Methemoglobinemia Induced by Transplacental Passage of Nitrites in Rats

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Increase in the nitrate levels in drinking water in many areas as a result of pollution from organic wastes or chemical fertilizers has given cause for concern as a result of the connection between high nitrate concentrations and the appearance of infant methemoglobinemia (GRUENER and SHUVAL 1970). High concentrations of nitrates and nitrites naturally found in certain vegetables or added to meat as a food preservative can also give rise to the same condition. The conversion of hemoglobin (Hb) to methemoglobin (MetHb) can be caused by nitrites which may be reduced from nitrates in water or food by intestinal bacteria (CORNBLATH and HARTMAN 1948). Since clinical methemoglobinemia from nitrates in water apparently appears only in infants, one possible solution proposed for areas with high nitrate water is to supply the infants with low nitrate content water from alternate sources. This measure will not exclude the risk of the exposure to nitrates in the prenatal state, i.e. the transfer of nitrates or nitrites through the placenta to the foetus, where methemoglobinemia may be induced.

The possibility of this occurring was tested on pregnant rats. Nitrites were given to pregnant rats in their drinking water or by injection and subsequently nitrite and MetHb were assayed in the foetal blood.

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

The transfer of nitrites to the foetus *in utero* and the production of MetHb was tested in the following experiment. Pregnant white albino rats were used. Each pregnant rat was weighed and anesthetized with ether. From 2.5 - 50 mg/kg of sodium nitrite was given orally or injected intraperitoneally to the pregnant rat and the kinetics of nitrites and MetHb in the dams as well as in the foetuses were measured. Blood was collected from the tail of the dam at regular intervals throughout the experiment. After opening the abdomen the foetuses were removed serially from alternate sides, at regular time intervals over a two

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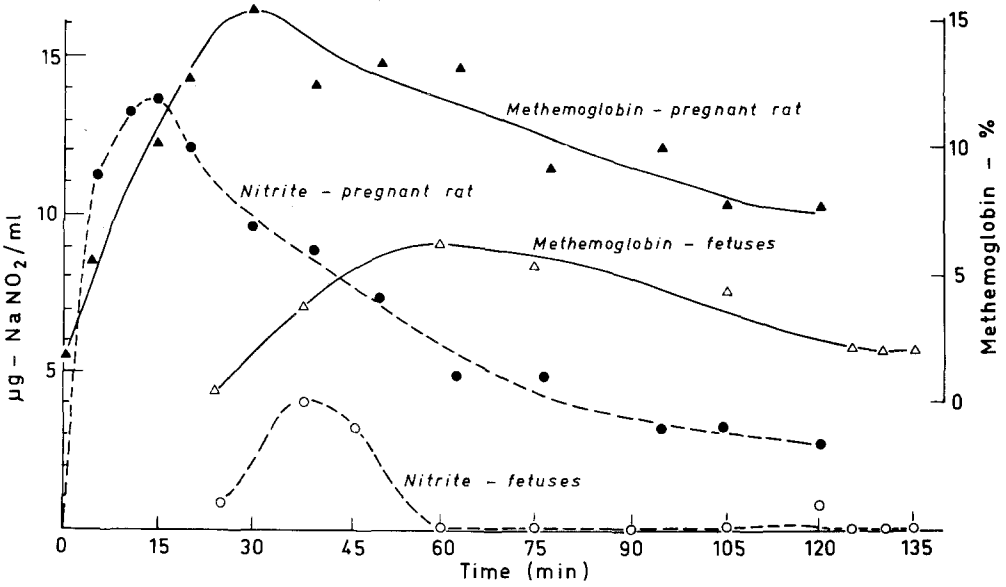
hour period, the umbilical cords being cauterized. The foetuses were washed in saline solution at 37°C and then decapitated. Blood was collected and the MetHb and nitrite levels were measured. The micromethods developed by our group for determining MetHb (HEGESH et al 1970) and nitrite (SHECHTER et al 1972) in blood enabled us to carry out these experiments with the small amount of blood available from each foetus.

The characteristic picture obtained is shown in Fig. 1. After a 30 mg/kg dose of $NaNO_2$ per os to the pregnant rat, nitrite levels rose in the foetal blood though with a lag of about 20 minutes behind the dam. This rise in nitrite in the foetus was followed by a rise in MetHb.

KINETICS OF NITRITE AND METHEMOGLOBIN IN BLOOD OF A PREGNANT RAT AND THE FETUSES (30 mg /kg $NaNO_2$ per os)



The possibility that the placenta was damaged during our experiments leading to increased permeability was excluded when sodium nitrite was given to normal pregnant rats after labor had started. The first foetus showed a normal MetHb level of 1.2% MetHb while those which were born after the chemical had been given showed a level of 10.1% MetHb and 1.2 µg/ml of sodium nitrite in their blood. All births were unassisted.

