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Reproduction in the Guinea Pig as Affected by Chronic Administration of Potassium Nitrate and Potassium Nitrite[1,2]

STUART D. SLEIGHT AND OSMAN A. ATALLAH[3]

Department of Pathology, Michigan State University, East Lansing, Michigan 48823

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Toxicosis produced by nitrates and related compounds is a problem in man and in domestic animals. The most prominent effect of nitrate and nitrite is the conversion of hemoglobin to methemoglobin. Nitrate must be reduced to nitrite before excessive methemoglobinemia occurs. Nitrites are therefore several times more toxic than nitrates. Pharmacologically, KNO_3 had been used as a diuretic, and much of this action is due to a direct irritant action on renal cells. The effects of nitrates and nitrites on reproduction have assumed considerable importance in recent years. Abortions in swine, sheep, and cattle have been attributed by Case (1957) to nitrate and nitrite poisoning. Experimental work emphasizing the effects of nitrates or nitrites on reproduction has been reported in cattle, sheep, swine, and rats. Simon *et al.* (1959) repeatedly administered potassium nitrate intraruminally to pregnant cows and thereby caused abortions. Davidson *et al.* (1963) produced one death and one abortion in a group of 40 cattle given varying levels of nitrates in feed. Winter and Hokanson (1964) reported that female cattle could tolerate quantities of nitrite sufficient to convert 40% to 50% of the total hemoglobin to methemoglobin with no apparent detrimental effects on maintenance of pregnancy. Eppson *et al.* (1960) and Setchell and Williams (1962) reported no apparent effects on reproduction in sheep. In swine, Tollett *et al.* (1960) reported that 5% levels of potassium nitrate in feed had no effect on the number of corpora lutea, the percentage of implantations, or the weights of ovaries, placenta, or thyroid gland. Abortions were not produced. Muhrer *et al.* (1956) reported that only 2 of 8 rats fed a diet containing 2% KNO_3 had normal litters. At necropsy, 4 had placental tissue indicating absorption or abortion.

In man, interest in this area has been on the problem of methemoglobinemia in infants due to nitrates or nitrites in drinking water. Typical of many reports of poisoning was a case described by Bucklin and Myint (1960). Schmitz (1961) postulated a relationship between high maternal methemoglobin levels and abortions in pregnant women.

There has been relatively little experimental work in laboratory animals such as the guinea pig, and basic information is lacking. The present experiments were attempts to determine the effects in guinea pigs of chronic administration of potassium nitrate and potassium nitrite in drinking water. The clinical, hematologic, and pathologic effects

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[2] Michigan Agricultural Experiment Station Journal Article No. 4282.
[3] Present address: Cairo University, United Arab Republic.

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were studied. This report emphasizes the reproductive aspects in the female guinea pigs used.

MATERIALS AND METHODS

Forty-six female guinea pigs were used. The chemical and concentration given, the number of guinea pigs per treatment, and the average number of days each group was given the chemical are shown (Table 1). Potassium nitrate was given in the water at concentrations of 300, 2500, 10,000, and 30,000 ppm. Concentrations of KNO_2 included 300, 1000, 2000, 3000, 4000, 5000, and 10,000 ppm. No more than 5 guinea pigs were in any one cage, and at least 1 was a male.

Food and water consumption was recorded. Guinea pigs were weighed weekly. A blood sample was obtained from a guinea pig of each group at approximately 2-week intervals and terminally from all guinea pigs killed. Hemoglobin and methemoglobin levels and differential and total leukocyte counts were determined by standard procedures. Serum nitrite levels were determined by the method of Diven *et al.* (1962). Levels of blood urea nitrogen were determined by utilizing an Auto-analyzer. Serum potassium levels were measured by flame photometry.

Those guinea pigs that died during the course of the experiment were necropsied as soon as possible after death. There was variability in the number of days the guinea pigs were given the chemicals since experiments were designed to determine pathologic effects of various lengths as well as levels of treatment. At necropsy tissue samples were collected from ovaries, uterus, cervix, thyroid, liver, spleen, kidneys, adrenals, skin, heart, and bone marrow. Tissue sections were fixed in 10% buffered formalin. Tissues were processed by routine paraffin technique and stained with hematoxylin and eosin.

RESULTS

Reproduction was not appreciably affected by KNO_3 at levels as high as 10,000 ppm in drinking water whereas, at 30,000 ppm, reproductive performance was poor (Table 2). One female (30,000 ppm) died and had 4 mummified fetuses *in utero*. Food and water consumption was essentially normal at all levels of treatment (Table 1) and weight gains were normal. There were no significant gross or microscopic lesions in the reproductive organs.

The most serious effects on reproduction were seen in females given 5000 or 10,000 ppm of KNO_2 in drinking water. No live births were recorded at these levels (Table 3). Reproductive disturbances were manifested in different ways. At 5000 ppm 6 fetuses were aborted, 3 were mummified and 1 was in the process of absorption at the time of necropsy (Table 3). One female (5000 ppm) died from apparent toxemia.

Histologic examination revealed placental, uterine, and cervical lesions especially in females in which the fetuses had died. Lesions were most extensive in females given 5000 ppm of KNO_2. Placental lesions were degenerative in nature and were characterized by necrotic foci with pycnosis, karyorrhexis, and calcification. Uterine and cervical lesions were typically inflammatory with accumulations of lymphocytes and neutrophils in the lumens, in the endometrium, and in the cervical epithelium.

