rates of manure and nitrogen fertilizer application." Agriculture Secretary Clayton Yeutter has repeated the same points, most recently at the national Farm Credit System meeting in Minneapolis in September, where he called ground water contamination an area of "reasonable concern."

The Minnesota legislature has adopted sweeping rules for preserving clean water and for penalizing agriculture practices that threaten designated environmental zones in the state. Similar measures are pending in other states. In some local areas, a prospective farmer must present an elaborate livestock waste management plan—usually too demanding to be accomplished—before the local authorities will permit him to produce food.

In Texas, environmentalist lobbying has resulted in unheard-of fines from $25,000 to over $90,000 per farm, levied by the Water Commission this September against several dairy farmers in the Fort Worth area, because of water pollution. In the cases in question, bacteria from manure runoff into local streams is the issue, and not nitrates. But the legal mechanisms and precedents could be used in many other situations and locales against farmers.

Georgia Sen. Wyche Fowler (D-Ga.) has introduced federal legislation that includes severe penalties, to restrict farming in the name of protecting the environment. His radical bill, the "Farm Conservation and Water Protection Act," may not pass Congress, but it is expected to be a key part of the 1990 new five-year farm bill.

Assault on agriculture in Europe

Parallel operations are being run in Western Europe to restrict farming in the name of protecting water purity, with equally devastating results on agriculture. To prevent potential nitrate contamination of wells and other ground water features, rules have been imposed on manure management, and synthetic nitrogenous fertilizer applications in West Germany. In one German state, a "water penny" tax has been imposed to be applied to water safety work.

The European Community's European Commission has designated "environmentally sensitive zones," including water purity issues, throughout some of the prime farmland regions of Western Europe, such as the lush Po Valley in Italy. Rules have been drafted by the governments of the EC member nations, which specify how few cows, pigs, and other livestock per hectare (2.47 acres) a farmer may be allowed to keep in these designated regions, in order to prevent manure runoff. The proposals call for only 2 milk cows, 16 fattening hogs, or 5 sows per hectare.

One town in the Netherlands has gone so far in self-styled efforts to protect its ground water from perceived agricultural pollution, that officials set an 8 p.m. to 6 a.m. curfew in which all cows must be herded indoors, to concentrate their droppings and prevent runoff. This laborious chore may well be the last financial straw for many farmers.

The scandal of all this is that there is no sweeping excess levels of nitrates in ground water, on either side of the Atlantic. In fact, one of the leading dietary sources of nitrates is not ground water at all, or even processed meats, but raw vegetables—and especially organically grown ones—such as spinach, carrots, celery, and beets. As the 1976 paper by Dr. Thomas H. Jukes below details, the danger does not lie in nitrates as such, but the mechanism by which nitrates are converted into cancer-causing nitrosamines. This process is poorly understood, and its relation to dietary intake of nitrates is now totally obfuscated by the hysteria whipped up by the friends of William K. Reilly.

On the larger scale, the absence of food supplies caused by the menace of fanatic environmentalism on farmers, will result in millions of people needlessly suffering and dying from malnutrition and even starvation.

Nitrates and nitrites as components of the normal environment

by Thomas H. Jukes

Dr. Jukes presented the following speech to the Meat Industry Research Conference on March 25-26, 1976.

The existence of life as we know it is completely dependent upon nitrogen. In July 1976, the Viking Space vehicle is scheduled to land on the surface of Mars to conduct a search for living organisms and to carry out other scientific measurements. No human beings will be present, of course, and the information obtained by the instruments on Viking will be transmitted by radio back to Earth. So far, nitrogen has not been detected in the Martian atmosphere. Horowitz concludes that if there are no functional amounts of nitrogen on Mars, there will be no possibility of life on that planet. Theories of the origin of life always include amino acids as an essential component of even the most primitive forms of life. These ideas have been greatly stimulated by the detection of small amounts of amino acids in meteorites, carbonaceous chrondrites from outer space. The role of nitrogen in the origin of life is discussed in the book by Miller and Orgel, *The Origins of Life of Earth*. The usual theory is that the primitive atmosphere, which had been built up by the escape of gas from the interior of the Earth, was dominated by water, methane, and ammonia. Free oxygen was absent, but carbon dioxide may have been present.

