawa and Gage, respectively.

Hucklesby et al. (1971) found that increase in nitrogen of the grain and straw of the treated plants over that of the controls was more than adequate to account for the uptake and assimilation of the 112 kg. N ha.⁻¹ applied May 9 (booting stage for all three varieties used). Since isotopic nitrogen was not used, this result is based upon depletion of an equivalent amount of nitrogen in excess of the applied fertilizer nitrogen. No residual nitrate was detectable in the aboveground vegetation or grain at maturity in any of the material of these two wheat experiments.

In contrast, the mature vegetation of soybean and three weed species contained appreciable levels of nitrate (table 20). The bulk of the nitrate for these four plant species was found primarily in the nonleafy tissue. The higher level of nitrate in the leaf tissue found in these species than in corn is partly because the midribs and veins were not removed.

It is fortunate that the cereal grains used so widely for food do not accumulate appreciable

⁵ Copy on file at Agronomy Dept., University of Illinois, Urbana.

TABLE 19.—Nitrate content of stalk and leaf material of corn at different stages of development as affected by supplemental nitrogen fertilization¹

Hybrid No.	Plant 58 days when sampled				Plant 82 days when sampled						Plant 120 days (maturity) when sampled									
	Nitrogen applied at—																			
	0 day		44 day		0 day		44 day		59 day		72 day		0 day		44 day		59 day		72 day	
	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf
	μg. NO ₃ ⁻ -N/g. dry weight ⁻¹																			
1	8,410	259	10,670	432	1,010	14	2,570	44	1,420	14	1,670	24	234	27	3,452	27	2,141	28	1,755	38
2	7,240	235	8,310	317	1,320	16	2,810	60	2,270	44	1,940	13	316	26	2,644	25	2,106	29	2,106	22
3	10,200	255	14,050	403	1,260	14	3,390	30	2,650	40	1,980	21	1,158	51	3,054	57	2,808	45	1,977	42
4	10,040	274	15,020	610	1,090	15	4,580	154	3,380	99	1,650	35	281	33	3,276	48	2,925	52	2,223	43
5	8,810	212	13,440	526	870	5	3,040	36	1,930	31	1,450	5	199	56	3,288	35	2,141	40	1,778	37
6	9,940	230	11,810	302	850	19	3,530	36	3,510	79	1,550	31	410	43	2,656	44	3,498	42	1,977	38

¹ Adapted from Deckard, E. L. (1970).

² All plots received 90 kg. N ha.⁻¹ before planting and 337 kg. N ha.⁻¹ were applied to the treatment plots, 44, 59, and 72 days after planting. These dates correspond to 14 days after tassel initiation, tassel emergence, and initiation of silking. All plots were irrigated (2 inches of water, overhead sprinkler) each time supplemental nitrogen was applied. Plant population was 59,000 plants ha.⁻¹.

TABLE 20.—Nitrate content of the total above-ground portion of soybean and three weed species at maturity and the distribution of nitrate between leafy and nonleafy tissue

Plant family and species ¹	Nitrate content and distribution	
	Mg. NO ₃ ⁻ -N/plant	Percent in leaves
<i>Setaria faberii</i> (foxtail)	78	9
<i>Glycine max</i> (soybean)	88	15
<i>Amaranthus hybridus</i> (pigweed)	117	18
<i>Abutilon theophrasti</i> (velvetleaf)	203	9

¹ Same experimental material as used for the data of table 18.

levels of nitrate. McNamara et al. (1967) used a modification of a sensitive and specific enzymic assay for nitrate developed by Lowe and Hamilton (1967) to survey 113 seed samples representing 37 different plant species. Seeds of 28 wheat samples and 20 oat samples had low (maximum 1.9 $\mu\text{g. NO}_3^-$ -N g. dry wt.⁻¹) or negligible levels of nitrate. The nitrate content of 11 corn grain samples ranged from 1.1 to 3.8 $\mu\text{g. NO}_3^-$ -N g. dry wt.⁻¹. Many of these samples of wheat, oats, and corn were known to have been produced on highly fertile soils, and some of the wheat and corn plants were given large amounts of supplemental nitrogen. Nitrate was not detected in polished rice or grain sorghum (1 sample each). Barley, navy, and lima bean seeds (1 sample each) had 4.6, 3.4, and 1.3 $\mu\text{g. NO}_3^-$ -N g. dry wt.⁻¹, respectively. Soybean seeds (10 samples) ranged from 8.6 to 23.8 $\mu\text{g. NO}_3^-$ -N g. dry wt.⁻¹ (these seeds were obtained from the U.S. Regional Soybean Laboratory, Northern Marketing and Nutrition Research Division, Urbana, Ill.). While cultural conditions used in the production of soybean seeds were not ascertained, it is presumed that they were produced without supplemental nitrogen. These data suggest that investigation of the effect of cultural conditions and varietal dif-

