HEALTH EFFECTS OF WATER QUALITY : A CASE STUDY

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ABSTRACT. When alien substances are found where they are not supposed to be, the usual reaction seems to invest money in a policy to get rid of them. Yet the first thing to do would be more appriopriately to establish whether such substances are dangerous or not for human health. In this case, nitrates concentrations above a "health warning" limit were found in drinking water supplied to a large urban area. The study of scientific literature did not show any direct relation between those nitrates and health. Even methemoglobinemia in infants does not happen without an infection, i.e. a bacteriological pollution. And it is easy to prevent. The hypothesis that nitrates increase the incidence of stomach cancer has not been proven to this day. However, the situation will be monitored.

Let me begin with a rather puzzling fact. Health consequences are seldom taken into account when pollution problems are considered, and more or less solved. Epidemiological studies are not easily found in the scientific literature on pollutants, although they are the surest way to know if or not a substance found in the environment, where it is not supposed to be, is dangerous or not for human health.

Yet one may believe that pollutants are not fought only because they stink, or are unsightly. One may also believe that money is spent mainly to make (or keep) the environment safe for human beings.

But these beliefs are not quite supported by a quick exploration of the real world, let alone a deep research through the rich scientific literature that describes its phenomena and their consequences. If any.

We had to study a peculiar problem of "pollution", that of nitrates in drinking water because we are in charge of a research on "demand" and "supply" in our regional health system. Being a governmental planning organization, ours is bound to be involved in any such study on environmental pollutants and their health effects, either directly or as a "user" of what has been found by life scientists. In this case, we were asked to study the "problem" because a decision was to be made by regional and local authority councils on whether wells supplying water with a "high" nitrate content were to be replaced or not. No other public body had a qualified staff that could be diverted for a few weeks to produce a preliminary survey of the literature on the health consequences of nitrates in water.

This was needed because concillors had already been asked to pay for the smaller part of the cost of a geological study of aquifers not yet "contaminated" by nitrates, to know if they could supply enough water to replace older wells. They answered that the data they had were not enough to make a decision.

They were right in being cautious. In fact, the evidence of any danger coming from nitrates in water was from a Ministry of Health circular of March 15, 1962 saying that "although no limit is fixed for nitrate concentrations in public water supplies, a rate above 10 mg/l of Nitrate nitrogen may cause troubles, particulary in newborn infants" (1) and from a British epidemiological study concluding that "in a town where the intake of nitrate was abnormally high for a prolonged period of time, the death rate from gastric cancer was also abnormally high" (2). The latter evidence came as a "raw" information, without any discussion of its scientific bases, from a survey of the literature on the elimination of nitrates from drinking water, where the chapter on health effects was very short indeed (3).

Then, why was the problem stated is such a way that the public was lead to think that the water in a large area was positively dangerous (4) ? In fact, the Water Authority did just what is had to do - but not in the right direction, as it should have alerted health officials, not local concillors. Communication channels through political representatives work well in small rural communities, not in urban areas where the lightest health warning message is bound to become a frightening one, due to mass media interpretations or neighbourhood rumours.

The report of the Water Authority mentioned (from the literature survey already quoted) (3) a number of infant deaths caused by methemoglobinemia. We read this with some skepticism as we had completed a survey of health problems in the area with high nitrate concentrations in water, and no one ever draw our attention to methemoglobinemia. We got in touch at once with the head of the largest paediatrics department in the area, and we were told that he never had seen a methemoglobinemia case since he was appointed nine years ago.

Nor had general practitioners or paediatricians of the very efficient miners health service seen one, according to the service's chief medical officer (in the area where nitrates cause concern the inhabitants are mainly coal miners).

So we began to wonder why the health problem either did not exist or was not perceived by physicians. We read all the publications that we could detect (sometimes across the Atlantic). We found that the problem has been studied in depth. First, what is methemoglobinemia ? The methemoglobin is a brown pigment which is produced as the blood hemoglobin is oxidized, to the rate of 3 % daily. A complex

enzymatic system keeps the methomoglobin level in blood at 1 % of hemoglobin. This is important, since methemoglobin is unable to carry oxygen - and when it reaches about 10 % of hemoglobin, the symptoms of methemoglobinemia appear, of which cyanosis is the best known. Methemoglobinemia was first described at the Belgian Royal Academy of Medecine in 1844. The case was probably a congenital one. Most of the other cases found in the literature have been caused either by industrial substances, or by drugs.

A clear relationship between water nitrates and the disease was established in 1945 (5). The author had seen a two-weeks old baby which had repeated methemoglobinemias. After the first one, "forty-eight hours later the baby again was taken to the hospital with similar signs, and the same treatment was employed (...). She was hospitalized for sixteen days and at the time of discharge weighed 4,545 grams (10 pounds), had normal stools and was taking her soy bean and water formula in a normal, healthy fashion.

Two days later the parents suspected the onset of the same condition and returned with her to the hospital. This time the signs evidently were not convincing, for the baby was not admitted or treated. The parents were assured that she was well and were told to take her home.

Much to everyone's disappointment, the baby was readmitted the next day suffering the worst attack of any. Two hours were required for the methylene blue treatment to relieve her distress.

It was realized that the only significant change in the infant's environment from hospital to farm home was in the water. Sulfhemoglobinemia being a remote possibility, the water was analysed but no sulfides were found. The water was not tested bacteriologically. Nevertheless, the parents were warned not to give any more well water, and a formula of acidified whole milk was substituted.

The infant's father was dissatisfied with this incomplete explanation and also was loath to accept the possibility that his daughter was abnormal. Therefore arrangements were made to have her admitted to the Children's Hospital of the University of Iowa. The father believed that a peculiar reaction occurred between the well water and the soy bean preparation, producing a poison which caused her distress. Hence he asked the admitting physician if it would be desirable to bring samples of the water and powdered formula to the hospital for analysis. An open minded attitude on the part of the latter in accepting this "cock and bull" theory as plausible resulted in the father's bringing the water, which yielded the answer to the problem(...).

Reports in the literature of infantile methemoglobinemia caused by bismuth subnitrate suggested that nitrates might be the causative agent

in this case. It was thought that the well water might contain toxic amounts of nitrates. An analysis of water confirmed this suspicion. An unboiled sample of water had a nitrate nitrogen value of 140 parts per million which is equivalent to 0.619 g. of nitrate ion to the liter (...).

The sample contained 0.4 mg. of nitrite ion to the liter.

A second sample of water collected from the well several days later and boiled the same length of time as that given the baby contained 0.530 g. of nitrate ion to the liter, which approaches the amount found in the first sample analyzed. Culture of water from the well revealed the water to be highly polluted, an an M.P.N. (most probably number of coliform organisms for each hundred cubic centimeters) of 240 was obtained. U.S. Public Health Service drinking water standards allow an M.P.N. of not more than 5.

The baby was sent home on the fifth hospital day, receiving a half-skimmed, acidified milk formula to which no water was to be added. When seen by the family physician at the age of 4 months, no evidence of any permanent central nervous system damage from cerebral anoxia was found".

In another case, "a sample of the well water used in preparing the infant's formula had a nitrate nitrogen value of 90 parts per million, which is equivalent to 0.388 g. of nitrate ion to the liter. This is roughly two-thirds the amount present in case 1. The nitrite ion content was 1.314 mg. to the liter (...). It would appear that the baby ingested approximately 0.20 g. of nitrate ion a day, roughly equivalent to 1/3 Gm. of potassium nitrate.

The well water was tested bacteriologically and found to be as badly polluted as the well in case 1.

Samples of blood from the mother and father, both of whom drank the unboiled well water, revealed no abnormal quantities of methomoglobin".

It was also found quickly that the pollution of rural wells was frequent in the United States at the time, and was bound to cause methemoglobinemia.

"Dr. Morgan J. Foster of Cedar Rapids, Iowa, states that he has seen 5 similar cases in his practice. All the infants tended to be irritable and had diarrhea. Four were treated with methylene blue, and 1 infant died before the methylene blue treatment was known. From his records he noted that all the infants were receiving diluted milk formulas and that they came from farms in southeastern Iowa.

