ABSTRACT

EXPERIMENTAL NITRATE, NITRITE AND
HYDROXYLAMINE TOXICOSIS IN
THE GUINEA PIG

by Osman Abdel-Aziz Atallah

Seven experiments utilizing 233 guinea pigs were conducted to study nitrate, nitrite and hydroxylamine toxicoses. Animals in their respective experiment were given untreated water or a varying concentration of KNO_3, KNO_2, $NaNO_3$, $NaNO_2$, $Mg(NO_3)_2$, NH_4NO_3 or hydroxylamine hydrochloride ($NH_2OH.HCl$) in water for 5 to 310 days.

Toxicity was evaluated by growth rate, food and water consumption, clinical signs, reproductive performance, gross and histopathologic changes and by hematologic and chemical determinations.

Certain lesions were observed with nitrate, nitrite and hydroxylamine in variable numbers of animals. These included vacuolar necrosis of the myocardium, hyperplasia of the pulmonary interalveolar septal cells, thickening of the media of pulmonary arterioles, thinning of the epidermis, and individualization and pyknosis of cells of the zona reticularis. Erythrocytic abnormalities such as basophilic stippling, hypochromasia, poikilocytosis, nucleation and macrocytosis were seen in all treated groups.

Nitrates were generally mild in their effects except for higher levels of KNO_3 and $NaNO_3$ which generally reduced food and water consumption, lowered growth rate or caused weight loss.

Mortality rate was increased among the newborn of females given KNO_3 or $NaNO_3$. Intrauterine fetal death occurred in females given 1.5% $NaNO_3$, and reproduction stopped with 4% KNO_3 or 2% $NaNO_3$.

Lesions, in addition to those previously mentioned, included endometritis and papillary endometrial hyperplasia and necrosis of the uterine-placental junction. Hematopoietic tissues were hyperplastic with low levels of treatment and hypoplastic with high levels. A few adrenal glands had partially atrophied zonae glomerulosae.

Nitrites were more toxic than nitrates, and $NaNO_2$ was apparently more toxic than KNO_2. Toxicity was reflected by an impaired growth rate in animals given 1 to 1.1% KNO_2 or 0.5 to 0.7% $NaNO_2$, or a loss in body weight in animals given 1.2% KNO_2. Water consumption was decreased at high levels of treatment. Live litter-size was zero at levels of 0.5% or more KNO_2 or $NaNO_2$ and less than normal at lower levels. Abortion, absorption or mummification of fetuses occurred. Some of the females given 0.5% KNO_2 failed to abort and died.

Hemoglobin levels were generally decreased and methemoglobin values were slightly increased in some animals.

Additional lesions included atrophic Leydig cells, partial aspermatogenesis with atrophied seminal vesicles and disintegrated spermatozoa in the epididymides. Many ovaries did not have mature Graafian follicles and had abnormal corpora lutea. Cervicitis, endometritis and ischemic and necrotic placentas were observed. Pulmonary atelectasis and compensatory emphysema and centrolobular hepatic fatty change were noted. The hematopoietic elements reacted in the same way as in nitrate toxicoses. Some adrenal glands were hemorrhagic.

Hydroxylamine hydrocholoride was more toxic than either nitrate or nitrite and induced a marked drop in hemoglobin values. A level of 0.1% decreased growth rate, and o.15% or more decreased weight. All animals given 0.2% $NH_2OH.HCl$ died within 44 days and those given 0.25% or more died within 19 days.

Abortion was noted near full term with 0.1% $NH_2OH.HCl$, and no pregnancies occurred at levels of 0.15% or more.

The lesions in the male and female genetalia, related to hydroxylamine hydrochloride, were similar to those noted in nitrite treated guinea pigs. Hydrothorax was observed as was atelectasis with compensatory emphysema. There was centrolobular hepatic fatty change. Hemochromatosis was prominent in the liver, kidney and spleen. Maximal splenic weight was 10 times normal. The examined

hematopoietic tissues were hyperplastic, the dermis had hyalinized dermal collagen and the colloid of most thyroid glands was vacuolar. Approximately 15% of the adrenal glands were hemmorrhagic and the zonae glomerulosae were partially atrophied in 25%.

EXPERIMENTAL NITRATE, NITRITE AND HYDROXYLAMINE TOXICOSIS IN THE GUINEA PIG

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DEDICATED

TO

MY MOTHER

AND

THE MEMORY OF MY FATHER

ABDEL-AZIZ ABDEL-HALEEM ATALLAH

1910 - 1956

WHO HAD NURTURED MY AMBITIONS AND INSPIRED

ME IN MY ACHIEVEMENTS IN THE

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INTRODUCTION

Nitrate toxicosis in man and animals is an important public health and economic problem. The necessity for experimental data is apparent because there have been few controlled experiments utilizing laboratory or farm animals.