ferences on nitrate content of soybean seed is warranted. Of all samples examined, the weed seeds had the highest nitrate content (195 samples with values ranging from 8.9 to 122.3 $\mu\text{g. NO}_3^-$ -N g. dry wt.⁻¹) than seed from other plant species.

In summary, experiments with several cereal plant species under "normal" conditions indicate that in the early vegetative stages, the leaf tissue can accumulate nitrate in relatively high concentrations (400 to 1,500 $\mu\text{g. NO}_3^-$ -N g. fresh weight⁻¹). This initial accumulation, under normal conditions, is attributable to available water and nitrate in the soil that favors high rates of absorption. Thus, in the early stages of development, absorption and translocation exceeds the capacity for nitrate reduction and assimilation. As the plant develops the concentration of nitrate decreases in both leafy and nonleafy tissue, with the more rapid decrease occurring in the leaf tissue. The decrease in nitrate content is attributable to the following several factors: (a) Rapid growth tends to dilute the nitrate concentration; (b) the capacity of the leaf tissue to reduce and assimilate nitrate is increased not only from having a greater amount of the proper enzymes but having an adequate supply of energy for the reductive steps; and (c) a reduction in available water and nitrate in the soil. In mature plants, nitrate accumulates primarily in tissue that exhibits a low capacity for reduction and assimilation (nonchlorophyllous stem and stalk structures). From studies with weeds and soybeans (Wells⁶, unpublished work), it appears that other plants exhibit a similar pattern of nitrate accumulation.

There are many metabolic factors that are associated with the accumulation of nitrate in plant tissue. Since nitrogen and carbohydrate metabolism are closely interlinked through the need for carbohydrate to provide the skeletons for formation of nucleotides and amino acids, and the energy for reduction of nitrate (Klepper, 1969) and synthesis in general, factors that are unfavorable for carbohydrate metabolism will also adversely affect nitrogen metabo-

⁶ See footnote 4, page 44.

lism. If the specific factors affect reduction to a greater extent than absorption and translocation, nitrate can be expected to accumulate. Some factors are known to specifically decrease nitrate reductase activity, while other factors are more indirectly related to the accumulation of nitrate. In the following section this will be discussed in some detail.

Nitrate Availability

While listing soil nitrate as a factor may seem obvious, the availability of soil nitrate available for absorption by the plant is most complex. Soil nitrate levels are extremely variable as nitrate can be lost by leaching, volatilization, microbial utilization, or simply unavailable to the plant due to location or lack of water. Nitrate is considered to be contained entirely in the soil solution; hence, the rate of replenishment (from organic or ammoniacal forms) may be more important than the amount of nitrate present at a given time (Bould and Hewitt, 1963).

Another truism is that nitrate must be present and available to the roots that are actively absorbing. Specific and direct data bearing on this point especially for field-grown plants are minimal. Barat et al. (1969) indicated that wheat plants in full head continue to absorb nitrate. The data obtained by Hucklesby et al. (1971) showed that wheat plants in the booting stage can effectively absorb soil applied nitrate. In contrast, the results of Sayre (1948) and the data in table 19 suggested that corn does not absorb appreciable amounts of nitrate after tassel emergence.

Drought and Temperature

Generally, when nitrate accumulates in plants grown under droughty conditions, high temperatures are usually an associated factor. Both high temperature and moisture stress have been shown to drastically reduce or inactivate nitrate reductase (Younis et al., 1965). Huffaker et al. (1970) demonstrated that nitrate reductase activity was much more adversely affected by very slight moisture stress than were certain enzymes of the photosynthetic system. These results suggest that either

high temperature or moisture stress has a more adverse effect on nitrate reduction than the absorption and translocation of nitrate; hence, the accumulation of nitrate by the plant. Obviously, for nitrate to accumulate under conditions of drought, sufficient nitrate and water must be present in the soil to permit absorption and translocation by the plant.