Dr. Roland Stahr, formerly of Fort Dodge, Iowa, reported 5 cases of

idiopathic cyanosis at the annual meeting of the American Academy of Pediatrics in San Francisco during May 1941. All of the infants had gastrointestinal disturbances".

This talented author at once established the new cause of the disease, noted that a gastrointestinal infection was also necessary (besides nitrates) to have methemoglobinemia, that it was an illness adults did not catch from water, and that diluted milk was also involved. He also discovered the right treatment, and the way to prevent methemoglobinemia :

"Members of our staff, after having learned of this condition, saw 2 babies who were cyanotic without obvious cause. Both infants improved when the well water was removed from the feedings. In retrospect it was realized that 2 other infants, both from the same family, had been seen in consultation because of a peculiar cyanosis. Checking back, it was found that they both were taking evaporated milk feedings diluted with well water. Both of them had recovered when feedings containing less well water were given.

The 2 infants who were the subjects or this report has gastrointestinal disturbances. Whether a separate cause brought about the diarrhea and vomiting in each case or whether the nitrate salts themselves or other constituents of the putrid water incited the diarrhea cannot be ascertained. Since the gastrointestinal symptoms were almost universal and in certain cases became more severe as the formulas were further diluted with the well water, it seems likely that the cause for the whole difficulty may have been in the water", as "the high nitrate water which the cyanotic infants ingested came from very undesirable wells. In many cases the wells were old, dug rather than drilled, had inadequate casings or none at all, and were poorly covered so that surface water, animal excreta and other objectionable material could enter freely. In every one of the instances in which cyanosis developped in infants the wells were situated near barnyards and pit privies. Some of the wells had trees growing nearby, and the roots had penetrated or broken down the casings. In four of the five wells the water was highly contaminated with coliform organisms". Therefore, "if the source is under suspicion, the physician will do well to prescribe a formula containing relatively little water. Thus, a diluted whole milk feeding would be preferable to a diluted evaporated milk mixture. A dried milk and water formula would be most dangerous, whereas an acidified, boiled, undiluted milk feeding would provide the greatest possible margin of safety".

But the abundant medical literature that was to follow showed that the author was overcautions when writing that " although no definite statement can be made, it would seem advisable to recommend that well water used in infant feeding possess a nitrate content no higher than 10 or, at the most, 20 parts per million".

Not only because the former limit is almost the "natural" nitrate concentration in underground water, but also because experimental observations were needed before such precision could be reached. Moreover, the condition was "not necessarily serious", although more frequent than was suspected at the time (6). The necessary observations were published in 1948 (7) :

"An "artificial well water" was prepared which contained 1 mg. of nitrate ion per cubic centimeter, supplied in the form of sodium nitrate. The solution was autoclaved before incorporation into the infants' formula (...).

After feeding 50 mg. of nitrate ion per kilogram per day to four infants ranging in age from 11 days to 11 months for periods of two to eighteen days, the highest level of methemoglobin obtained was 0.75 Gm. per cent (5.3 per cent of the total hemoglobin). No cyanosis was evident. The dosage of nitrate ion was then doubled to 100 mg. per kilogram per day. The increased amount of nitrate was fed to four infants, 2 days to 6 months of age, for periods of six to nine days. The only noteworthy level of methemoglobin, 1.3 Gm. per cent (7.5 per cent of the total hemoglobin) was obtained in an infant 10 days of age eight days after the nitrate solution was added to his formula. Again, no cyanosis was evident.

Then 100 mg. of nitrate ion per kilogram per day were fed to infants who had previously been cyanotic due to the ingestion of well water containing nitrate and in whom treatment with methylene blue had been successful in relieving the cyanosis. The highest level in these babies, who were 6 and 7 weeks of age, was 11 per cent of the total hemoglobin, and this time cyanosis was apparent, but not marked.

Therefore, it appeared that there were other factors in addition to the quantity of nitrate ion ingested that determined whether of not an infant became cyanotic. We decided to investigate first such factors as bacterial well water contaminants and gastrointestinal flora with regard to nitrate reduction and then age, gastric acidity, and the level of intestinal absorption of nitrate (...).

In the well waters described in the literature and in the samples which we analyzed, bacterial contaminants were found. Ours were all of the so-called nonpathogenic varieties and were chiefly Aerobacter aerogenes, aerophylic diplococci, and Escherichia freundii. All strains produced nitrite from nitrate.

The saliva, gastric juice, and stools of our cyanotic infants were cultured. Aerophilic streptococci and A. aerogenes were found in the saliva and gastric secretions of one infant. In another case, green streptococci, Staphylococcus aureus, and Escherichia coli were grown from the gastric juice, and a green streptococcus from the saliva. All of these organisms were then grown on simple peptone liquid media,

one-half of which contained small amounts of sodium nitrate and one-half of which served as a control and contained no source of nitrate. In twenty-four to forty-eight hours, each tube containing the nitrate and one of the above organisms gave a strongly positive α-naphthalamine sulphonic acid test for nitrite. Therefore, all of the organisms found in the mouths and upper gastrointestinal tracts in two of our cyanotic babies were capable of reducing nitrate to nitrite.

In view of the above findings, some of the contaminated well water was fed to one of the previously cyanotic infants whose methemoglobin level was 0.1 Gm. per cent following therapy. After seventy-two hours, the methemoglobin level rose to 1.4 Gm. per cent or 12.1 per cent of the total hemoglobin, and the infant was cyanotic. Well water, after being sterilized by boiling for fifteen minutes, was fed to a two-month-old infant with meningitis and to another of the treated methemoglobinemia babies. The former's methomoglobin level dit not exceed 0.2 Gm. per cent (2 per cent of the total hemoglobin) after sterile well water for two days, at which time the feedings had to be discontinued. The previously cyanotic infant developped methemoglobin levels of 1.26 Gm. per cent (11.1 per cent of the total hemoglobin) after six days and 1.0 Gm. per cent (9.5 per cent) after four days of sterile well-water feedings on two separate occasions. The infant who had been cyanotic continued to have positive gastric juice cultures for A. aerogenes and aerophilic streptococci while he was receiving the sterile well water.

In addition, three infants were fed sterilized well water plus interval feedings of a suspension of A. aerogenes. Two of the infants were prematures ; each developped levels of 8.5 per cent methemoglobinemia in twenty-four hours, at which time the feedings were discontinued because one of the infants developped diarrhea. The third infant fed sterile well water and A. aerogenes had a strongly acid gastric juice and did not develop a noteworthy level of methemoglobin despite five days of nitrate feeding (...).

Examination of the gastric juice of the infants who developed appreciable levels of methemoglobinemia either on well water or nitrate feedings revealed no free acid to be present and the pH to be greater than 4.0. All of the babies were less than 2 months of age.

In order to determine whether or not the gastric acidity had an effect on the survival of the nitrate reducing bacteria, the organisms which were cultured from the well water , saliva, gastric juice, and stools of one of our cyanotic infants were grown in culture media of various pH. All of the organisms including A. aerogenes, Esch. freundii, and Esch. coli grew in media of pH. 5 to pH 7. None grew in media of pH. 4.0".

The conclusion of the observations was extremely neat - "only infants

who have a gastric juice pH higher than 4.0 and nitrate - reducing bacteria in the upper gastrointestinal tract develop methemoglobinemia from oral ingestion of water containing nitrate".

Therefore "nitrates are not harmful in themselves. They produce disease only when reduced to nitrites in the intestine ; and the intestinal bacteria which cause this reduction work only at a very high pH value. This reduction to nitrites seems to occur only in the intestinal tract of infants during the first few weeks of life.

To such infants, the menace involved in the use of waters of high nitrate content in their feeding formulas, is by no means a negligible one. Surveys in Minnesota in 1947-1949 revealed 139 cases with 14 deaths, which would roughly indicate an incidence of one case per 1 000 live births per annum. The concentration of nitrate nitrogen in well waters which has been found to be associated with the disease has almost always been over 20 p.p.m. and in the majority of instances, over 40 p.p.m. It is generally agreed that in the areas where methemoglobinemia exists, water from private household well supplies containing more than 10 p.p.m. of nitrate nitrogen should not be used for infant feeding" (8).