Different concentrations of nitrites caused similar kinetic pictures differing only in their timing and MetHb levels. Table 1 shows that the threshold of the effect was at a sodium nitrite dose of 2.5 mg/kg. The increase in effect was steep with increased dosage.

TABLE I

Blood Nitrite and Methemoglobin Levels After Injection of Different Doses of Sodium Nitrite

$NaNO_2$ dose mg/kg	Peaks of MetHb Level as percent of total Hb		Peak of nitrite level in blood as $NaNO_2$ µg/ml	
	mother	foetus	mother	foetus
-	0.9	1.2	0.0	0.0
2.5	3.4	1.9	3.9	traces
5.0	5.0	2.7	6.9	traces
10.0	11.9	5.1	8.9	0.4
15.0	17.0	7.9	10.8	1.2
20.0	33.2	13.3	21.7	5.9
25.0	40.4	19.2	25.6	6.9
30.0	60.2	27.2	32.5	9.4

Pregnant rats exhibited a higher susceptibility to nitrites than non-pregnant rats in chronic and acute experiments.

In chronic feeding experiments pregnant rats exposed to 2000 mg/1 of $NaNO_2$ in drinking water or about 200-250 mg/kg/day developed severe anemia with a mean of 10.3±1.5 g% Hb as compared with non-pregnant rats exposed to the same levels on nitrites having means of 14.2±0.9 gm% Hb. The control kept on tap water showed a mean of 14.3±0.8 g% Hb. All pregnant rats (five) died within one hour when injected with doses of 60 mg/kg while non-pregnant females survived such doses.

In general, nitrites given per os led to somewhat lower levels of MetHb as compared with comparable doses given sub-cutaneously but the kinetic picture was similar in both cases. The finding that the MetHb peak could be detected in the foetus 45-60 minutes after injecting $NaNO_2$ in the dam but not in the newborn several hours after birth indicates that the MetHb reductive mechasnism in the foetus and newborn rat is highly effective. MetHb recovery rates were measured in rats after ceasing the consumption of water which contained $NaNO_2$. The time which it takes for the MetHb to be reduced to 50% of its initial level was found to be around ninety minutes (GRUENER and SHUVAL 1972), independent of the initial concentration of MetHb. NADH-dependent MetHb reductase is claimed to be the main pathway of MetHb reduction (SCOTT 1968). The enzyme acting in the red blood cells was measured in adult rats and in foetuses. Similar determinations were made in humans and human cord blood, Table 2 shows that the rat foetuses have MetHb reductase activity some ten times higher than adult rats have or that found in human cord blood, while human adult blood exhibits one and half times the activity of the cord blood.

TABLE II

Methemoglobin Reductase Activity in the Foetus

and the Pregnant Female - Rats and Humans

	n	Methemoglobin reductase mμmoles/min/mg Hb
Pregnant rats	6	1.86±0.34
Rat foetus	10	17.4 ±1.3
Human pregnant females	49	2.38±0.78
Human cord blood	69	1.58±0.54

Enzyme activity was checked in blood sample of 10 μl which was incubated in a mixture that contains 50 μmoles citrate buffer (pH4.7), EDTA 300 mμmoles, $K_3 Fe(CN)_6$ 56 mμmoles, MetHb 288 mμmoles. Total volume is 0.6 ml.

The reaction is started by adding 120 mμmoles of NADH. The reduction of MetHb to oxyhemoglobin is followed at 577 nm (Unicam SP 1800, band width of 1nm). Calculations are based on the value of 42.0 for $\Delta\varepsilon M$ at 577. Molecular weight of hemoglobin is taken as 66.000. Hb was measured at 540 nm according to the cyanomethemoglobin method.

These findings point to the possibility that the human foetus might have a weaker defensive mechanism to the intra-uterine exposure to nitrites than that detected in rats.

The results underline the possible risk of intra-uterine methemoglobinemia when water or foods containing nitrates or nitrites are consumed during pregnancy. It should be noted that certain foods such as spinach can contain as much as 4000 ppm of nitrates, a major portion of which can be reduced to nitrites on storage under certain conditions (EISENBERG et al 1970). 200ppm of nitrites can legally be added to many "corned" meat products. It is, however, premature to extrapolate from these acute animal experiments to the situation that may exist with humans consuming MetHb inducing chemicals in water or food. A study of MetHb levels in infants' cord blood where the mothers come from areas with high and low nitrate levels in their drinking water is in progress to help clarify this question.

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