Food and water consumption was maintained at or near normal at all levels of treatment (Table 1). The rate of weight gains was normal except for those guinea pigs

TABLE I

AVERAGE FEED, WATER, KNO_3, AND KNO_2 CONSUMPTION BY GUINEA PIGS

Concentration of chemicals in water (ppm)	Number of females	Average number of days given chemical	Daily feed consumption (g/100 g body wt.)	Daily water consumption (ml/100 g body wt.)	Daily intake of chemical (mg/100 g body wt.)	Daily intake expressed as nitrate or nitrite nitrogen (mg/100 g body wt.)
Control						
0	4	191	8.9	32.5	0	0
KNO_3						
300	6	166	7.2	29.7	8.9	1.2
2,500	3	194	7.1	29.7	74.2	10.2
10,000	3	143	8.0	36.7	367.9	50.7
30,000	3	204	7.5	27.3	819.0	113.0
KNO_2						
300	3	240	8.1	36.4	10.9	1.8
1,000	3	176	6.6	27.2	27.2	4.5
2,000	3	165	12.1	47.1	94.2	15.4
3,000	5	123	9.1	37.1	111.2	18.2
4,000	6	100	8.7	29.0	119.2	19.2
5,000	4	135	8.4	29.8	149.0	24.4
10,000	3	118	6.1	35.2	352.0	57.7

TABLE 2

REPRODUCTIVE PERFORMANCE OF GUINEA PIGS GIVEN KNO_3 IN WATER

Conc. of KNO_3 in water (ppm)	Number of females	Average number of days given KNO_3	Litters produced	Live births	Relative[a] percent reproductive performance	Fetal deaths			Percent of fetal loss
						Stillborn	Aborted	Mummified or absorbed	
0 (control)	4	191	8	31	100	1	0	0	3
300	6	166	12	33	81	11	0	0	25
2,500	3	194	6	19	80	3	0	0	14
10,000	3	143	5	15	86	1	0	0	6
30,000	3	204	2	2	8	0	0	4	67

[a] Relative percentage was calculated on the assumption that the controls had 100% reproductive performance. The number of females in each group, the number of days the chemical was given, and the total live births were taken into consideration.

TABLE 3

REPRODUCTIVE PERFORMANCE OF GUINEA PIGS GIVEN KNO_2 IN WATER

Conc. of KNO_2 in water (ppm)	Number of females	Average number of days given chemical	Litters produced	Live births	Relative[a] percent reproductive performance	Fetal deaths			Percent of fetal loss
						Stillborn	Aborted	Mummified or absorbed	
0 (control)	4	191	8	31	100	1	0	0	3
300	3	240	8	31	106	3	0	0	9
1,000	3	176	6	17	79	3	0	0	15
2,000	3	165	4	12	60	1	0	0	8
3,000	5	123	8	20	80	4	0	0	17
4,000	6	100	4	10	41	2	0	2	29
5,000	4	135	4	0	0	0	6	4	100
10,000	3	118	1	0	0	0	2	0	100

[a] Relative percentage was calculated on the assumption that the controls had 100% reproductive performance. The number of females in each group, the number of days the chemical was given, and the total live births were taken into consideration.

given 10,000 ppm of KNO_2. At this level the rate of gain was about one-seventh normal. Weight losses took place after parturition in several females given higher levels of KNO_2. These losses were associated with the subacute endometritis and cervicitis.

Hemoglobin levels were slightly depressed at the higher levels of treatment with KNO_2. Recorded methemoglobin levels did not exceed 20% of the available hemoglobin, and the highest levels were in those guinea pigs given 10,000 ppm of KNO_2.

There were no significant alterations in serum nitrite, blood urea nitrogen, or serum potassium in any of the blood samples tested throughout the experiment.

DISCUSSION

Conception took place at all levels of treatment. Since males were treated similarly, their fertility was apparently not impaired. Reproductive disturbances in the female were dose related and particularly evident at 5000 and 10,000 ppm of KNO_2 and 30,000 ppm of KNO_3. Abortion, fetal absorption, fetal mummification, and maternal death were important manifestations.

Hypoxia of the fetus due to maternal methemoglobinemia is hypothesized as a cause of fetal death. In the present study, recorded methemoglobin levels did not exceed 20% of the available hemoglobin, but there were only limited numbers of blood samples taken. Fetal death could occur following a relatively short period of severe methemoglobinemia. One might also relate the placental and uterine lesions to fetal death but, in our opinion, most of these lesions developed after the fetuses had died.

The relatively normal reproductive performance by guinea pigs given levels of KNO_2 as high as 3000 ppm and KNO_3 as high as 10,000 ppm indicated considerable tolerance by the guinea pig to these chemicals.

SUMMARY

Guinea pigs were given KNO_3 in doses ranging from 300 to 30,000 ppm in the water and KNO_2 in amounts from 300 to 10,000 ppm for periods ranging from 100 to 240 days. Reproduction in the female was impaired by 30,000 ppm of KNO_3 with reproductive performance only 8% of that of the controls. Fetal losses were 100% in females given 5000 and 10,000 ppm of KNO_2. Reproduction was maintained at lower levels of treatment: Male fertility was apparently not impaired since conception took place at all levels of treatment. Food and water consumption and weight gains were normal except for a diminished rate of gain at 10,000 ppm of KNO_2. Uterine and cervical inflammatory lesions and degenerative placental lesions were present in females in which the fetuses had been aborted, mummified, or absorbed.

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