The above statement was based on the first attempt to gather epidemiological data (9).

"Thousands of rural wells in many states yield water containing much higher concentrations of nitrates without methemoglobinemia being sufficiently prevalent to lead to the reporting of cases. Accordingly, it is evident that the problem remains to determine the current extent of methemoglobinemia, the distribution and range in the nitrate content of ground waters, and the cause of high nitrate content of such waters, so as to permit conclusions being reached as to the permissible upper limit of nitrates in water fed to infants".

A large survey, covering the United States, was undertaken in 1949-1950. However, only "a total of 262 cases was noted, not including the indefinite number of cases reported from Iowa, "several" cases in South Dakota and the 3 questionable cases in California, Georgia, and Virginia. Minnesota reports 139 and Illinois 75 of this total. It will be noted from Table 1 that a total of 29 deaths was reported, including 13 in Minnesota, 11 in Iowa, and 6 in Illinois. With the exception of the "indefinite" reports from California, Georgia, and Virginia, it is evident that these cases and deaths were reported from a group of north central states" (see table 1).

"A special effort was made to secure precise information as to the nitrate content of waters actually fed the 262 infants developing methemoglobinemia (...). 37 of these waters, specifically mentioned as being associated with reported cases, had concentrations less than 49 p.p.m.".

TABLE 1

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

State	Methemoglobinemia Reported Cases	Reported Deaths	Number of Cases Associated with Indicated Ranges of NO_3-N Conc. (P.P.M.) 0–10	11–20	21–30	31–50	51–100	100+	No. of Cases for Which Data Are Available	Supplementary Reference
California	1	0	0	0	0	0	1	0	1	
Georgia	6	3	—	—	—	—	—	—	0	Frith [37]
Illinois	75	5	0	4	2	2	12	31	25	
Indiana	1	0	0	0	0	0	1	0	1	
Iowa	Several	11	0	2	0	0	1	1	2	
Kansas	13	3	0	0	1	1	2	8	12	Metzler [38]
Michigan	7	0	0	0	0	0	0	7	7	
Minnesota	139	14	0	2	25		53	49	129	Beach et al.,[3]
Missouri	2	0	0	0	0	0	0	2	2	
Nebraska	22	1	0	1	0	4	9	8	22	
New York	2	0	0	0	0	0	1	0	1	
North Dakota	9	1	0	1	1	0	0	6	8	
Ohio	0	0	0	0	0	0	0	0	0	
Oklahoma	0	0	0	0	0	0	0	0	0	
South Dakota	Several	0	—	—	—	—	—	—	—	
Texas	0	0	0	0	0	0	0	0	0	
Virginia	1	0	0	0	0	0	1	0	1	
	278+	39	0	5	36		81	92	214	
Per cent of Total			0.0	2.3	16.8		37.8	43.1	100.0	

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

But "detailed epidemiological and technical data connected with the cases associated with water found to contain less than 50 p.p.m. nitrate nitrogen when the samples were collected," were not available. Moreover, it was carefully stated that "many uncertainties prevail, such as that samples of water collected after cases were reported may have contained a lower concentration of nitrates than when the water from the same well was consumed by specific infants".

One may infer that a higher concentration was also possible, since nitrate levels may change both ways.

In fact, "the nature of the information secured (...) as to the nitrate content of natural waters, whether or not associated with cases of methemoglobinemia, prevents its statistical analysis. But the replies from 37 states, however, disclose that minimum values were zero to one p.p.m., that average values were between 0.25 and 19.0 p.p.m. and that minimum and maximum values were 5.0 to 980.0 p.p.m. nitrate nitrogen (...).

The high concentration of nitrates reported in ground waters tributary to rural wells does not seem to be associated with nearby sources of pollution, although poorly constructed, shallow dug wells are mentionned most frequently in connection with the study of specific cases. Weart reports that the organic nitrogen content in the humus in the upper 40" of soil amounts to 16.000 lbs. per acre. Obviously, the leaching of soluble nitrates from humus, as nitrifi-

cation progresses, accounts for the high nitrate content of ground waters. At the same time, the wide distribution of organic nitrogen in humus and also the extent of ground waters containing high concentrations of nitrates, is in marked contrast to the relatively few cases of methemoglobinemia reported. This seems to indicate that other factors have a part in determining whether infants develop methemoglobinemia even when fed formulae prepared with water containing high concentrations of nitrates".

And as "there (was) definite evidence that a large number of rural wells, especially in the north central portions of the country, yield waters containing more than 50 p.p.m. nitrate nitrogen without cases of methemoglobinemia being reported, even when waters containing up to 500 p.p.m. nitrate nitrogen were involved", it was concluded that it was "impossible (...) to select any precise concentration of nitrates in potable waters fed infants which definitely will distinguish between waters which are safe or unsafe for this purpose".

This is an important statement, since our problem was to know whether or not there was enough evidence showing that nitrates were dangerous for newborn infants at concentrations above that indicated in the Ministry of Health circular - or at any known level.

The available literature has not shown any further advance of scientific knowledge in the establishment of a "safe" limit of nitrate concentration.

The first survey of literature was published in 1951 (10). It confirmed the findings of 1945 (5) and of the "epidemiological" survey just quoted, that methemoglobinemia was induced by water nitrates especially if the newborn infant already was suffering from gastrointestinal disturbances (11). It also confirmed that "the permissible nitrate-nitrogen concentration in water which may cause infant methemoglobinemia when used in a feeding formula is dependent on the individual's susceptibility, the increase in nitrate-nitrogen concentration due to boiling the water, the quantity of boiled water consumed per day per unit weight of the infant, the duration of exposure to high nitrate water, and possibly other factors (7, 11,12)."

Prevention of methemoglobinemia was found to be largely a matter of education, "if the infant's parents and the doctor in the case are informed of the problem, steps are usually taken to prevent nitrate-induced methemoglobinemia".

This policy was already being developed : "the testing of the private well water supply for nitrate content should become an integral part of the prenatal examination in rural areas. Well water containing more than twenty parts of nitrate per million should be condemned for use in infant feeding.

The importance of breast feeding at least in the first two or three months should be stressed, since there has been no report of a proven case in a breast fed baby.

If these measures were publicized and adopted by state and provincial public health bodies, well water methemoglobinemia would become a rarity in a much shorter time and with far smaller expenditure of public funds than would be involved in large-scale reconstruction of hundreds and thousands of wells" (13).

Rural water wells were the only concern of this policy, as "no case of methemoglobinemia has ever been attributed to a public well water supply, although there are several such supplies in Illinois with nitrogen contents of over 100 p.p.m." (8).

This is no longer true, as cases have been reported in the United States (for 63 mg. of NO_3 per liter concentrated to 73 mg. by excessive boiling) (14), Germany (15), and France. In a convalescent home for mothers and newborn infants, methemoglobinemia was induced by a concentration as low as 33-40 mg/l NO_3 (when measured) (16). In another case (17), the concentration was still lower - 30 mg/l. This recalls two cases found in Minnesota, which were apparently due to a nitrate concentration of only 20 mg/l (although for the bulk of the cases found in this survey the concentration was in excess of 100 mg/l) (18).

However, the number of methemoglobinemia cases is certainly very small indeed, compared to the number of infants fed water with a high concentration of nitrates. An epidemiological research completed in California in 1972 shows that methemoglobin levels do not rise without another cause - an infection of the digestive tract - besides nitrates.

"The primary area of study included two communities of 15,000 and 5.000 population, five miles apart, in the south central area of California known to use groundwater with varying levels of nitrate. The area is at an elevation of a little over 350 feet, the rainfall averages 6,44 inches per year and the temperature ranges from an average low of 38°F during the winter months to an average of 90°F in the summer months. The economy of the area is dependent on agriculture,particularly the production and shipping of cotton, grapes, field crops and citrus fruits. In 1967, nitrate concentrations in the groundwaters in this vicinity were studied considering in detail the sources, seasonal variations and water table variations (...).