Nitrates and evolution

Next to the origin of life, probably the most important single event in the evolutionary history of the Earth was the appearance of photosynthesis. This started about 2 billion years ago, and it led to the introduction of oxygen into the terrestrial atmosphere. As a result, nitrates appeared in the biosphere because of their formation by the action of lightning on nitrogen, oxygen, and water in the atmosphere. At first, the predominant photosynthetic organisms were blue-green algae. These supplied free oxygen, formed from carbon dioxide and water. Nitrogen within the interior of organisms mostly remains at the level of ammonia. Organisms that lived before photosynthesis appeared on the Earth obtained chemical energy from various organic molecules by anaerobic fermentations in the absence of molecular oxygen. There are many bacteria, termed obligate anaerobes, that use such reactions exclusively, and, to these organisms, oxygen is poisonous. Indeed, patients with gas gangrene are placed in oxygen chambers to kill the infective *Clostridia*. Such bacteria are considered to be the evolutionary descendants of primitive microorganisms that have survived by occupying ecological niches, such as the deep mud of lake bottoms, which the oxygen of the atmosphere does not reach. However, most forms of life now on Earth are descended from ancestors that adapted to the entry of oxygen into the atmosphere, caused by the appearance of chlorophyll in the biosphere about 2 billion years ago. This adaptation was made possible by the evolutionary development of enzymes that use molecular oxygen, such as the enzymes in mitochondria that participate in oxidative phosphorylation.

During the development of such enzymes, organisms also had to adjust to the presence of nitrates in the environment produced by thunderstorms from the oxygen that entered the atmosphere as a result of photosynthesis. Some forms of life, including all the green plants, developed enzyme systems that reduced nitrates to ammonia. Other organisms developed excretory systems, especially kidneys, for removing nitrates and nitrites that entered the body following the consumption of food materials obtained from plants. At some time during the past 2 billion years, two broad classes of soil bacteria appeared, known as *Nitrosomonas* and *Nitrobacter*, that are widely distributed in soil. *Nitrosomonas* oxidizes ammoniacal compounds to nitrites which are then oxidized to nitrates by *Nitrobacter*. This process is called nitrification. The nitrate that is produced as the end product can be destroyed by reduction to molecular nitrogen by other bacteria or it may be absorbed from the soil by higher plants that transform it to ammonia by two steps, first, reduction of nitrates to nitrites, and, second, reduction of nitrites to ammonia.

Nitrates are therefore evolutionary intruders into living organisms. Nitrates are formed from nitrogen and atmospheric oxygen. They are, indirectly, by-products of photosynthesis that are used by green plants as precursors of ammonia and hence of amino acids. Nitrates have no nutritional value for animals, which excrete them through the renal portal mechanism. There are some bacteria that produce nitrous oxide by reduction of nitrates.

One of the paradoxes of terrestrial nitrates is the fact that, given the pH and oxidation reduction potential of sea-water, practically all nitrogen should be present as nitrate ions in sea-water rather than as nitrogen in the atmosphere (see **Table 1**). It is assumed that this is prevented by denitrification, carried out by living organisms. In the steady state, the rate of production of free nitrogen, mostly by denitrification, should equal the rate of fixation. Exposure of animals to nitrates takes place primarily through the consumption of plants containing them, and through nitrates in water that the animals drink.

Where nitrates are found

The most famous accumulations of nitrates are, of course, the deposits in Chile. Nitrate salts have also been found to be widespread in Antarctica. These have been studied along with margin of the Ross Sea and the Ross Ice Shelf. Claridge and Campbell (1968) proposed that these have accumulated by precipitation from the atmosphere, which they have reached from the upper, very thin layer of the ocean. Compounds of nitrogen, during transport in the air, are oxidized to nitrates. These reach the Antarctic continent, and fall to the ground with snow which either sublimes, or melts and evaporates. In Antarctica, there is no leaching or biological activity. In most other parts of the world the nitrates are either leached away or are used by living organisms. The main exception is Chile where the huge nitrate deposits were for many years, one of the principal sources of nitrogenous

TABLE 1

Comparison of primitive and contemporary atmospheres

Primitive atmosphere (before photosynthesis)

No oxygen (less than 0.1% of present level)

Fixed nitrogen present as $NH_3 \rightleftharpoons NH_4^+$ from aspartic acid (Miller and Orgel)

Contemporary atmosphere

$N_2+O_2+H_2O \rightleftharpoons HNO_3$

$$K=\frac{(HNO_3)}{(N_2)0.5(O_2)1.25}=0.0425^\circ$$

$HNO_3 \rightarrow N_2$ and NH_3 by living organisms fertilizers for other countries.