The objectives of this present work were to study the clinical, hematologic and pathologic effects of nitrate, nitrite or hydroxylamine toxicity in guinea pigs with particular emphasis on pathologic aspects and reproductive performance. Another objective was to test the difference among the combinations of nitrate or nitrite with sodium, potassium, magnesium and ammonium.

Results of such a study should provide valuable basic information, and indicate areas which need further study in the guinea pig, other animals and man.

REVIEW OF THE LITERATURE

The general subject of nitrate toxicosis was reviewed by Briggs (1964). He cited other review articles and listed more than a hundred papers dealing with this problem in livestock. Though of interest, these papers were not directly related to the topic under discussion. Only references considered pertinent will be cited.

History:

The earliest reference to nitrate toxicosis was published in 1895 by Mayo according to Huffman (1963). Newsom (1937) reported a condition in cattle which he called oat hay poisoning and which was attributed to high levels of nitrates.

Mechanisms involved in nitrate toxicosis:

General information. Simon et al. (1959) stated that nitrite was 10 times as toxic as nitrate. Dietary nitrates were reduced to nitrite, hydroxylamine and ammonia in the gastrointestinal tract (Bradley, Beath and Eppson, 1939; Bradley et al., 1940; Diven et al., 1962). The rate of reduction of nitrate to nitrite was controlled by the consistency, ingredients, color, volume and pH of the ingesta. A sterile solution of nitrate maintained its toxicity over 8 days, while

a nonsterile solution did not (Davidson, Daughty and Bolton, 1941).

Effect on the cardiovascular system. The action of nitrate and nitrite on the cardiovascular system was reported by several workers (Goodman and Gilman, 1965; Holtenius, 1957; Beckman, 1958). These drugs relaxed smooth muscle by direct action without nerve intervention. The postarteriolar vessels and the splanchnic arterioles were the most susceptible to nitrate action. The generalized effects of nitrate were a fall in blood pressure, increased organ volume, increased blood flow through capillaries, and diminished pulse pressure. The systolic blood pressure was lowered more than the diastolic. The local effects of dilated blood vessels were increased intraocular tension and disturbance of vision, increased intracranial pressure, increased blood flow in coronary arteries and decreased cardiac work. The action of nitrate on blood vessels was variable as mentioned by Holtenius (1957). The blood vessels which reacted first and to the lowest doses were those of the head, neck, brain and meninges. Larger doses dilated the coronary vessels and visceral vessels. Nitrate reduced renal glomerular filtration but tubular function remained normal.

Effect of nitrate on respiratory, gastrointestinal and urinogenital tracts. Beckman (1958) indicated that nitrate action on those systems was through the relaxation of the smooth muscles. The action was accentuated by alcohol ingestion.

Effect of nitrate on hemoglobin. Several authors (Bradley, Beath and Eppson, 1939; Bradley et al., 1940; Riggs, 1945; Kendrick, Tucker and Peoples, 1955; Eppson et al., 1960; Garner, 1961; Diven et al., 1962) indicated that nitrates are not toxic unless reduced to nitrite. The nitrite ion oxidized the ferrous component of hemoglobin to ferric thereby producing methemoglobin which did not carry oxygen. Methemoglobinemia and anoxia were considered by Simon et al., (1958) to be the trigger mechanism for all the pathologic lesions induced by nitrate toxicosis. A nonfatal methemoglobinemia for a resting animal could be fatal if the animal were forced to exercise according to Holtenius (1957) and Eppson et al. (1960). Kleine and Orten (1962) stated that several drugs were able to produce methemoglobinemia. These drugs included chlorate, acetanilid, nitrite, nitrobenzene, antipyrine, iodine, phenacetin, trional and sulfonamides.

Effect of nitrate on nutritional status. The conversion rate of carotene to vitamin A was disturbed by dietary nitrate (Bloomfield et al., 1961; Smith et al., 1962; Pugh et al., 1962; Reddy and Thomas, 1962; Sell, Robert and Waddell, 1963; Olson, Nelson and Emerick, 1963). The syndrome of vitamin A deficiency in nitrate treated animals with abundant dietary carotene was noticed. Dietary nitrite was more important than nitrate in reducing liver and plasma