Slightly more than half the infants born in the area were examined at least once. Those who participated and those who dit not were similar in ethnic origin and in area of residence. While there may be a selection bias, we were unable to identify any pattern

which could be influenced by it (...).

Less than three per cent were on dry milk formula and less than three per cent were breast-fed. Commercial formulae generally contain 50 mg/l of Vitamin C, the infant thus receiving 1,5 mg per ounce of formula per day (...).

Babies who have minor illness appear to have higher methemoglobin levels than healthy babies of the same age. One-third of the infants with elevated values (above 4 %) had respiratory illness, but the four highest values observed were from infants with diarrhea.

Since it is anticipated that standards for the community water supplies might be based on the total nitrate-nitrite-nitrogen content, the intake of infants was calculated similary on the basis of total nitrate-nitrite-nitrogen intake in milligrams per liter. This was calculated from the history of ingestion and the measured levels in water and formula (...).

Among ill infants, elevated methemoglobin levels are f ve times as frequent in the 5.0 to 9.9 mg per day nitrogen intake group as those with an intake below 5 mg.

Bacteria in significant numbers were found in the water and formulae available to infants based on coliform, fecal coliform and standard plate counts. Immediate and flushed samples from the household supply, bottled water, boiled tap water, and the formula often revealed considerable contamination. The least contamination was found in the immediate and flushed samples of the public community water supply (...).

Many variables play an important role in the determination of methemoglobin levels. The role of age is seen in the clustering of elevated values in the 31-60 day group. No infant over 90 days had a methemoglobin over 3.0 per cent. The majority of illness was due to respiratory infections. However, the highest methemoglobin elevations were in infants with diarrhea. For example one infant had 30 days of loose stools. This infant had a high nitrate exposure and the highest methemoglobin level, 10.7 %. Among the other infants there was no consistent correlation. The only infant hospitalized with diarrhea had a methemoglobin level of 8.4 per cent but had been using bottled water continually.

The presence of bacteria in water and formula may present as much of a problem as the presence of nitrate (...).

Compared to the effects of age and state of health in elevation of the methemoglobin level (over 4 % MHb) the effect of ingesting nitrate-nitrite-nitrogen in excess of 5 mg. per day from community water

supplies is detectable but not impressive. Bacterial contamination of formulae may contribute to such elevations (19)".

Now, what about cancer risks ? The epidemiological survey quoted at the beginning of this paper (2) must be read carefully. At Worksop, a mining community where drinking water contained 90 mg/l nitrate "at least since 1953", the observed numbers of deaths from liver cancers were 5.56 an 5.72 times higher than expected, for men and women respectively. However, the number of cases was very small - 10 and 8, and an analysis of the aetiology of each case could have been possible through the health history of each patient. Carcinogenic habits such as alcohol consumption (19) and eating of smoked food could have been studied.

Deaths were also higher for oesophagus (x 1,34 and x 1,25) and stomach (x 1,31 and 1,93) cancers. Both are usually correlated with alcohol drinking (19). For the oesophageal cancer, an epidemiological study was published more than twenty years ago. It explained clearly why cases in a Bantu township in East London were clustered around disused brickfields - where people brewed "cidiviki" from mealimeal porridge (adding... acetylene to it, in order to improve its narcotic effects) (20).

The hypothesis about stomach cancers is that nitrosamines are produced in the infected urinary bladder (21). They are dangerous. In animal experiments, a single dose of a nitroso compound may produce tumors (22). Target organs in animals are the liver, the intestines and possibly kidneys and lymphoid organs (23). There are no data on the target organs in man (2).

Stomach cancer is probably related to the quality of food. It is almost the only cancer showing a persistent trend to decrease - in developed countries where the diet composition and food conservation methods are improving and thus reducing the exposure to carcinogens (25). The major exception to this trend is Japan, where a raw fish (Sanma Hiraki) treated with nitrite may be a carrier of carcinogens (26), although in 1976 a sophisticated epidemiological study still retained well water as a factor to be studied further. But nitrate concentrations in water from rural wells had not been investigated (27).

These reports seem to show that ascorbic acid has a protective action against stomach cancer. Lettuce and fruits reduce the risk (26). This is confirmed by other reports. The formation of nitrosamines from nitrites is inhibited by vitamin C (28).

Smoked food has been suspected for a long time as a carcinogen and may explain the high incidence of stomach cancers in Scandinavia and Eastern Europe (29).

Other epidemiological studies to investigate the origin of gastric cancer cases, and to test the hypothesis of nitrates as causes have been completed in Chile and Colombia.

The Chile study (30) does not keep the promises of its detailed title. It is unclear if and how nitrates reached the human body. Quantities actually ingested were impossible to estimate. Other water pollutants, such as arsenic, which is a known carcinogen, were not taken into account. Hospital files were not searched for health histories of relevant patients, although the epidemiological research is on autopsies only. So the diet of these patients remains unknown, as is their level of alcohol intake. Nor is social class given, at least as a substitude of diet data. Regional morbidity and mortality differences were not studied, and the research took place in Santiago, far away from the provinces where nitrates are abundant.

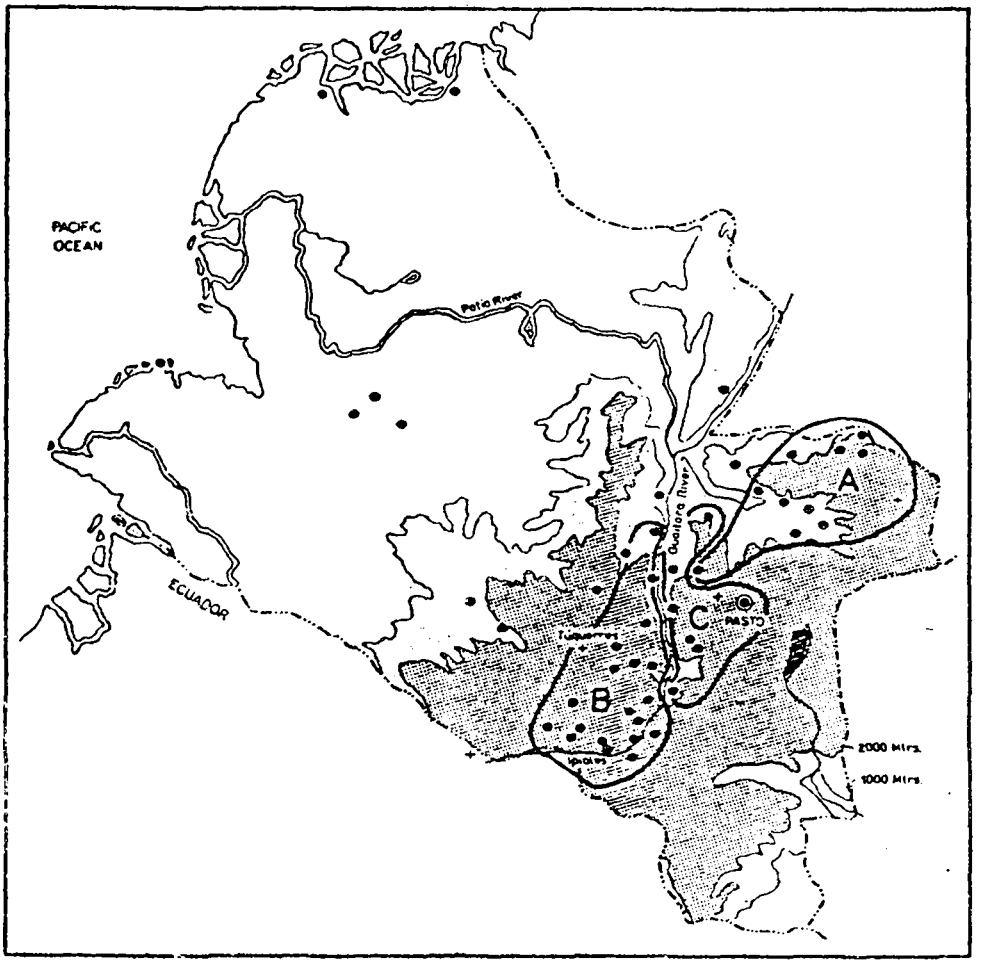
The Colombian study, made in the Narino province is much more sophisticated. "Samples of water were collected from 173 sources (wells, streams, and aqueducts) scattered throughout the populous mountain region of Narino. For selected sources, samples of water were collected at 2 months intervals to evaluate seasonal variation (dry vs. wet season) in nitrate levels ; because no important seasonal differences were found, our data summarize the findings from all samples.