The mean concentration of nitrate in the soil of the Chilean desert is between 0.1 and 0.2%, which is within the range of that of the soils of other deserts. This is in accordance with the theory that nitrates originate primarily from atmospheric processes, including the oxidative decomposition of organic remains, volcanic activity, and so on. However, the individual Chilean deposits contain up to millions of tons of minerals with 10% or more nitrates. The concentration of nitrates to form these deposits has been postulated to occur by capillary moistening of salt pans. The nitrates did not come from guano because in this case, phosphates would also be present, and this is not so. Mueller (1968) considers that the nitrate deposits are formed in two stages. The first is the primary genesis of nitrate through electrical discharges in the atmosphere, action of nitrifying bacteria on organic matter, atmospheric oxidation of organic remains in the soils, effects of cosmic rays and ionizing radiation of the atmosphere, hydrosphere, and soils, oxidation of products of forest fires and other combustions in the atmosphere, oxidation of ammonium salts produced by volcanic action, springs, and leaching out from rocks, production of nitrates by volcanic action, and nitrate formation through the action of radioactive substances on recent fossil organic matter. The second is concentration from salt pans that takes place by capillary transport in areas on the North Chilean desert with a yearly precipitation of less than 10 millimeters.

The world fixation of nitrogen was estimated in 1974 as 237 million tons. The mechanisms by which this was fixed were 63% by natural processes on agricultural, forested, and unused land, 2% by industrial fixation, 9% by combustion, and 4% by lightning. Oceanic fixation accounted for less than 1% of the total, but this is admittedly not a reliable estimate.

With the public apprehension about the toxicity of nitrates, it is well to remember that nitrates are the primary source of fixed nitrogen for green plants, to which nitrites are toxic. The production of nitrates takes place to reach high concentration in muck or peat soils by breakdown of vegetable matter. The second source of fixed nitrogen is ammonia in the soil, either from natural or synthetic sources.

Nitrate and ammonium ions present in the soil are absorbed by the roots of plants. The source may be either chemical fertilizers, decayed plants, or decayed animal residues, breakdown products of bacteria containing nitrogen compounds fixed from the atmosphere, or nitrates from thunderstorms carried down by rainwater. Absorption of nitrates is followed by reduction and incorporation into organic nitrogen compounds such as amino acids, compounds containing pyrrole rings, nucleotides, and coenzymes. Prior to reduction, nitrate may accumulate in plants at high levels especially if the soils are rich in nitrates. The first step in utilization of nitrates by plants is reduction to nitrites. This is catalyzed by an enzyme system containing molybdenum. Nitrites are then reduced to ammonia by the nitrite reductase system in which ferredoxin and a protein-containing iron-porphyrin are involved. The ammonia produced by the reduction forms glutamic acid by reaction with alpha keto-glutarate. Fresh plant materials, such as vegetables, contain substantial quantities of nitrates but only very small quantities of nitrites.

Dietary sources of nitrates

According to White (1975) the estimated average daily ingestion of nitrate per capita in the U.S.A. is 86 milligrams, principally from an average daily intake of 306 grams of vegetables. The amount must vary enormously according to the type of vegetable eaten, and the nitrate content of the vegetable. Lettuce, beets, celery, and spinach supply about 45 milligrams of nitrates at a consumption of 33 grams daily, about half of the amount supplied by all vegetables. Fruits, milk, bread, and water make minor contributions. The nitrate taken in the form of food is recycled through the saliva. During this procedure, it is partially reduced to nitrite by bacteria in the mouth, so that saliva supplies an average of 9 milligrams of nitrites daily compared with 2.4 milligrams supplied by cured meats. This was shown by Tannenbaum (1974) who has also demonstrated the formation of nitrosamines in saliva. The other ingredients of the diet make minor contributions to nitrite intake.

Nitrates are more toxic to human beings than they are to dogs or rats. A fatal dose for an adult human being is in the range of 8 grams to 15 grams. However, no toxic effects are produced by a dose of 1 to 1.5 grams. The "no effect" level for dogs is about 500 mg per kilo of body weight, corresponding to 35 grams for an adult human being weighing 70 kg. No effect was produced by feeding dogs a diet containing 2% of sodium nitrate for more than 100 days (Fassett, 1973). Rats are even more resistant: 5% of nitrates in the diet produced only slight growth depression in one experiment. This level is 100 times the amount allowed in meat.