Light

Light has long been known to enhance the reduction and assimilation of nitrate, although the effects are complex. The level of nitrate reduction in leaf tissue varies diurnally and is influenced by the intensity of illumination (see the review by Beevers and Hageman, 1969, for full details). There is good evidence that light influences the level of the enzyme *per se*.

Kannangara and Woolhouse (1967) reported that there is a direct and absolute requirement for photosynthetic CO₂ fixation for the induction of nitrate reductase in leaves of *Perilla*. This requirement does not appear to hold true for all tissue, as the enzyme can be induced in the dark in various tissues. Thus, the effect of light would appear to be indirect, either by providing energy for synthesis of the enzyme or in some way increasing and stabilizing polyribosome formation (Travis and Key, 1970), or altering membrane permeability so that transport of nitrate is enhanced. Adequate light would also insure photosynthate for reductive energy via carbohydrates (Klepper, 1969) and carbohydrate skeletons for amino acid and nucleotide formation. Shade or inadequate light can lead to the accumulation of high levels of nitrate in plant tissue (Knipmeyer et al., 1962).

Mineral Deficiencies

Molybdenum is a constitutive part of nitrate reductase (Evans and Hall, 1955; Anacker and Stoy, 1958). In most plants, molybdenum is thought to have minimal additional metabolic functions; therefore, its deficiency leads to the accumulation of very high concentrations of nitrate in leaf tissue (Hewitt and Jones, 1947; Mulder, 1948; and Wilson, 1948). There is some evidence that iron is a constituent of the enzyme of higher plants (Anacker and Stoy,

1958) and conclusive proof that it is a part of the enzyme of *Neurospora* (Garrett and Nason, 1967). Several workers have also shown that phosphate stimulates nitrate reductase activity (Hewitt and Nicholas, 1964). However, deficiencies of iron or phosphate do not lead to the excessive accumulation of nitrate noted for molybdenum deficient plants. This is probably due to the deficiencies of iron and phosphate that would affect metabolic energy generation systems and metabolism in general as well as affecting nitrate reductase activity per se; thus absorption, transport and reduction of nitrate would all be decreased in varying amounts depending on conditions and species. Manganese deficiency has also been associated with the accumulation of both nitrate and amino acids. These excesses are probably indirect deficiency effects, as it seems logical that the main effects of manganese deficiencies are on photosynthesis and respiration. These effects would be complex and diverse. Deficiencies of other elements, such as potassium, could conceivably cause similar complex alterations of metabolism.

Endogenous Metabolites

The inhibition of nitrate reductase or repression of its synthesis by endogenous components could lead to the accumulation of nitrate in the plant. However, it is not known whether the inhibitors or repressors of nitrate reductase that are effective in vitro are responsible for accumulation of excessive amounts of nitrate in vivo. Both cyanide (Hewitt and Nicholas, 1964; Maranville, 1970) and cyanate, a derivative of carbamyl phosphate (Schrader and Hageman, 1967) will inhibit nitrate reductase, in vitro. Filner (1965) reported that certain amino acids will repress the synthesis of nitrate reductase in cultured tobacco pith cells. Smith (1969) showed that ammonia and certain amino acids will repress the enzyme in *Chlorella* and barley roots but not in leaf tissue of higher plants. With both *Chlorella* and barley roots, Smith expressed the opinion that ammonia per se, or derived from the various amino acids, or a metabolite of ammonia (glutamate) may be the specific repressor in all cases. It does not seem logical that these metabolites and their regulatory

effects would lead to excessive accumulation of nitrate in "normal" plants.

Herbicides

Sugarbeets (Stahler and Whitehead, 1950) and broadleaf weed species (Willard, 1960) treated with herbicidal levels of 2,4-dichlorophenoxy-acetate (2,4-D) were shown to accumulate nitrate. No record of nitrate accumulation has been reported for members of the *Graminae* family, similarly treated. An explanation for this difference in response was given by Beevers et al. (1963). They found that nitrate reductase activity was decreased in cucumber plants sprayed with herbicidal levels of 2,4-D solutions, while the opposite effect was noted with corn seedlings. With both species, nitrate content varied inversely with enzyme activity. Reproducible results were obtained with growth chamber plants, while much greater variability was encountered with field-grown plants.