Specimens of urine and saliva were collected from a sample of residents in four municipios ; two (Guaitarilla and Guachucal) are in the region delineated as high risk for stomach cancer and two (Tambo and Tangua) are in the low-risk region (...).

A fifth set of urine and saliva specimens was collected in Guachucal for comparison with specimens obtained from residents of a low-income district (barrio) in the city of Cali (...).

The populations from which patients and controls were drawn represented essentially a single socioeconomic stratum, and economic status was not controlled in the computation of relative risks. Most of the Narino population is very poor and practices a traditional form of agriculture. Few individuals have a monthly family income in excess of 750 pesos (approximately 25-30 dollars)" (31).

As the British and Chilean studies seemed to associate gastric cancer with nitrates in water supplies, these were systematically examined. However, no marked excess prevalence of intestinal metaplasia (IM) of the gastric mucosa and chronic atrophic gastritis (CAG) were found among users of well waters (the only ones rich in nitrates) in the high-risk areas of Narino. "The sources of water were readily and reliably ascertained, and inaccurate histories can be discounted as a reason for failure to find an effect. We must accept the presence of an



1. Map of the Narino province, Colombia, depicting altitude, location of the 51 communities (municipios) where investigations took place, and the areas at high (A,B) and low risk to stomach cancer (From Cuello et al., J. Natl. Cancer Inst., 57, 1016).

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elevated prevalence of IM and CAG among residents of high-risk areas who customarily drink nitrate-free water. Hawksworth et al. (32) found elevated levels of urinary nitrate (an index of nitrate intake) among people in Guaitarilla who did not drink well water, which suggests the presence of other sources of nitrates such as locally grown foods. The poor road and transport facilities ensure that a high proportion of the food consumed in the mountainous region of Narino is grown locally. In Guaitarilla, Hawksworth (32) found 18 µg nitrate nitrogen/g in the soil and 31 µg nitrate nitrogen/g in locally grown green vegetables. Inasmuch as commercial fertilizers have

been recently introduced and are not used extensively, they are probably not the source of nitrates in the samples of soil and vegetables".

In fact, it was impossible to demostrate that gastric cancer patients had consumed more nitrate-rich water or foods than had controls, or even that they excreted nitrate in larger amounts than the latter.

The similar geographic distribution of wells producing waters with high nitrate concentrations and of gastric cancer cases were therefore quite independent phenomena.

The authors were more successful with the investigation of foods as carcinogens. "At the time of examination, a trained interviewer completed a questionnaire about occupation, family income, residence history, sources of water supply, methods of meat preservation, type of cooking utensils, and consumption of foods and beverages. A qualitative fourpoint scale (very much, regular use, infrequent use, and not at all) was used to rank consumption of foods and beverages. The replies about foods were later checked for internal consistency by asking the respondent to rank in order the most important foods in his diet (...).

Foods found in a preliminary survey by a nutritionist to be the most important in the local diet were included in the questionnaire. Observations on the gastric mucosa were made without knowledge of diet and residence history ; thus the data could be used to identify differences in food consumption between individuals with and without IM-CAG, and between high- and low-risk areas. Three foods (corn, wheat, and cabbage) showed a substantial excess consumption in the high-risk area ; three others (lettuce, acelgas, and capulies) showed the reverse relationship. In a comparison of individuals positive and negative for IM-CAG, four foods were identified with significant differences at the 5 % level in rate of use-corn, lettuce, lima beans, and moras (a berry resembling the North American blackberry) (...). Many people in Narino practice the traditional Indian method of cooking corn in water and wood ashes ; this method enhances the availability of certain amino acids and protects against pellagra (...).

The prevalence of IM-CAG remained significantly low among regular users of lettuce after adjustment for age and birthplace (relative risk, 0.52), and the findings gave the distinct impression fo a protective effect in both high- and low-risk areas.

The association between moras and IM-CAG persisted after control for age and birthplace. Because use of moras, a native berry of high acid content, is widespread throughout Narino, this association requires elaboration in future field work.

Control for birthplace and age reduced the association between lima beans and IM-CAG to non signifieant dimensions. In the absence of other information incriminating lima beans, this food may be dropped from the list of active candidate foods.

Of the three foods suggesting evidence of a link with IM-CAG, two (corn and moras) appeared positively associated and the third (lettuce) was negatively associated (...).
The variation in prevalence of IM-CAG by the joint level of use of lettuce and moras appeared to represent the sum of two independent effects. The results for lettuce and corn raised the possibility of more subtle relationships. The reduced prevalence of IM-CAG among regular users of lettuce was confined to persons not using corn as a staple food ; this raised the possibility that a protective effect of lettuce might not be operative in the presence of high consumption of corn. The prevalence of IM-CAG was high for all nonregular users of lettuce, irrespective of corn consumption status. These tentative, essentially speculative, observations indicated that further tests by the collection of additional data were needed.

Because a number of foods were screened in this survey, chance factors alone could have resulted in the apparent association of a few foods with IM-CAG. This may be the reason for the finding on moras. For lettuce and corn, however, collateral evidence permits one to place more credence in the present results. Case-control studies of stomach cancer conducted among native- and foreign-born whites in Buffalo, New York ; Japanese in Hawaii and Japan ; and Norwegian populations at home and abroad have consistently reported that controls eat lettuce more frequently than do patients with stomach cancer (27, 33). The repeated detection of a protective effect for lettuce in several populations studied in various ways requires attention to possible mechanisms. For example, nitroso coumpounds synthesized in vivo have been proposed as a mutagen-carcinogen for gastric carcinoma, and vitamin C in lettuce has been shown to inhibit formation of nitroso compounds (26). Other candidate antioxidants are the tocopherols, which are abundant in lettuce. Corn has been retained for study because of its importance in the diet of the high-risk area and the need to consider the effects of alkali cooking. The latter may be a variation on a general epidemiologic observation for stomach cancer. Because of the diversity in foods eaten by many high-risk populations, clearly no single food or class of foods can be identified as common to all such populations. However, one consistent theme can be discerned High-risk populations practice some form of cheminal manipulation of foods, such as the salting and smoking of fish and meat and/or salting of vegetables for preservation and storage. Alkali cooking is in this general category. The pratice of salting meat in the high-risk communities of Narino has already been noted".

Moreover, "the data revealed a slightly lower prevalence of precursor

lesions among current smokers or drinkers of alcoholic beverages. This probably reflected a tendency to discontinue use of items that exacerbated gastric symptoms".

Genetic factors could not be ruled out. "The higher prevalence of IM-CAG among persons in blood group A (51 % compared with 39 % for group 0) was consistent with many observations on an excess liability of individuals in blood group A to stomach cancer. An association of blood group A with stomach cancer, confined to diffuse-type carcinomas, had been noted previously in Colombia" (34).

This level of sophistication, and the comparison with the other epidemiologic studies, show how carefully research programmes have to be designed and implemented. This is not a new fact.

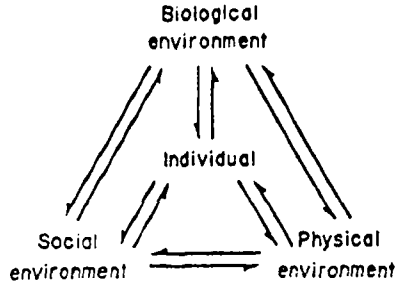
"All research begins with an idea, and the nature of the idea is dependent upon preceding knowledge, the "state of the art" at the time. Ideas for epidemiologic investigation may come from a variety of sources, depending on the background and the interest of the epidemiologist (...).