The effect of nitrate on ruminants was reported in a classic experiment by Mayo and co-workers in 1895. The animals showed tremors, diuresis, and collapse, and their blood was dark in color. The cattle had been feeding on cornstalks that contained 25% of potassium nitrate by dry weight. The corn had been grown on soil containing large amounts of nitrates because of heavy application of manure. Similar symptoms were produced by administering potassium nitrate to cattle at about 1.3 grams per kilograms of body weight. The toxicity was actually caused by nitrites which had been formed by reduction of nitrates, in the rumen by bacteria. Ruminant animals are therefore particularly susceptible to nitrates which cause poisoning by the formation of nitrites after ingestion.

The main hazard from nitrates is to infants, and most of the deaths and injuries have come from nitrates in well water. Because of the absence of hydrochloric acid in the stomach of infants, bacterial reduction of nitrates to nitrites readily takes place. Reduction of nitrates to nitrites may also take place in vegetables that are allowed to stand at room temperature after they have been harvested. The principal offender is nitrate-rich unprocessed spinach. The changes do not take place in frozen or canned spinach.

The public is justifiably apprehensive over carcinogenic hazards from nitrosamines. These are inextricably linked with nitrates and nitrites, because nitrates are reduced to nitrites in the digestive tract, and nitrites can react with secondary amines *in vivo* to form nitrosamines. In addition to their role in forming nitrosamines, nitrites are also pharmacodynamically active as vasodilators and hence they can produce death in a dosage as low as 1 gram. Nitrites also react with hemoglobin to produce methemoglobinemia. Keating and co-workers (1973) reported a case of methemoglobinemia in an infant who had been fed nothing but carrot juice, containing 525 parts per million (ppm) nitrate and 775 ppm of nitrite. These are approximately the same proportions as found present in Florida carrots grown in a peat muck area of the Everglades that had received no nitrogen-containing fertilizer. No coloring or preservatives were applied to these carrots. Presumably nitrite was formed from nitrate in the carrots after harvesting. Some of their findings are summarized in **Table 2**. The investigators found that California carrots purchased at the same time from the same bin contained only 22 ppm of nitrate and 0.2 ppm of nitrite. They also found that so-called "organic" carrots contained an average of 320 ppm of nitrates as compared with 72 ppm of nitrates in conventionally produced carrots that had received varying amaounts of nitrate fertilization. The "organic" carrots therefore contained 60% more nitrates than the average of cured meats. The infant recovered following intravenous injection with methylene blue. The control in this case was a twin brother who had received commercial milk formula and who was asymptomatic.

TABLE 2

Nitrate and nitrite content of family and locally purchased materials

(in parts per million)

Material	Nitrate	Nitrite
Carrot juice fed to infant	525	775
Similac (made with tap water 1:1)	2.0	0.2
Carrots purchased 3 days later (same shelf):		
Florida	265	270
California	22	0.2
Carrots:		
Conventional growth	71.7	0.2
(Range)	(39–108)	
Organic growth	320	0.2
(Range)	(220–405)	

Source: G. Mueller, 1968, *Nature*, 219:1131.

Hill and co-workers (1973) studied the death rate from gastric cancer in an English city in which the public water supply contained high concentrations of nitrate, about 93 ppm as compared with 15 ppm of nitrate in control towns. The daily intake of nitrate from drinking water in this city was estimated as 92 milligrams. You will recall that the average daily intake in the U.S.A. from vegetables is estimated at about the same amount, namely 86 milligrams. Stomach cancer deaths in females were 60% higher than expected, but there was no significant difference in males. However, deaths from all forms of cancer were lower in the city under study, Workshop, than in the other communities. The sample is a small one, since only 43 cases of stomach cancer in females were recorded during the nine-year period.

Correa and co-workers (1975) have proposed an hypothesis that drinking water containing high levels of nitrate may give rise to stomach cancer because of conversion to nitrite which in turn produces nitroso compounds. The mutagenic effects of these, according to this proposal, eventually give rise to cancer. There is no evidence that the nitrate content of vegetables has increased in recent years; the levels present in various vegetables vary greatly. Beets may contain up to 3,000 ppm, about 15 times as much as in cured meats, which contain about 200 ppm. Nitrate in drinking water is probably easier to control than nitrate in vegetables, and a level of less than 45 ppm in drinking water is recommended.

