Studies reported here are part of a program to reevaluate current nitrate and nitrite standards for water and food. Included are field studies among infants in areas with varying concentrations of nitrates in water supplies as well as experimental studies on laboratory animals. Findings are reported and discussed. The detection of irreversible electrical brain activity changes in rats receiving low doses of nitrites in drinking water is of particular concern.

Epidemiological and Toxicological Aspects of Nitrates and Nitrites in the Environment

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Introduction

Nitrates and nitrites are frequently ingested by large segments of the population in drinking water and food. The ever-increasing concentrations of nitrogen salts in water are mainly a result of pollution by organic solid and liquid wastes and inorganic chemical fertilizers used extensively in agriculture. Nitrates and nitrites are found as natural components of many vegetables such as spinach and rhubarb but also are used widely as food additives in sausages and other forms of processed meat.

Infant methemoglobinemia resulting from the consumption of water with high nitrate concentrations was first recognized clinically by Comly in 1945.[1] Since then about 2,000 cases, including many fatal poisonings in infants resulting from ingestion of water containing nitrates have been recorded in various countries throughout the world.[2] The Public Health Service Drinking Water Standards[3] recommend that water consumed by infants not contain over 45 mg/l of nitrates as NO_3. The World Health Organization has made a similar recommendation. These standards were originally based on limited epidemiological evidence gathered by Walton in 1951,[4] indicating that no cases of methemoglobinemia had been reported in the United States when water containing less than 45 mg/l of NO_3 was consumed. More recent studies in Europe have, however, reported on clinical and subclinical cases of the disease in infants consuming water having less than 45 mg/l of NO_3.[5]

Recently there has been pressure to relax the nitrate standard in drinking water in areas exposed to increasing nitrate pollution of ground water in which overt cases of infant methemoglobinemia are rarely reported. The Department of Health in California has issued administrative orders which require surveillance of infants' health in communities supplying water containing up to 90 mg/l NO_3 while recommending the discontinuation of the source only at higher concentrations. The W.H.O. Recommended Drinking Water Standard for Europe - 1970 more or less have followed suit. The 1971 International Drinking Water Standards of the W.H.O. retained, however, the recommended maximum limit of 45 mg/l.

Allowable concentrations of nitrates and nitrites have been set in the U.S. by the Food and Drug Administration at 200 ppm for most "corned" meat products. This standard is supported by toxicological work carried out prior to the establishment of the rigorous testing procedures currently practiced.[6] Methemoglobinemia, in some cases fatal, has been reported from the consumption of meat products containing nitrates in excess of allowable concentrations as well as from the consumption of unadulterated spinach naturally containing high concentrations of nitrates.[7]

The series of studies reported upon here are part of a broad spectrum program to re-evaluate the current nitrate and nitrite standards for water and food, and include epidemiological field studies among infants in areas with medium-high and low concentrations of nitrates in the water supplies as well as various experimental studies in laboratory animals. Of particular interest was the possible presence of chronic sub-clinical methemoglobinemia in infants in the areas of Israel having medium-high concentrations of nitrates in the water supply but few overt clinical cases, as reported on by Knotek and Schmidt in Czechoslavakia.[5]