Science deals with the question, "how ?" and not with "why ?". The concept of cause embodies the latter, and as such is somewhat metaphysical. There are few, if any simple causes in biology. There are, instead, complex situations and environments in which the probability of certain events is increased. The appearance of cancer following exposure to chemical or viral agents merely means that the incidence of this type of cancer has been increased over the incidence that would have occurred without the exposure. With description of intermediate morphological, biochemical, and other changes associated with pathogenesis, the relationship of the exposure to the development of cancer becomes increasingly better understood, and eventually accepted as causal.

The reactions occurring between exposure to the mycobacterium and pulmonary tuberculosis are as multiple and complex as the processes between cigarette smoking and pulmonary cancer. These are misrepresented by the usual oversimplified formula : agent + host = disease. Rather, as Goldberger (35) describes, a field is required to indicate the processes (see Fig. 2).

In this formulation, the host, an open biological system, interacts with the environment as part of one system. The interaction can be either spatial or temporal, and all components are involved and affected, although the effects may be quantitatively quite disproportionate. The task of epidemiology and of preventive medicine is to identify these relationships, with special emphasis on relationships that can be most readily terminated, reversed, or altered.

Actually, acceptance of a causal relationship in its usual sense is



2. The "health maintenance system" of man reacts to biological, social and physical environment stimuli (from Goldberger, 1965).

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based upon a consensus of opinion by technically qualified individuals and the public, and is determined by the sum total of the presumably relevant information available at the time. Recent preoccupation with these discussions arises, in part, from the reluctance of important economic enterprises based on tobacco to accept the overwhelming demonstrations of tobacco's health hazards. Absolute proof is a philosophical impossibility, as David Hume established in the eighteenth century. An excellent discussion of strong inference that applies to epidemiology as well as to molecular biology is provided by Platt (36). In research, it is more relevant to perform the fewest, most decisive experiments than to immobilize ressources of personnel and time in scientific trivia (37)".

The British study that first suspected that nitrates in water might be carcinogenic is certainly not to be put among scientific trivia. It was mainly a "pilot" experience and it will be completed by a new study (38), which will certainly be more sophisticated. It starts from the fact that dietary nitrate comes principally from vegetables, and in smaller amounts from cured meats and from drinking water.

Maybe there is a "safe" level of nitrosamines, smaller doses being metabolized by the liver (23). Animal studies have shown that excess nitrates over the normal level of secondary amines are necessary for the production fo nitrosamines by bacteria.

Moreover, as with methemoglobinemia, there may be more than one factor for carcinogenesis to be started. Bladder infection and gastric achlorhydria may be among such necessary factors. Maybe a "safe" level of nitrates in drinking water could be determined from the results of the new study (39).

Maybe one has not to expect too much from such research. Biologists and physicians emphasize the limits of present knowledge. They see promising ways for new research. But they also show that premature

hopes could be broken in front of obstacles that certainly exist.

"In consideration of the causation of cancers of the alimentary canal, an immediately plausible hypothesis is that diet is involved. The evidence is confused and complicated but there are some pointers to further investigation. It may be speculated, for example, that the progressive fall in mortality from gastric and colo-rectal cancers reflects dietary control and stringencies in the depression of the '30s and in the last war and that the present hint of an increase is linked with the end of rationing. Or changes in the improved storage of food, or dietary fads and fashions, could be invoked.

Animal experiments established fairly convincingly that caloric restriction gives a reduced incidence of cancers of the alimentary canal. It is well known that certain normal or abnormal constituents of our diet can cause cancer in experimental animals. Examples are aromatic amines, nitroso compounds, polycyclic hydrocarbons and mycotoxins (notably aflatoxin). Some protective factors have been identified : vitamin A is protective in squamous epithelium and vitamine C may act by inhibiting nitrosamine formation.

Diet studied in man gives a very confused picture because of the complex nature of diet and the interactions with genetic and environmental factors ; migrant studies, international comparisons and case-control studies all add to it. As an illustration of the tangle : WHO data suggest that eating a lot of fish is associated with a high incidence of gastric cancer. But further investigation shows that Iceland and Japan provide extreme values and all other countries show no significant correlations.

Case-control studies* give confused pictures in gastric cancer ; little in the way of a recurrent theme can be picked out except that a protective effect of vegetables (especially fresh or raw) and fruit has frequently been suggested. In at least three studies, milk appeared to have some protective effect. So far is has not been possible adequately to investigate by epidemiological means the interesting question of the role of nitrosamines because of the difficulty in identifying populations having markedly contrasting intakes of cured meats but otherwise comparable. It is interesting to speculate, however, that the increased incidence of gastric cancer in patients with pernicious anaemia might the related to increased nitrosamine formation by the increased bacterial activity in the achlorhydric stomach (...).

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*A case-control study tests a hypothesis by identifying for each patient a number of controls matching the patient's attributes - such as age, sex, social class - as closely as possible.

Apart from the obvious difficulties of remembering, perhaps at many year's remove, what we ate, the study of the relation between diet and cancer is complicated because - as already pointed out - otherwise comparable subjects tend to have roughly comparable diets. And there is a complex interplay of risk and protective factors. In view of these problems it is perhaps not surprising that so little has emerged from case-control studies in colon cancer.

Special circumstances or populations can be identified, but the outcome is rarely simple. For example, Californian Seventh Day Adventists show only half the US national incidence of colo-rectal cancer. But not only do they not eat meat, they do not smoke or drink or use condiments. A large prospective study now in progress should help to clarify these effects.

The relation between diet and disease is an intriguing one. The importance of fresh vegetables and the protective roles of vitamins A and C merit assessment. It is, however, notoriously difficult to change eating habits (although if high-risk groups could be simply identified the effort might be more worthwhile). A study of the effects of wartime diet ought to be relatively easy, requiring only a search of mortality data. And in institutes, such as mental hospitals, with long-stay patients and uniform, well-documented diets, interesting studies could be carried out. Perhaps the greatest problem, however, is the complex interactions of different factors, which encourage the weaving of intellectual cats-cradles of hypothesis and counter-hypothesis.

There are hints of a genetic component in the indidence of gastrointestinal cancer. An example is the way incidence in migrant populations retains a slight bias towards the ancestral country's. (Of course, the extent to which racial (...).

It is important to try to distinguish between genetic and other familial factors. In principle, it should be possible to dissect out the relative contributions of genetic and other family (ie, environmental) influences ; for example, in man-and-wife studies. In stomach cancer there seems to be a two to threefold excess in blood relatives but the spouse is found not to be at higher risk. However, the aetiological factor could act early in life so that the evidence in favour of a genetic causation is blurred. Twin studies seem - on the sparse data available - to show higher concordance in identical twins than in fraternal ones.

A number of instances of high incidence of gastric cancer seem to be correlated with occupation. Coal mining is an example ; in a study which compared coal mining and other counties of Utah, a four-fold excess of stomach cancer in men (and a two-fold excess in women) was found in the coal mining areas. Other studies indicate a significantly raised, though small, risk ratio. The risk ratio varies with length of exposure and rises the longer the follow-up ; this is

well shown by studies of the effects of amosite (asbestos) dust. The connection between stomach cancer and occupation persists even after correction for social class effects. The common feature of most occupations showing an excess of gastric cancer seem to be dust (of whatever nature) ; in addition to coal and amosite miners, dockers, textile workers and warehousemen are all at risk. It would be interesting to know whether 'dust' is intrinsically carcinogenic or merely a vehicle for a carcinogen.

This problem merits continued analysis although it illustrates the danger of such exercises : they provoke exercises of imagination. For example, the correlation between occupational dust exposure and cancer could be conjuctured to be tied up with the common factor of beer drinking.

There is evidence for excess gastric cancer in rubber workers (which may go some way to explain - with dust from the potteries perhaps - why the area around Stoke-on-Trent shows a high incidence). Another interesting high incidence group is of workers involved in war-time munitions manufacture (...). Correlations have been suggested between gastro intestinal cancer and smoking and peptic ulcer (...). The question of a carcinogen in bracken has been repeatedly posed, both in connection with, variations in the sources of drinking water - from areas with a lot of bracken or little - or in the incidence in cattle fed on bracken ; this latter correlation is supported by some American studies of people who use young bracken fronds as a salad vegetable. The importance of alcohol in the causation of gastrointestinal cancer merits further investigation.