The main source of nitrite is saliva. When vegetables and vegetable juices that are high in nitrates are consumed, the salivary nitrite concentration may increase. Tannenbaum reported levels of more than 400 ppm in the saliva of volunteers who had consumed one portion of "organic" celery juice containing 240 milligrams of nitrates (1976). This is several times as high as the maximum level, 125 ppm, that has recently been proposed for nitrites in cured meats. Most samples, about 93%, of cured meats contain less than 50 ppm of nitrite, and only 0.1% of such samples were found to contain more than 200 ppm.

Nitrites in bacon give rise to nitrosopyrrolidine on frying at 340°F. The approximate content is in the neighborhood of 10 parts per billion. This is one-fortieth the level of another carcinogenic nitrosamine, nitrosonornicotine, in unsmoked tobacco.

Can we reduce dietary nitrates?

No quantitative information is available on the roles played by environmental factors, including air, water, and food, as sources of nitrosamines. There is no obvious way of eliminating nitrates from the diet. Nitrates form nitrites in the digestive tract, and these nitrites are potential sources of nitrosamines. The addition of antibiotics to the diet could be one way of attempting to stop the formation of nitrites from nitrates in the digestive tract. Laboratory animals receiving high levels of antibiotics in experimental diets tend to live longer than controls. However, the prejudice against adding antibiotics to foods is so great that this procedure will never be tried as a way of reducing the formation of nitrites. Reduction of the nitrite content of bacon and addition of erythorbate, seem to be desirable procedures, but there is no way of predicting that these steps will markedly reduce the net supply of nitrites.

Food labeling regulations present a very serious problem because they list only the additives, not the "natural" ingredients of foods, that may be far more toxic than additives.

Is there any way of reducing the nitrate content of vegetables? The nitrate content of plants is increased by high temperature, drought, shading or cloudiness, deficiencies of phosphorus, potassium, or calcium and excessive amounts of ammonia in the soil as supplied by manure, chemical fertilizers, and legumes. One study showed that the nitrate content of vegetables was about the same in 1964 as it had been in 1907. Excessive use of liquid ammonia as a fertilizer has brought about increased nitrate content in forage crops that are consumed by animals. The same thing has happened from overuse of barnyard manure. The Dow Chemical Company has produced chlorinated pyridine compounds that are active against nitrifying bacteria. The substances inhibit the growth of *Nitrosomonas* bacteria that normally oxidize ammonia to nitrite. Since nitrate ions are easily lost from the soil by leaching, or are often converted to atmospheric nitrogen, the addition of the chlorinated pyridine compounds help to conserve ammonia-nitrogen in the soil by preventing its conversion to nitrates.

The simplest way to reduce dietary nitrate intake is to eat less lettuce, celery, spinach, and beets. These four vegetables account for about one-half of the supply of nitrates of vegetable origin per capita in the United States and about 42% of the nitrates from all food sources. During cooking, nitrates in vegetables are so soluble and diffusible that they readily pass into cooking water. The practice of draining cooked vegetables and throwing away the cooking water is often criticized because it leads to loss of some of the vitamin and mineral content. However, this practice is an excellent way of getting rid of a major part of the nitrate content of spinach. Tannenbaum has pointed out that several studies have shown that eating vegetables has a negative effect upon the risk of gastric cancer.

Nitrous acid in high concentrations has a mutagenic effect on tobacco mosaic virus particles. However, no mutagenic effect of nitrous acids on intact organisms has been detected.

Present trends in enforcement and public pressures keep the food industry continually on the defensive, especially as regards the use of intentional food additives. For some reason, this seems to spill over on contaminants such as aflatoxins, and the food industry and the Food and Drug Administration (FDA) frequently are criticized for the presence of aflatoxins in peanut butter. When we point out that many toxicants occur in natural foods, a customary reply is that this circumstance makes it all the more necessary not to "add to the burden" of harmful substances to which consumers are exposed. In my opinion, this attitude is illogical. It is far more important to evaluate the relative impact of all potentially injurious substances according to their relative effects. In this respect, food labeling regulations present a very serious problem because they list only the additives, not the "natural" ingredients of foods, that may be far more toxic than additives.

Summary

Nitrates are inseparable from human diets because of their universal occurrence in foods of plant origin.

Nitrates are reduced to nitrites by bacteria that occur in the mouth. For this reason, human saliva always contains nitrites.

Nitrites can react with secondary amines and other compounds in foods to form nitrosamines. These substances are mutagenic and carcinogenic. Nitrosamines are also formed during the frying of bacons.

Nothing is known of the extent to which nitrosamines formed in the body from salivary nitrites may be mutagenic or carcinogenic.

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