Field Studies

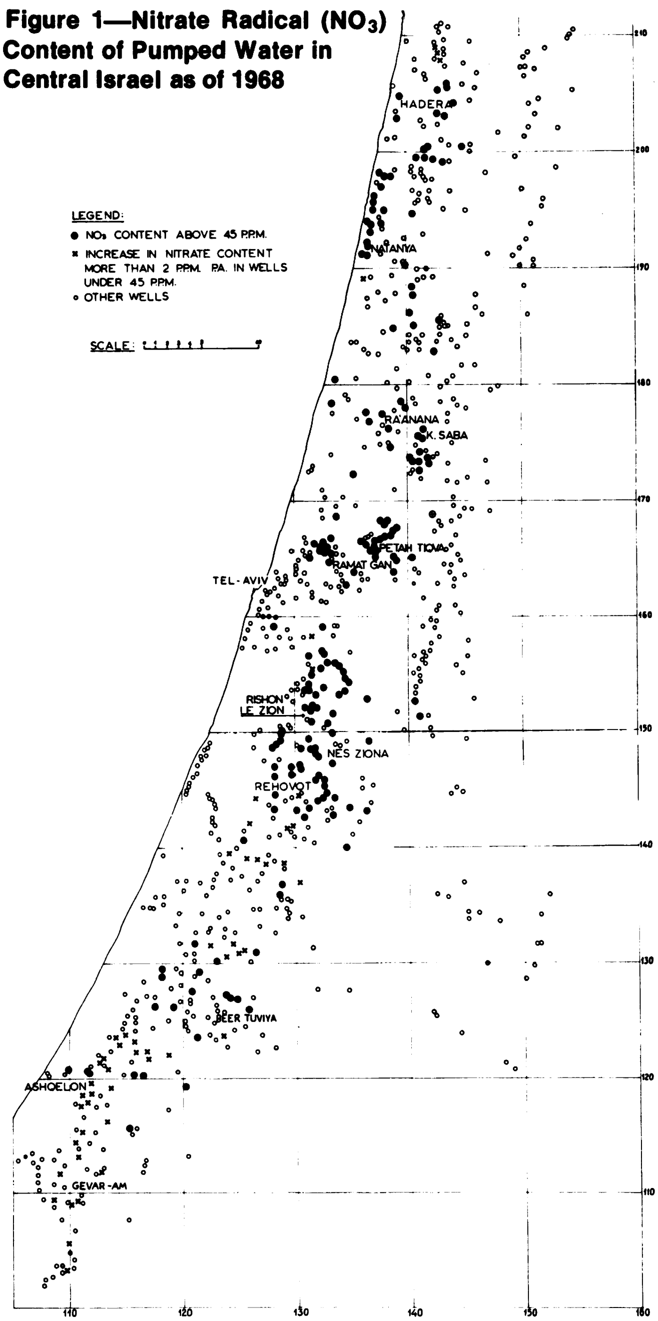
2,473 infants were studied in communities with medium-high and low concentrations of nitrates in the drinking water. The study area is on the coastal plain of Israel and includes the towns of Rehovot, Rishon le Zion, and Nes Ziona as well as a number of small agricultural settlements near Natanya (see Figure 1). The nitrate concentration is most of the water supply wells in these communities varied from 50-90 mg/l. However, since the integrated water supply systems in each community are served by a number of wells of different nitrate content, the actual NO_3 concentration at the tap in a given home may vary from hour to hour depending on the pumping rates and water demand. Infants were examined at well-baby clinics run on a routine basis by the Ministry of Health, or by the Kupat Holim-Medical Insurance scheme, so that it was not practical to determine the exact concentration of nitrates at the home tap of the infant examined at the time of examination.

For the purposes of this analysis all 1,702 infants from the study area are considered as one group with a mean of about 70 mg/l. Jerusalem, with 5 mg/l of nitrates in the water supply was chosen as a control area, and 758 infants were examined at Municipal Health Department well-baby clinics.

A 0.2 ml fingertip blood sample was taken from each infant studied by project staff nurses and examined for hemoglobin and methemoglobin (MetHb) in the laboratory in Jerusalem. An accurate and precise micromethod for testing MetHb was developed to meet the needs of this study and similar field studies. The method is a modification of the classical Evelyn and Malloy[8] method and has been reported upon previously.[9] Blood samples are stabilized so that they can be taken in distant communities and transported to the laboratory for assay within 24 hours rather than within less than an hour as required by the conventional procedure. This method can show differences in MetHb as small as .1% which is essential in a study aimed at detecting slightly raised levels of MetHb in a healthy population where normal MetHb levels are expected to be about 1% of total hemoglobin.

A detailed questionnaire was filled in by the project nurse for each infant, based on information supplied by the mother and the files of the well-baby clinic. Details on age, sex, weight, ethnic background, health status and nutritional regime were recorded and together with the laboratory results were punched on cards for computer analysis. Particular attention was given to intake of tap water with milk formula or from other sources.