In their review, Beevers and Hageman (1969) reported that several workers have found that treatment of plants with sub-herbicidal levels of 2,4-D alone, or fortified with micronutrients, increases crop yields and grain protein for barley. However, effects of treatments on nitrate accumulation were not given.

Subherbicidal levels of simazine have been reported to increase the growth rate and nitrogen content of plants cultured on suboptimal levels of nitrate or when grown at low temperatures (Tweedy et al., 1967; Ries et al., 1967). Field trials where six different plant species were treated with subherbicidal levels of simazine were reported to show that appropriate treatments increased crude protein production, per acre, from 10 to 52 percent (Ries et al., 1968). It was stated that simazine treatment enhanced nitrate uptake from the media. The data indicated that in some instances the nitrate content of the tissue was higher. The rationale of the simazine effect is that simazine increases nitrate uptake by the plant and that the higher level of nitrate in the tissue induces higher levels of nitrate reductase. Again, as with the 2,4-D treatments, the effects on growth, yield, nitrate and protein content for a given crop

varied with simazine level and environmental conditions.

Workers at the University of Auburn have shown that plants treated with atrazine (a related triazine) have a larger shoot-to-root ratio of nitrogen and dryweight than comparable controls. It has been proposed that the subherbicidal levels of triazine are inhibiting the photosynthetic process, thereby reducing the supply of carbohydrate available to the root and thus altering the shoot to root ratios.⁷

TIME TRENDS OF NITRATE ACCUMULATION

Foods and Feeds

In the past decade, public concern has been aroused over the possible health hazards of foods and feeds of high nitrate content, to both humans and animals. This concern has basis in the following series of well-publicized events that occurred in the mid-1950s to mid-1960s: (a) Several individuals died from breathing silo gases (NO , NO_2 , N_2O_4) produced from ensilage of high nitrate content (Wright and Davison, 1964); (b) loss of livestock was associated with feeding forages or ensilage of high nitrate content (Wright and Davison, 1964; Hanway et al., 1963; Wright, 1963; Case, 1970); and (c) infant mortality resulting from the ingestion of spinach of high nitrate content was publicized in both scientific and popular press (Simon as reported by Lee et al., 1970). In this latter case, however, it was shown that death was caused by nitrite produced by bacterial action after the canned spinach was opened. Although nitrate per se can be toxic to animals, the major hazard of high nitrate feeds normally results from the conversion to nitrite by microflora of the digestive tract. This is especially true for ruminants. In a comprehensive survey of the toxicity of nitrates of foods, Phillips (1968) concluded that the nitrate content per se of foods currently consumed by humans older than 3 months poses no major health hazard.

Articles appearing in the popular press, health culture magazines, and a few poorly researched scientific articles have led part of the

In summary: (a) The increase in yields and protein content achieved with the sublethal levels of herbicides emphasizes the importance of nitrate reductase in increasing grain and grain protein production; (b) nitrate reductase is interwoven with metabolism as a whole; (c) herbicidal effects on nitrate reduction are probably indirect as observed through changes in overall metabolism and, hence, are complex and variable.

general public to believe that high nitrate content of foods and feeds is a new phenomenon and directly related to the use of inorganic nitrogen fertilizers. These views can be refuted by a review of the literature. Schimper (1888) reported that shaded leaves contained a much higher content of nitrate than comparable leaves grown in the sun. Mayo (1895) reported the loss of livestock fed cornstalks of high (2.6 to 3.5 percent NO_3^- -N g. dry wt.⁻¹) nitrate content. Producers of truck crops have always used highly fertile soils in which organic matter was the major source of nitrogen until the development of the nitrogen fertilizer industry after World War I. Nitrates will be produced in high amounts by microbial action in soils supplied with amounts of organic nitrogenous matter adequate for profitable truck crop production. Thus, it is not surprising that Richardson (1907) found high levels of nitrates in various market vegetables, both fresh and canned.

With respect to human foods, two recent surveys (Jackson et al., 1967; Lee et al., 1970) indicate that there appears to be no evidence that there has been any appreciable change in nitrate content of vegetables from 1907 to the present date. Two specific examples taken from the review of Lee et al. (1970) illustrate this point (table 21). As indicated by these two reviews, there is a paucity of data on the nitrate content of human foods.