In general, well-planned epidemiological studies can be interesting and useful. But, in terms of cost-benefit and cost-effectiveness, they may be expensive. It may also be asked how much time can be justifiably spent on following up small group of workers ; but the studies have interest in establishing mechanisms of carcinogenesis and providing clues to more general indentification of risk factors, and, at least with industrial hazards, there are legal and moral sanctions that can be brought to bear with some prospect of reducing subsequent exposure of workers. We have no conviction at all that the identification of dietary factors will significantly change exposure to them unless some economic factor brings the forces of commercial persuasion into the side of the angels (40)".

However high the cost of epidemiological studies, it will certainly be lower than any "public health" investment misdirected because of wrong data - and the replacement of a public water supply system might be such a wrong decision if it was made on the existing, inconsistent evidence of any contribution of nitrates to increase the incidence of gastric cancers. Concerning the aetiologic hypothesis about nitrosamines" many drugs, such as the analgesic aminopyrine and the antidepressant nortriptylene, are readily converted either to a N-nitroso derivative, or to a simpler nitrosamine. Nitrates and nitrites have

long been recognised to be useful as food preservatives, and meals containing them can cause dramatic rises in gastric nitrite for up to an hour. The fasting gastric nitrite level is particularly high in pernicious anaemia. Dietary nitrate readily gives rise to nitrite in the saliva. The saliva of smokers is particularly rich in thiocyanate, which can catalyse very effectively the nitrosation of secondary amines. The reported protective effect of vitamin C might be associated with a capacity to reduce nitrosamine formation.

At present the evidence is insufficient to show a persuasive association of nitrosamines with human gastric and colo-rectal cancers, although gastric cancers seems to be more common in areas where there is a high nitrate (eg from agriculture use) content of water supplies. At least two studies are planned or in progress in the UK in such areas (Worksop and East Anglia). Nitrosamines have been found in high concentrations in the urines of Egyptian bladder cancer patients.

Apart from the dietary implications of nitrosamines, nitrogen oxides are powerful nitrosating agents and may be serious environmental pollutants from combustion.

An understanting of the way nitrosamines are formed in the gut could be important, for example, in developing pharmaceuticals. And further exploration of the relation between molecular structures and carcinogenic potency might be of interest in the development of chemotherapeutic agents. Work on nitrosamines seems, on the whole, to be well represented at a variety of levels but opportunities should be taken to support work directly related to the significance of nitrosamines in the aetiology of human tumours. The obvious hypothesis of a relation with preserved food would not be easy to test because of the difficulty of establishing significant variations within groups of otherwise comparable subjects.

Another approach to the analysis of aetiology is to combine metabolic and epidemiological techniques ; an interesting example of the application of 'metabolic epidemiology' is in the study of the ways in which certain clostridia among gut bacteria seem to convert dietary components and intestinal secretions into carcinogens (40)".

After this review, one may safely share the opinion of the director of the U.S. Environmental Protection Agency's Criteria and Standards Division, Office of Drinking Water - the hypothesis that nitrates in water can be converted to nitrosamines and therefore contribute to the incidence of cancer has not been proven to this day (41).

As cancer risks are as yet unproven, there are no reasons to replace existing water wells in our region, at least from this point of view.

And what about methemoglobinemia risks ?

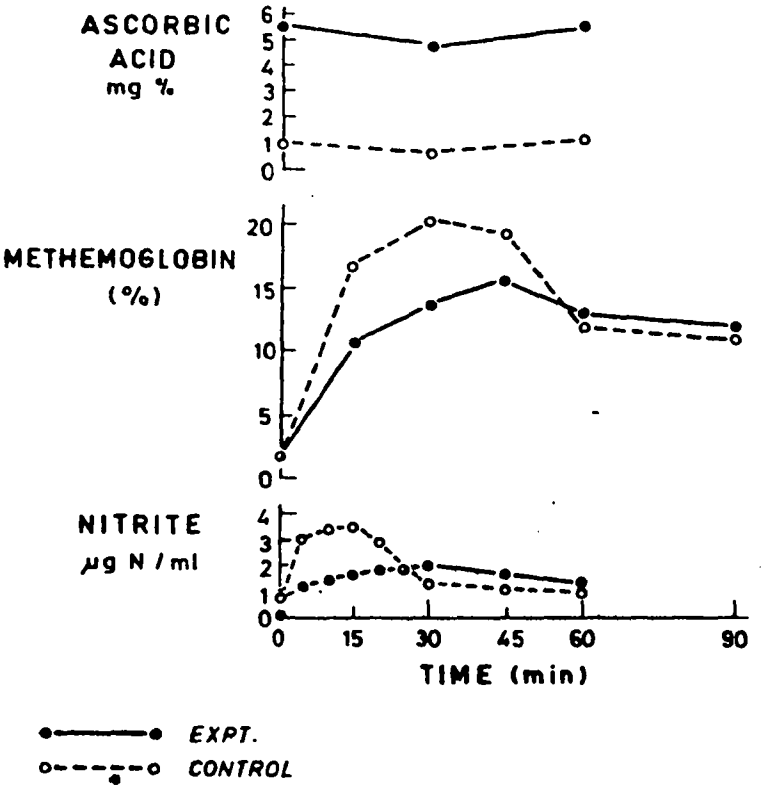
These are well known, and are not related directly to nitrates in water, as an infection of the digestive tract in necessary to cause the disorder (5,6,7). Moreover, even when there is an infection, the disease may be prevented by a suitable diet.

The best diet is, of course, breast feeding, at least during the first two or three months, when the methemoglobinemia risks exist (13). If this is not possible, undiluted milk feeding would provide a similar, total, safety (5). In France, prevention of methemoglobinemia is already a well-established policy, as baby feeding formulae are generally diluted with bottled, "mineral" water. This is an expensive policy (42).

In Israel, citrus and tomato juices are usually included in baby diets. There is some evidence that their vitamin C reduces methemoglobin levels, although the study mentioning this fact is rather unconvincing because of its methods. No relationships between nitrate intake and methomoglobin levels have been established (43). Laboratory studies on rats are more convincing (see figure 3), but one has to believe even better results could be obtained in newborn infants, in order to prevent methemoglobinemia (44).

Another prevention method has been experimented in Czechoslovakia, where methemoglobinemia was a major problem. From 1948 to 1960, 314 cases were reported. In 40 communities of the Prague area, 115 cases were found from 1953 to 1962. These represented 2 % of all babies born during that period (5,800). Out of the 115 cases, 69 (60 %) were severe, 46 (40 %) light. For the latter, substitution of pure water for that containing nitrates was sufficient to stop the disorder. For the severe cases, hospitalization was necessary, and there were 10 deaths (8 % of total cases) (it seems that methylene blue was not used in that country to treat methemoglobinemia). Zdenek Knotek and Pavel Schmidt thus concentrated their attention on dried milks as a necessary factor for the disorder "and succeeded in proving their content of spores of the B. subtilis group, almost in pure culture. These sporulating microbes intensively reduce nitrates to nitrite (...).

The finding that all infants with clinical or inapparent methemoglobinemia were fed on the dried-milk preparations (...) was important. These dried milks - commonly used for artificial feeding of infants - contain, as all the other dried-milk products do, the sporulating microorganisms of B. subtilis. It is not possible to supress these microbes in the production of the dried milk or to avoid deterioration of the product.



3. Ascorbic acid intake and nitrite and methemoglobin levels in rats administered 20 mg/kg Na NO_2 at time zero (from Shuval and Gruener, 1977, p. 87).

None of the infants with clinical or inapparent methemoglobinemia was fed on the dried buttermilk (...). As we have proved, this preparation does not reduce nitrates to nitrites.