Figure 1—Nitrate Radical (NO_3) Content of Pumped Water in Central Israel as of 1968



Results of Field Study

There are essentially no differences between the mean MetHb levels in the study group and the control group as shown by Table 1. The mean MetHb level for the entire population studied was 1.04% with a standard deviation of .72%. For the most susceptible age group of 1-60 days the mean MetHb for the study group is 1.38% while being 1.30% for the controls. The mean for both groups is 1.33%. The differences are not significant. For all ages, the mean MetHb per cent in the control population is actually higher than that of the study population, but this is not statistically significant. However, on further analysis it was shown that 31.5% of the infants up to 60 days of age in the study population have MetHb levels higher than 1.7% as against 21.9% for the controls.

Only 6% of the infants studied received powdered milk formula made up with tap water. The remaining 94% of the infants were either breast-fed and/or received whole cow's milk. Those infants fed with powdered milk formula in the study area showed somewhat higher MetHb per cent than those fed exclusively on other types of milk (see Table 2). There are only 36 infants in this category and the differences are not significant.

49% of the infants in the 1-60 day age group consumed either citrus or tomato juice or both. For the 61-90 day group 80% and over 91 days 91% consumed such vitamin C rich foods. The mean consumption for the total population studied was 87%. In Table 3 the MetHb levels in infants with or without vitamin C rich foods in their diet are presented. The 1-90 day age group of the study population consuming citrus or tomato juice show somewhat lower MetHb levels as compared to the non-consumers. In the control group and in those over 91 days in age there were no differences.

In the 1-90 day age group infants reported to be suffering from diarrhea on the day of examination or within the last month showed higher MetHb in both the study and control area (see Table 4). In the study area, 1-90 day infants with diarrhea had a mean of 1.78% MetHb as compared to 1.16% in infants not suffering. Infants of 1-90 days with diarrhea from the study area showed higher MetHb than those from the control area. The numbers in this category are small and the differences are not significant.

There were essentially no differences among infants above 91 days in age.

Table 1—Methemoglobin in Infants in Areas With High and Low Nitrate Concentrations in Drinking Water

	Mean NO_3 Mg/1	1-60 days n	1-60 days MetHb%	61-90 days n	61-90 days MetHb%	91+ days n	91+ days MetHb%	All ages n	All ages MetHb%	S.D.
Control area	5	96	1.30	75	1.24	556	.97	758*	1.11	.72
Study area	50-90	71	1.38	188	1.14	1426	.99	1702*	1.01	.72
Total		167	1.33	263	1.17	1995	.98	2473*	1.04	.72

* Total includes infants with age unknown.

Table 2—Methemoglobin in Infants Drinking Powdered Milk Formula and Only Other Forms of Milk

	Powdered Milk n	Powdered Milk MetHb%	Only other Forms of Milk n	Only other Forms of Milk MetHb%
Control area	111	.98	664	1.14
Study area	36	1.17	1666	1.00
Total	147	1.01	2310	1.04

MetHb is significantly higher in the first 60 days of life in both study and control populations (Table 1). Table 5 shows that the mean for both groups is 1.33% for the 1-60 day group, and 1.17% for the 61-90 day group. Infants over 90 days show MetHb levels of about 1%, with the mean for the total population studied of 1.04%. The same patterns are found with present weight as shown in Table 6.

Results of Laboratory Studies

Many questions concerning the toxicological effect of exposure to nitrates and nitrites in drinking water or food can be elucidated only by experimental studies carried out under laboratory conditions. Some of the major findings of our toxicological investigations on nitrites will be reported upon here.

Chronic Exposure of Rats to Sodium Nitrite in Drinking Water

Previous chronic studies by others have not shown significant MetHb levels or other pathological findings in rats consuming water containing nitrites as high as 2000 mg/l.[10] Our studies of the kinetics of MetHb disappearance after the administration of nitrites showed that the MetHb level is reduced by 50% every 90 minutes. We have also shown that rats being nocturnal animals consume 80% of their water at night and that by 10 A.M. they may no longer show significant MetHb levels, even when consuming water high in nitrites. Our measurements showed a peak in water consumption and MetHb level in the middle of the night. In designing our chronic studies we adjusted the "day" and "night" hours in the animal rooms so that blood examinations would be taken at about the rats "midnight," so as to detect the expected maximum daily MetHb concentration.