The nitrate content of cornstalks reported by Mayo (1895) is far in excess (threefold) of the highest level of nitrate observed in cornstalks by Deckard (1970). Deckard's plants were

⁷ Personal communication from Mason Carter, (1970).

TABLE 21.—Nitrate content of two vegetables

Vegetable and source	NO ₃ ⁻ content			Analysis by
	High	Mean	Low	
	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>	
<i>Beets:</i>				
Market	8,062	2,552	930	Richardson (1907)
Grown	-----	1,333	-----	Wilson (1949)
Grown	4,500	-----	600	Smith (1966)
Fresh and canned	1,654	1,197	671	Jackson et al. (1967)
<i>Spinach:</i>				
Market	3,809	1,892	310	Richardson (1907)
Canned	1,949	1,100	266	Do.
Market	2,353	-----	1,600	Wilson (1949)
Frozen	-----	3,636	-----	Do.
Fresh and frozen	739	524	238	Jackson et al. (1967)
Canned	550	473	396	Do.
Canned	1,435	886	505	Phillips (1968)
Frozen	2,463	-----	429	Do.
Fresh	2,330	-----	1,501	Do.

grown in fertile soil that were given supplemental inorganic nitrate fertilizers (427 kg.-N ha.⁻¹) and irrigation. These data strongly argue that the current use of inorganic nitrogen fertilizers has not led to marked increases in nitrate content of feeds.

As pointed out by Jackson et al. (1967) and as shown in the section on Nitrate Absorption, Reduction, and Accumulation in Plants, page 35, there are many variables that contribute to the accumulation of nitrate by plants. However, in spite of these variables, the available data do not indicate that the use of inorganic nitrogen fertilizers has increased the nitrate content of foods or feeds. In contrast, as stated by Kurtz and Smith (1966), the extensive use of inorganic fertilizers has caused a marked increase in both yield and quality of feeds and foods.

Soil and Water

Nitrate in the root zone is extremely variable depending on the timing of sampling in relation to recency of fertilization, crop growth, and rainfall or irrigation. It may vary from almost none to 200 or more pounds of N per acre during the season. Such fluctuations are needed for good crop growth and for prevention of leaching of nitrate. With the increased use of commercial fertilizers, there has been a growing

tendency for nitrate to accumulate deep in the root zone. More soil testers are now recognizing the importance of subsoil nitrate and are insisting on deeper sampling to improve their predictions of the amount of fertilizer N to use on crops. This awareness may be evidence that nitrate is accumulating in areas with intensive agriculture.

We have also noted the large decreases of soil organic matter associated with cultivation. These losses mean that one source of nitrate is becoming of less importance and that the deficit needed for good crop yields must be made up with fertilizer N and increased cycling of N in crop residues and animal wastes.

Whether there is a trend toward higher nitrate concentration in surface waters is impossible to evaluate because of the biological reactions in which it is involved. Increases in nitrate in lakes and streams may simply be a manifestation of better supplies of dissolved oxygen because of progressively lower inflows of raw or partly treated sewage. For these reasons, the authors of this paper cannot conclude that there is any trend in nitrate in surface waters, although they would not deny that the total N and total soluble N in many lakes and rivers may have increased significantly. It should be noted that the 10 p.p.m. NO₃⁻-N re-

lated to Public Health standards for water is far in excess of the 0.3 p.p.m. total soluble N sometimes regarded as sufficient for eutrophication.

Nitrate in ground water appears to be much more stable biologically than that in surface waters, but its concentration is subject to wide fluctuations caused by dilution, mixing, and stratification. To have evidence of a trend, we must have sampling of sufficient frequency and extend over enough time to warrant a conclusion. The best evidence of increasing nitrate concentration is the statistical study of Nightingale (1970) who showed that nitrate was increasing under both the agricultural and urban areas of the Fresno-Clovis area in California. The calculations and core analyses of Stout and Bureau (1967) for the Grover City-Aroya Grande Basin in California would also point to a trend of increasing nitrate in ground water.