Two infants hospitalized with severe anomalies of the nervous system were studied. The first infant, of female sex, aged 35 days, body weight 2,500 gm, was fed 6 times a day on the 10 % solution of the dried milk. The infant was examined clinically and blood samples were taken for methemoglobin. The level of methemoglobin in the infant

observed had already risen to 2,11 % (probably because of reduction in the enzyme activity in the infant since this methemoglobin concentration was ascertained before the infant became ill, and was found to be the same after recovery). Apparently, for the infant observed, this methemoglobin concentration was at a physiological level and constant. Afterward, 500 mg Na NO_3 in tea were given and, as indicated in Figure 5, blood samples were taken for the examination of methemoglobin and the infant was also clinically examined. On the following day another 500 mg. Na NO_3 was administered. On the first day of Na NO_3 intake, the amount of methemoglobin reached 3.83 % after 6 hours and after 12 hours it returned to the original value. On the second day it increased after 6 hours following another dose of nitrate to 5.03 and after 12 hours it returned again to the original value. The infant was slightly cyanotic around the mouth and the acral portions of the body ; before the rise of methemoglobin its pulse rate increased (...).

Nitrates were not converted to nitrites in the doses of 200-1000 units/ 10 ml milk. It can be supposed that efficiency of nisin will be greater if the condition of its maximal solubility (low pH) is observed, as was not the case in our experiments.

On the third day the infant was given dried buttermilk, and after a week of this artificial feeding 3 more doses of 500 mg Na NO_3, in 3 days were given. When the feeding was changed to the dried buttermilk, repeated administration of nitrates was not accompanied by any clinical signs of methemoglobinemia and the level of methemoglobin ranged between the limits of the original values.

The second infant (of male sex, 72 days old, body weight 3.700 gm, fed 6 times a day on the 10 % solution of the dried buttermilk) was given daily doses of 50 mg Na NO_3 in tea for 4 days, and the methemoglobin level in blood was observed. It dit not exceed the original value of 0.86 %.

Thus, when the infants were fed on dried buttermilk and given nitrates the methemoglobin remained at physiological levels.

This fact was confirmed by the case of an infant in an institution in the vicinity of Prague, who became ill with nitrate alimentary methemoglobinemia, when fed on dried milk diluted with water of nitrate concentration exceeding 50 mg/lit. On the 15th day of hospitalization, nitrate methemoglobinemia with dyspepsia was evident. For treatment of dyspepsia, dried buttermilk, diluted with (bottled) water, was introduced. The infant began to consume larger doses of artificial feeding, its body weight increased and the signs of the disease disappeared".

Later on the nitrate concentration of the bottled water was found do be 100 mg/1. Although the NO_3 intake had at least been doubled, the baby recovered quickly.

The preventive effects of buttermilk may be explained by its microbial flora, as it differs from other milks only from this point of view. Specimens of three milks were diluted with water containing 600 mg/l NO_3 and put in an autoclave for two hours to destroy their microbial flora. A specimen of buttermilk was also prepared in the same way and submitted to the same treatment. It was found that nitrates were transformed to nitrites in all three milks, even without the bacteria of lactic fermentation. Only in buttermilk nitrates were not reduced. It was therefore evident that the substance effective in preventing methemoglobinemia that existed in dried buttermilk was independent of the vitality of bacteria, as it persisted after their destruction.

"It is known that S. lactis contained in the buttermilk produces nisins exerting an antibiotic effect and able to prevent the growth of sporulating microorganisms. For this reason we carried out another experiment in which 10 ml dried milk was diluted with water containing 600 mg NO_3 and nisin was added (English preparation Nisaplin-Research, Dir. Alin and Barret, England, 1 gm = 1 000 000 units), diluted in the physiological solution ; after 15 hours reduction of nitrates to nitrites was estimated with the Griese reagent (...).

Studies carried out in vitro and in vivo indicate that the microorganisms of B. subtilis get into the digestive tract almost in pure culture with the artificial feeding on types of dried milk capable of reducing nitrates to nitrites. Growth of these organisms is not prevented when the milk is diluted directly before the feeding of the infant. Their growth can occur during the course of digestion and is supported by continually new intake of sporulating microorganisms. Nitrites, thus formed from nitrates in water, are absorbed and retained in the blood. The consequence of their presence in blood is the rise of methemoglobin over normal values (...).

The finding of the preventive effect of the buttermilk is important ; our experiments with nisin suggest that this is related to the amount of microbes of lactic fermentation and the antibiotic effect of their production of nisin in the dried buttermilk. This finding explains the effects of the dried buttermilk on the intestinal bacterial flora in infants and suggests other dietary effects of this food (...).

Artificially fed infants in areas with high concentrations of nitrates in the water are rather often affected with inapparent methemoglobinemia. Consequently it is necessary to assume that artificially fed infants in such areas are in the state of intoxication with nitrites. For this reason medical treatment of the methemoglobinemia does not suffice and it is necessary to prevent the formation of nitrites in the ingestion tract of infants. It would be logical to use water of lower nitrate content for the dilution of the milk. But in the affected areas this is often impossible, as they are relatively large and all wells contain nitrates (...).

The use of dried buttermilk as a basis for artificial feeding in the first 3 months of life is a suitable preventive measure. As the buttermilk used contains 1.4 % fat, we need not fear any lack of fat in such a food intake (45)".

Thus, the disease may be prevented by feeding infants under three months, who are at risk,

1. at the breast (13)
2. with undiluted milk (5)
3. with buttermilk (45)
4. with dry milk diluted with bottled water without nitrates.

The last one is the French solution. It is expensive (42), while the other policies cost nothing although they require medical assistance, which is also needed to prevent all kinds of perinatal risks.

Prevention policies that cost nothing are, of course, a very cheap solution, distribution while the replacement of water wells and of part of a water system would cost million of dollars.

At what concentration of nitrates in water supplies shall the attention of physicians be drawn to the risk of methemoglobinemia ? The old, scarce epidemiologic evidence gathered a quarter of a century ago (9) is still the "scientific" base of water quality standards, including the new ones which are to be enforced soon for the European Economic Community. These are based on two "risk" thresholds, 50 mg/l of nitrates for methemoglobinemia, 100 mg/l for... cancer - although there is as yet no evidence that nitrates contribute to the incidence of cancer of the digestive tract. Moreover, it seems difficult to establish relationships between nitrate concentrations and health risks (46).

However, limits are being fixed. One hopes that they are not unforceable, and that communities will not be obliged to spend huge sums of money to replace water wells that are not really dangerous, if a prevention policy is carefully implemented by local physicians. The French "limit" is a mere warning, which is quite sensible. And one also may tbinck that California health authorities are not too bold in recommending the discontinuation of a source only when nitrates are above 90 mg/l, and in requiring the surveillance of infants' health. A similar policy has been adopted in Israel, where low nitrate water is to be made available to infants under one year (43).

But as cases have been noticed at concentrations as low as 30 mg/l/ NO_3 (16,17), it would seem sensible to issue warnings to health officials and physicians when nitrates in water supplies reach this

(low) level. 20 mg/l/NO_3 may also be considered a "safe" limit according to evidence (18). But no investment in new water supplies can be decided on the mere observation of nitrate concentrations in existing wells.

The origin of nitrates shall also be better known, in order to protect aquifers from fertilizers and polluted water.

Moreover, the health effects of nitrate must be monitored. This is what we will do. A preliminary epidemiological study has been completed (47). Although it did show that cancer incidence was higher where nitrate concentrations were lower, it must be followed by another research based on the national computer register of cancer deaths. Informations will be exchanged with the British team in charge of the new epidemiological research.

Another problem shall be monitored - methemoglobinemia in patients with chronic renal failure (48) - although there is only one such case in the literature, caused by a family well. Its water contained 94 mg/l and produced the disease in a 56-year old home dyalisis patient. However, anemia secondary to chronic renal failure may have predisposed this patient to develop a severe form of methemoglobinemia : in a patient with a hemoglobin level of only 5.7 g/100 ml, the oxygen-carrying capacity of the blood was to be diminished much more than in a nonanemic individual. Such a patient would probably show the symptoms of methemoglobinemia at levels of 2 g methemoglobin/100 ml of blood (49). Moreover, the incidence of cancers is higher among dyalisis patients than in the population as a whole. Physicians have to follow strict water quality standards for dyalisates - aluminum (48), copper (50,51), calcium and magnesium (52) and possibly fluoride (53) being also dangerous for chronic renal patients. Water quality standards are enforced through treatment systems that may also be used, if necessary, for drinking water.

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