Five groups of male 3-month-old rats were used

Table 3—Methemoglobin in Infants With and Without Citrus or Tomato Juice in Diet

	Age 1-90 days with n	with MetHb%	without n	without MetHb%	Age 91+ days with n	with MetHb%	without n	without MetHb%
Control area	65	1.28	106	1.27	452	1.03	104	1.09
Study area	226	1.19	33	1.30	1366	.97	73	.98
Total	291	1.21	139	1.28	1818	.98	.77	1.04

Table 4—Methemoglobin in Infants With and Without Diarrhea

	Age 1-90 days with n	with MetHb%	without n	without MetHb%	Age 91+ days with n	with MetHb%	without n	without MetHb%
Control area	33	1.43	137	1.23	186	.99	369	1.06
Study area	20	1.78	239	1.16	165	1.01	1254	.96
Total	53	1.56	376	1.18	352	1.00	1635	.98

Table 5—Methemoglobin as a Function of Age

Age in Days	n	MetHb%
1-60	167	1.33
61-90	263	1.17
91-120	235	1.07
121-180	393	.95
181-270	461	1.00
271-360	411	.94
361-450	277	.99
451-540	161	.97
541+	57	.86
Unknown	48	-
All ages	2473	1.04

Table 6—Methemoglobin by Present Weight

Wgt in Kgs.	n	MetHb
0- 4.0	38	1.37
4.1- 5.0	204	1.30
5.1- 6.0	303	1.13
6.1- 7.0	341	1.08
7.1- 8.0	408	.99
8.1- 9.0	431	.96
9.1-10.0	317	.93
10.1-12.0	323	.99
12 -	38	.97
Unknown	70	-
Total	2437	1.04

with 8 per group. The first group "A" received normal tap water was the control. The four other groups received drinking water containing sodium nitrite at the following concentrations: "B" - 100, "C" - 1000, "D" - 2000 and "E" - 3000 mg/l.

Rats were weighed monthly and samples of tail blood were taken for determination of MetH and Hb. After 24 months, the animals were sacrificed and examined for gross pathology as well as for histological differences.

In summary, the findings of this study which can be considered only as a pretest for the full scale chronic toxicity experiments in progress are as follows: a) There were no significant differences in growth and development or mortality between the controls and the experimental group. b) There were no significant differences in Hb levels between the controls and experimental group. c) The MetHb levels of experimental groups C, D and E were significantly raised throughout the study and averaged respectively 5%, 12% and 22%. The MetHb level of "B"—receiving 100 mg/l of sodium nitrite was slightly raised above the controls for the first 60 days of the study only. Afterwards the level of "B" and "A", the controls, were identical. d) Examinations for blood glucose, pyruvate and lactate did not show any differences between the controls and experimental group. e) Pathology: At the end of 24 months' exposure to nitrites, the five groups were sacrificed. The animals were anesthetized with ether, bled, and their internal organs were inspected, weighed, and fixed in 10% neutral formaline.

Histological examinations were done on tissues from the heart, lungs, kidneys, liver, spleen, pancreas, adrenals and some brains.

The last three organs showed no pathological features in any of the rats. The liver and spleen were frequently congested, while the kidneys sometimes showed focal inflammatory and degenerative changes. The main pathological changes in the experimental group were noted in the lungs and heart.

In the lungs, the bronchi were frequently dilated, their walls infiltrated with lymphocytes, while the mucosa and muscle were often atrophied. Frankly purulent bronchial exudate also occurred. Interstitial round cells and fibrosis were sometimes encountered, emphysema was the rule. These changes, while present in one or two rats of the control group and in group "B" (100 mg/l NO_2), were found with increasing frequency and severity in the last three groups treated with higher NO_2 doses.

In the heart, there were small foci of cells and fibrosis in some animals, while a mode diffuse interstitial cellularity with pronounced degenerative foci was frequent in the highest NO_2 groups only. The oil red O stain showed no increase lipid deposits in the hearts of the experimental animals.

The intramural coronary arteries provided the surprising feature of this study. In most of the control animals the blood vessels showed some degree of thickening and often even a marked hypertrophy and narrowing. In the experimental groups, and especially in group E, who received about 250-350 mg/kg of $NaNO_2$ for 2 years, the coronaries were thin and dilated, their appearance not what is usually seen in animals of advanced age.