The data of Gruener and Shuval (1970) also indicate increasing nitrification of the water on the central and southern Israeli coastal plain. Much of the data available indicate that drainage and percolating waters are higher in nitrate than the infiltrating water, but such enrichment does not necessarily mean that the water table is changing concentration because of dilution by water lower in nitrate coming from other sources. Although the evidence indicates that nitrate concentration may be increasing in some urbanizing areas with highly intensive agriculture, the conclusion that nitrate in ground waters is increasing generally cannot be justified.

The question about the time trends of nitrate accumulation in ground water probably could not be answered now, even if we had started intensive sampling back in 1945 when Comly showed that nitrate caused methemoglobinemia in infants. In humid regions, such as our Eastern and Southeastern States, where leaching of nitrate can be great if there is a nitrate source, the residence time of the ground water is probably too short to allow nitrate to accumulate (Carlson, 1964). In the writers' opinion, the hazard of nitrate accumulation in leached environments appears to be very low, a statement sup-

ported by the lack of a significant number of cases of infant methemoglobinemia and livestock disorders in high rainfall areas. In contrast, the Midwestern and Plains States present an entirely different picture. The rate of water recharge from deep percolation is so slow that the possible nitrate pollution of aquifers from our modern technology will take decades. However, once nitrate gets into the aquifer, decades will be required to replace the water with low nitrate water. Fifty to 100 years might be required to establish a time trend, considering the heterogeneity of aquifers. By the time the trend was established, a dangerous situation could be in the making that could not be corrected in a time shorter than it took to create it.

A third situation can be identified, and that is the "closed" water basin where the rate of recirculation of the water between the aquifer and the surface is rapid because of pumping for both domestic and agricultural supply, and the rate of recharge in part of the basin is high because of intentional recharge with waste water and leaching of agricultural soils to maintain a favorable salt balance in the root zone. These basins occur in parts of the West, particularly in California, and in other places in the World that are short of water and ground water is used extensively.

The time trends of nitrate enrichment of ground water that have been noted in areas such as the Fresno-Clovis area in California (Nightingale, 1970) and in Israel (Gruener and Shuval, 1970) are areas where a rapid circulation of water between the aquifers and the surface occurs on account of pumping. At least in California, the impact of man on nitrate accumulation is complicated by the large amount of nitrate of "geologic" origin that occurred in the profiles before irrigation or recharge was started. Glandon and Beck (1969), in summarizing their studies of nitrate in California drainage, stated that "High residual nitrates found in soils account for greater quantities of nitrogen in tile drainage than that contributed by fertilizers." It is of interest to note the projections of salts and nitrates in the San Luis

Drain being constructed as a master drain for the San Joaquin Valley waste waters to convey them to San Francisco Bay. The projection for 1970 was that the drainage would have 7,000

p.p.m. dissolved salts and would fall to 3,000 by the year 2020. However, nitrate nitrogen in the drainage is expected to remain at 20 p.p.m. (Price, 1969).

AN APPRAISAL

Our evaluation of the available information on nitrate in soil, water, foods and feeds is that the potential for nitrate accumulation does not pose a threat of an environmental crisis. There is no indication of widespread upward trends of nitrate concentrations in foods, feeds, surface or ground water.

Based primarily on a survey published by the National Institute of Environmental Health Sciences (Lee et al., 1970) and an authoritative article in the Canadian Institute of Food Technology (Phillips, 1968), the levels of nitrate currently found in foods pose no major health hazard to human adults. In isolated cases, nitrate content of water and foods (e.g. spinach) is toxic to infants (under 3 to 6 months). In many of these cases, the toxicity resulted from microbial contamination that converted nitrate to nitrite. However, even these limited number of cases can be eliminated by more extensive testing for nitrate in water and foods consumed by humans. This can be achieved by increased surveillance of nitrate levels of water and foods and by educational programs sponsored by National and local health agencies.

Contrary to opinions expressed by environmental alarmists and lay opinion, the enhanced use of inorganic nitrogenous fertilizers has not lead to increases in nitrate of foods and feeds. The reasons for this are that the cereal grains used so extensively for food and feeds inherently do not accumulate nitrate, and truck crops have also been produced on fertile soils. In general, soil organisms are equally adept at producing nitrate from organic or inorganic forms of reduced nitrogen. Moreover, the accumulation of nitrate in foods and feed crops depends more on the species and environmental adversities prior to harvest, such as drought, poor light, and high temperature, than they do on the

available N supply of the soil. Knowledge of these factors related to the accumulation of nitrate by food and feed plants, and the ease of assaying for nitrate, should minimize the use of foods or feeds possessing excessive levels of nitrate.