The findings in the lungs and heart, although per se non-specific, may be directly related to the experimental procedures, but intercurrent etiological factors must not be excluded. The lack of the usual aging changes in the coronary arteries is noteworthy and will be further investigated.

In each group tissue was taken from the heart for electronmicroscopic examinations. Full details of the pathological findings are to be reported upon elsewhere.

The Effects of the Administration of Sodium Nitrite in Drinking Water on Pregnant Rats and their Newborn

Two experimental groups were used, each containing twelve pregnant rats. Group I was given 2000 mg/l sodium nitrite and Group II, 300 mg/l in their drinking water. The control groups, of seven pregnant rats, received water without added nitrite.

The pregnant rats exhibited higher susceptibility to nitrites than non-pregnant rats. They showed higher MetHb levels than non-pregnant rats receiving the same doses.

Pregnant rats receiving 2000 mg/l in their water (Group I) developed enemia with Hb levels of 10.3 ± 1.5 gm % as compared to 14.2 ± 0.9 gm % Hb in non-pregnant controls.

There was a pronounced effect on mortality, among newborn rats of dams receiving 2000 mg/l (Group I) and 3000 mg/l (Group II) in their water, particularly in the three-week period up till weaning.

The litters in the control group contained 10 fetuses, 9.5 in Group I and 8.5 fetuses in Group II. The general mortality was 6% in the control, as opposed to 30% in Group I and 53% in Group II. Birthweights were similar with 5.5 gm. for each group. However, after birth, pups in Group I and II lagged behind the controls in their growth rates. For example, after 1 week the mean weights were 16.5 gm. in the control group, 12.0 gm. in Group I and 9.5 gm. in Group II. After 21 days (at the end of the period of giving nitrites to the dams), the mean weight in the control group was 51.5 gm. as compared to 29.5 mg. in Group I and 18.5 gm. in Group II.

Apart from the weights, a characteristic difference observed in the pups of the experimental groups was that the fur thinned and lost its luster. After separation from their dams and being put on water, there was an improvement in growth in the experimental groups. At 62 days the control group attained a mean weight of 213 gm., Group I, 181 gm. and Group II, 172 gm.

During the whole period from birth to weaning, the pups showed no abnormally high MHb. The mean hemoglobin of the pups from the experimental groups was low—about 20% less than that of the current group.

Induced Methemoglobinemia by Transplacental Transport of Nitrites in Rats

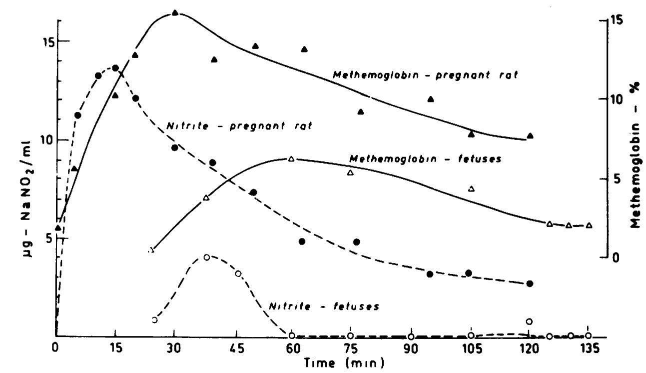
Since clinical methemoglobinemia from nitrates in water apparently appears only in babies, one possible solution proposed for areas with high nitrate water is to supply the infants with bottles of low nitrate content water from alternative sources. This measure will not exclude the risk of the exposure to nitrates in the prenatal stage, i.e., the transfer of nitrates or nitrites through the placenta to the fetus, where methemoglobinemia may be induced. Pregnant women can also consume nitrites in "corned" meat products.

The possibility of this occurring was tested on pregnant rats. Nitrites were given in drinking water or by injection to the pregnant rats and subsequently nitrites and methemoglobinemia were tested in the fetal blood.[11]

Suckling rats whose dams got nitrites in the drinking water only after giving birth showed no rise in MetHb levels. By contrast, their dams showed high MetHb levels. This demonstrates that nitrites are apparently not transferred in appreciable amounts to the pups via the milk.

The transfer of nitrites to the fetus in situ and the production of MetHb was tested in the following experiment. Pregnant white albino rats were used in this experiment. Each pregnant rat was weighed and anesthetized with ether. Suitable quantities of blood were collected from the tail at regular intervals. After opening the abdomen, the fetuses were removed serially over a two-hour period, the umbilical cords being cauterized and the fetuses washed in saline solution at 37° C, and then decapitated. Blood was collected and the initial MetHb and nitrite levels were determined. From 2.5 - 50 mg/kg of sodium nitrite were given orally or by injection to the pregnant rat and the kinetics of nitrites and MetHb in the dam as well as in the fetuses were measured. The micro-methods developed by our group for determining MetHb and nitrites in blood enabled us to carry out these experiments with the small amount of blood available from each fetus.[9 12]

Figure 2—Kinetics of Nitrite and Methemoglobin in Bloou of a Pregnant Rat and the Fetuses (30 mg /kg NaNo₂ per os)



The characteristic picture obtained is shown in Figure 2. Nitrites rose in the fetal blood though with a lag of about 20 minutes behind the mother. This rise in nitrite was followed by a rise in MetHb. The kinetic picture was similar between the dam and the fetuses. Different concentration of nitrites caused similar effects differing only in their timing and MetHb levels. The possibility that the placenta was damaged during the experiment and that this led to increased permeability was excluded when sodium nitrite was given to the pregnant rat after the birth had started. The first fetus showed a normal MetHb level of 1.6% but those who were born after the chemical had been given showed levels of 10.1% MetHb and 0.3 ug/ml of sodium nitrite in their blood. The births were unassisted.

The threshold of the transplacental transfer effect was at a sodium nitrite does of 2.5 mg/kg. The increase in effect was steep with increased sodium nitrite dosage. Pregnant rats exhibited a higher susceptibility to develop methemoglobinemia from nitrite doses than non-pregnant rats in acute or chronic experiments.

These experiments demonstrate the transfer of nitrites through the placenta and the production of MetHb in the fetus. The low levels of methemoglobin found in pups 12 hours after birth from dams receiving nitrites in their water can be explained by the rapid recovery of the methemoglobinemia even in the newborn rats. In mature rats, the time needed for the reduction of half of the MetHb level is about 90 hours. The results underline the possible risk of intrauterine methemoglobinemia when water or food containing nitrates or nitrates is given during pregnancy. It is, however, difficult to extrapolate from these acute animal experiments to the situation that may exist with humans consuming low levels of MetHb inducing chemicals. A study of MetHb levels in infants cord blood among mothers from high and low nitrate areas is planned to help clarify this question.

Behavioral Studies in Mice Chronically Exposed to Sodium Nitrites in Water

In an effort to develop sensitive tests to detect possible effects of sub-clinical methemoglobinemia, behavioral studies with mice were undertaken.[13] Groups of 57 black 6J male mice were given nitrites in their drinking water at doses aimed at producing MetHb levels varying from slightly above normal to 15% which can be considered in the sub-clinical range. $NaNO_2$ doses in water were 100, 1000, 1500, 2000 mg/l. Controls received tap water. The level of motor activity was determined in a special activity box designed for psychological studies with mice.[14]

Analysis of the results shows a decided and significant reduction of overall motor activity in the groups receiving the highest levels of nitrites. There is a significant inverse relation between MetHb level and activity with a coefficient of correlation of .65. An effort to counteract the methemoglobinemia was made by giving the group receiving the highest level of nitrites (2000 mg/l) vitamin C. The effect was to reduce the MetHb levels close to normal but the activity level of the group so treated remained low and about equal to the equivalent group receiving no antidote.

These experiments seem to indicate that nitrites have some form of sedative effect on the mice treated, not necessarily associated with the development of methemoglobinemia. The E.E.G. studies of rats treated with nitrites were expected to throw some light on these findings.

Brain Electrical Activity Changes in Rats Resulting from Chronic Exposure to Sodium Nitrite in Water

Studies were initiated to determine whether there were any detectable brain electrical activity changes in rats exposed to varying levels of sodium nitrite in their drinking water.[15] It was the specific objective of these studies to develop sensitive neurophysiological parameters to measure the effects of nitrite consumption leading to sub-clinical methemoglobinemia.