There is no indication of widespread upward trends of nitrate concentrations in surface or ground water. Absence of general trends, however, does not mean that nitrate is not accumulating in the shallow ground waters of some areas with highly intensive agriculture and an expanding suburban population lacking sanitary sewer systems. Areas of nitrate accumulation occur in the San Joaquin Valley and some coastal valleys of California, the North Central States of the United States, the coastal areas of Israel, and in northern Europe. The nitrate has multiple sources, some ancient and some new. Their relative contributions are largely unknown.

Since nitrate in ground water has many sources and its movement to and residence time in the ground water depends on the hydrology of the area, each basin or watershed must be examined as an entity. Nitrate in ground water is only one of the aspects of sanitary engineering and water-related pollution control measures that must be considered on a district basis. Certainly, Statewide or Nationwide controls on use of fertilizer are not justified.

Computer models are being developed to better understand the dynamics and the equilibria of nitrate in water basins, but the hydrological and chemical data needed for use in models are difficult to get. A simple input-output balance of N for a basin can be calculated from quantities that can be measured, but lack of data on denitrification in soils, profiles above water tables, and in water, plus the lack of quantitative in-

formation on inputs by N-fixing organisms makes the balance sheet approach entirely speculative for predicting the potential accumulation of nitrate.

Nitrate concentration in streams and lakes is highly erratic because of absorption by photosynthetic organisms, N mineralization in the bottom sediments, N fixation by algae, and denitrification reactions resulting from pollution with organic waste. With the cleanup of the Nation's waters by microbial oxidation of municipal wastes in treatment plants rather than in waterways, increases in nitrate in surface waters are possible as streams or lakes recover from organic pollution. Whether this will happen will depend on whether organic production in the water body resulting from eutrophy can be reversed. Such an increase in nitrate should be regarded as evidence of improving health of the water body rather than further deterioration.

Nearly all studies show that fertile soils produce subsurface drainage or water in transit to the water table that is much richer in nitrate than the rainfall or the irrigation water used. Similar studies are lacking on soils of low N fertility. Some nitrate leaching may be a necessity for a highly productive agriculture whether it is in a humid region where some percolation is unavoidable or in an arid, irrigated region where some leaching is needed to avoid salt accumulation. This nitrate may come from nitrate accumulated in the profile as a consequence of its past history, from the oxidation of high levels of organic matter or animal waste additions, and from fertilizers. Data do not exist that allow a separation of the relative inputs of these sources. A logical question is why does it matter if there must be some leaching to have a highly productive soil.

The significance of this downward movement of nitrate to underground water quality depends on the characteristics of the aquifer, the relative rates of recharge with water of low and high nitrate concentration, and the residence

time of the water in the aquifer. Strong arguments can be made for having each intensively cropped area surrounded by sufficient pasture or forest land (green belts) that will yield percolate with a low concentration of nitrate. Their spatial relationships depend on the hydrology of the region. It is indeed possible that even higher rates of N fertilization should be used on land with low percolation and erosion hazard so that more land can be returned to grass and forest for overall environmental improvement. Since much of our farmland has already lost much of its native organic N, thereby impairing its capacity to produce unless fertilized, we must use increasing amounts of fertilizer to utilize the full potential of our better varieties and water resources. The "balance of nature" can be better restored by using higher rates of N fertilizer than by using less.

We can probably accomplish the most in preventing nitrate accumulation in water by directing more attention to the concentrated sources of N supply. These include sewage plant effluents, concentrated livestock and poultry feeding operations, and nitrate-rich drainage, such as that of the San Luis drain in the San Joaquin Valley. Possibilities for eliminating much unwanted nitrate are the culture and harvest of algae in drainage and effluents, and managed denitrification in treatment plants, and on land that receives large amounts of animal waste.

Finally, almost every facet of the nitrate problem needs further investigation from the nitrate standards in water that will avoid methemoglobinemia to determination if there is any form of fertilizer N that can be used that will not yield some nitrate to the water table, and yet be economic and permit near maximum yields. Much greater team work is needed among scientists and engineers of all specialties, but in particular, hydrologists and agronomists need to work together in reducing the potential hazards of a productive agriculture.

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