Electrodes were implanted on the cortex of 4 groups of 3-month-old male rats. Group A, the controls, received tap water; Group B, 100 mg/l; Group C, 300 mg/l; and D, 2,000 mg/l of $NaNO_2$. For auto-controls E.E.G. recordings were made on each rat several times before starting the regime of nitrites in their drinking water. After exposure to nitrites regular E.E.G. recordings were made. After 2 months, nitrites were removed from the drinking water. E.E.G. recordings were taken at intervals for another 4½ month period. Analysis of the results shows that there is an increase in the frequency of the E.E.G. waves in the experimental group D over the controls, while groups B and C showed lower frequencies than the controls. Rats in all the experimental groups showed paroxysmal outbursts not appearing in the control group or in their own pre-nitrite E.E.G. recordings. On ending of nitrite intake the electrical outbursts disappeared only in Group B which received 100 mg/l in their water, which nevertheless showed diffused spikes and sharp waves. All other groups showed continuation of the same type of E.E.G. outbursts over the recovery period as appeared during the exposure to nitrites. This appears to indicate some form of irreversible brain electrical changes resulting from chronic exposure to nitrites even at the 100 mg/l level.

This study will serve as a pretest for a longer, more detailed study of these phenomena, during which it is hoped to further define the characteristics of the E.E.G. changes, and their cause.

Discussion

Field Studies

The fact that there are no significant differences between the mean MetHb levels in the 1,720 infants in the study area with a mean nitrate concentration of 70 mg/l in the drinking water as compared to the 758 infants in the control area does not support the findings of subclinical methemoglobinemia reported by Knotek and Schmidt[5] in Czechoslovakia. A possible explanation for this lies in the differences in infant feeding practices found in Israel where only 6% of the infants included in the study received appreciable amounts of tap water together with formula prepared from powdered milk. Preliminary measurements of fluid intake in infants 2-3 months of age indicate that even during hot dry periods their intake of tap water given as a supplement is about 20% of their total daily liquid intake, which is mainly made up of mothers' milk or cows' milk. Apparently all of the infants studied by Knotek and Schmidt received appreciable amount of tap water rich in nitrates since infant feeding was almost universally based on milk powder formula. Another factor may be the widely practiced feeding of vitamin C rich foods such as citrus and tomato juice to infants. Both foods are inexpensive and available during most of the year in Israel. 87% of the infant population studied consumed such vitamin C rich foods which are known to be an effective antidote to methemoglobinemia. In Central Europe it can be assumed that such vitamin C rich food supplements are expensive and not always in supply so that they are much less frequently used.

The finding that infants in the study area receiving powdered milk formula or no vitamin C rich food supplements had slightly higher MetHb than their controls is supportive of this hypothesis, although the number of infants in each category was small and the differences are not statistically significant.

The higher MetHb levels in infants (1-90 days) reported to be suffering from diarrhea in both the study and control area supports clinical findings in Israel of an association between infant diarrhea and methemoglobinemia.[16] The fact that in the study area the level of MetHb in infants with diarrhea was higher than similar infants in the control area may be suggestive of an additive effect resulting from the higher exposure to nitrates in water. But there again the number of infants in those categories is small and the differences not statistically significant.

The finding of higher MetHb levels in the first 60 days of life in both study and control groups supports similar findings by Shearer et al.[17]

One conclusion from this study is that no apparent public health problem associated with infant methemoglobinemia was detected in the study area, despite the fact that most of the wells were supplying water with nitrate concentrations above that generally recommended by public health authorities. However, it would be premature to extrapolate from these findings as to the situation that may exist in other areas where infants consume appreciable amounts of tap water together with milk formula and where vitamin C rich foods are not widely given as diet supplements to such young infants.[18]

Laboratory Studies

Previous work on the toxicology of nitrates and nitrites has not given rise to any serious concern[6,10] and both compounds are widely used as legally approved food additives. 200 ppm of $NaNO_2$ are allowed in sausages and other "corned" meat products, despite the fact that $NaNO_2$ is many fold more toxic than $NaNO_3$.

Nitrates are also widely found as a natural component of many foods.[7] In our own studies[19] we detected as much as 4,850 ppm of NO_3 and 233 ppm of NO_2 in a local variety of spinach. Samples of beets, cauliflower, cabbage, rhubarb and radishes were all found to contain over 1,000 ppm of NO_3. Under certain conditions of preparation and storage a portion of the nitrates can be reduced to nitrites by bacterial flora:[7, 19] The nitrite form is more toxic by far. Numerous cases of methemoglobinemia, some fatal, have been reported on in connection with infants who ate spinach.

Phillips[7] calculated that in a typical Canadian meal, adults may consume some 313 mg of nitrate, from various fresh foods or about 4.5 mg/kg. Under normal circumstances this is not considered detrimental. If all of that, or a major portion, was converted to nitrites prior to eating, or by stomach flora after eating, the effect might be toxic.

Our findings of distinct pathology in the heart and lungs of rats chronically exposed to 2,000 and 3,000 mg/l of $NaNO_2$ in their drinking water for a two-year period, providing a dose of about 250-350 mg/kg is striking although difficult to extrapolate to humans, both because the pathology was detected only at massive dose levels and since there is always the possibility of species specific toxic effects.

The fact that nitrites can be passed through the placenta of rats and cause raised levels of MetHb in the fetus, as well as other irreversible effects in newborn rats, may give rise to concern particularly since this effect was still detectable at $NaNO_2$ doses of 2.5 mg/kg. Such doses of nitrites can be consumed by humans under not particularly extreme conditions. The most disquieting finding is the fact that we have detected irreversible brain electrical activity changes in the E.E.G. recordings of rats exposed to 100 and 300 mg/l $NaNO_2$ in their drinking water for a period of 2 months. The level of exposure to nitrites in these experiments comes uncomfortably close to commonly found levels of nitrates in food and water. Here, too, we must suggest caution in extrapolating the results of such small scale preliminary experimental studies with $NaNO_2$ in rats to the possible effects on humans. But these latter electrophysiological findings, together with the other experimental studies reported upon here, suggest that if these results are confirmed by more extensive retests now in progress there may well be a need to re-examine the present standards for nitrates and nitrites used as food additives or for that matter allowed in drinking water.

Summary and Conclusions

2,473 infants in areas with medium-high (50-90 mg/l as NO_3) and low nitrate (5 mg/l as NO_3) concentrations in drinking water were studied in an effort to determine whether there is any association between methemoglobin (MetHb) levels and nitrates in drinking water.

No differences were found between the mean MetHb level in the study and control areas.

A possible explanation for this finding lies in the fact that only 6% of the infants consumed appreciable amounts of tap water together with powdered milk formula. The remainder were breast-fed or consumed whole cow's milk. 87% of the infants were fed vitamin C rich foods such as citrus or tomato juice which are known for their anti-MetHb effects. It cannot be assumed that there is no danger from nitrates in drinking water in areas where infants consume larger amounts of tap water with powdered milk formula and/or little vitamin C rich foods.

MetHb levels are highest in the first 60 days of life with a mean of 1.33%. The mean MetHb level for the entire infant population studied was $1.04 \pm .72\%$.

Rats chronically exposed to 2,000 and 3,000 mg/l $NaNO_2$ (250-350 mg/kg) in their drinking water for two years showed distinct pathology in the heart and lung tissues as compared to controls.

Pregnant rats given acute doses of $NaNO_2$ varying from 2.5 - 50 mg/kg show transplacental passage of the chemical with the production of MetHb in the fetuses.

Rats born of dams chronically exposed to nitrites in drinking water during the gestation period showed high mortality rates and poor growth and development as compared to controls.

Mice chronically exposed to 1,000-2,000 mg/l $NaNO_2$ in their water showed lowered motor activity as compared to controls.

Rats with electrodes implanted on the cortex were chronically exposed to nitrites in their drinking water in concentrations ranging from 100-2,000 mg/l of $NaNO_2$. All experimental groups showed major brain electrical activity changes as shown by E.E.G. recordings after two weeks as compared to their own controls and the control group. On cessation of exposure to nitrites after 2 months, brain electrical activity changes in E.E.G. recordings remained, suggesting some form of irreversible damage.

The findings of these small scale preliminary toxicological studies are being rechecked in more extensive tests, but, if found to be correct, would suggest that standards for allowable concentrations of nitrite and nitrate in food and water should be re-examined in light of possible toxic effects on man.

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