vitamin A stores. The destructive rate of nitrate or nitrite on carotene and the depletion of vitamin A storage were affected by the kind and pH of the ingesta and the molecular ratio of nitrite to carotene. Emerick and Olson (1962) stated that the depleting action of nitrite on hepatic vitamin A storage was stopped by subcutaneous injection of vitamin A. Smith (1961) suggested that the alteration of protein enzymes, required to convert carotene to vitamin A, was responsible for the disturbance in carotene and vitamin A metabolism in nitrate toxicosis. The metabolic disturbances were attributed to the anoxia caused by nitrite according to Winter and Hokanson (1964). O'Dell et al. (1960) observed a vitamin E deficiency syndrome in rats fed potassium nitrate. Certain authors related dietary nitrate to disturbed thyroid function (Bloomfield et al., 1961; McIlwain and Schipper, 1963). The interrelationship among iodine, nitrate and vitamin A was reported by Yadav et al. (1962) who indicated that adequate iodine in the diet counteracted, to some extent, the nitrate depletion effect of storage of vitamin A in the liver. Bloomfield et al. (1962) found that rats given nitrate in the diet had a lowered iodine uptake by the thyroid gland after 7 hours on the diet. This lowered iodine uptake became normal after 1 week of treatment. After 5 weeks, the nitrate-fed rats fixed more iodine than did the controls.

Effect of nitrate on reproduction. Abortion was the outstanding sign of nitrate toxicosis in swine (Case, 1954;

Tollett et al., 1960; Case, 1963) and cattle (Thorp, 1938; Muhrer et al., 1956; Case, 1957; Simon et al., 1958, 1959; Ebert, 1959; Bjornson et al., 1961). Aborted swine fetuses were mostly near full term and some were born weak (Case, 1954). Tollett et al. (1960) reported that nitrate at certain levels in the diet of swine had no effect on the number of corpora lutea and percentage of implantation and no effect on the weight of ovaries, placenta or thyroid gland. Bovine fetuses were aborted at 3 months of age or after according to Simon et al. (1958). Some full term fetuses were small and stunted (Simon et al., 1959). The cattle aborted without any impending signs. A few animals had retained placenta but abortion did not interfere with later estrous cycle regularity or conception rates. Davidson et al. (1963) reported a low conception rate in cattle given a nitrate-containing diet. Winter and Hokanson (1964) stated that hydroxylamine hydrochloride (60 mg./kg. body weight) fed daily to pregnant heifers had no effect on pregnancy. Abortion and absorption of fetuses were reported in rats fed a nitrate-containing diet (Muhrer et al., 1956).

The responses of various species to different levels of nitrate and nitrite are summarized in the following chart:

Doses of nitrate and nitrite and responses in various species:

Drug	Level	Animal	Results and References
KNO_3	25 to 30 Gm. per os.	Ewe	Death. Bradley et al. (1940). Holtenius (1957).
	1.84 to 3.17% in ration	Swine	Depressed gain in body weight. Tollett et al. (1960).
	30 to 32 mg. nitrite/ lb.	Swine	LD 50. Nelson (1965).
	4 Gm. per os.	Man	Minor degree of gastro-intestinal irritation. Holland (1917).
	8 to 32 Gm. per os.	Man	Some died and others survived. Holland (1917).
	1 Gm./kg. body weight.	Bovine	Death. Holtenius (1957).
$NaNO_3$	0.6 Gm./kg. body weight in ration.	Cow	Death. Egyed and Miller (1963).
	0.3% in diet.	Rat	Depleted liver vitamin A store. Emerick and Olson (1962).
	300 to 330 mg./kg. body weight was given subcut.	Mice	Died within 30 minutes. Bodansky (1951).
	35 mg./kg. body weight was given orally.	Cat	LD 100. Died within 60 minutes with 88 to 100% methemoglobinemia. Bodansky (1951).
	170 mg./kg. body weight was given orally.	Rabbit	LD 100. Died within 60 minutes with 86 to 94% methemoglobinemia. Bodansky (1951).
$NaNO_2$	0.15 to 0.17 Gm./kg. body weight in ration.	Bovine	Minimum lethal dose. Garner (1961).
	1.1 to 2 Gm./kg. body weight in diet.	Male Rat	Death. Rieman (1950).
	0.46 to 1.2 Gm./kg. body weight in diet.	Female Rat	Death. Rieman (1950).
	214 to 216 mg./kg. body in diet.	Rat	LD 50. Rieman (1950).

Factors predisposing to nitrate toxicosis:

Species. The occurrence of nitrate intoxication was reported in many species with inter- and intraspecies variability in susceptibility (Newsom et al., 1937; Thorp, 1938; Olson and Moxon, 1942; Riggs, 1945; Case, 1957; Garner, 1961). Feeding of wet oat hay killed some cattle and had no effect on other cattle, guinea pigs, rabbits and ewes. Swine were more susceptible than cattle, cattle were more susceptible than sheep and sheep were more susceptible than horses. The difference in susceptibility could be related to differences in the pH of the stomach contents (Olson and Moxon, 1942).

Ration and water consumption. Apparently the pH of the ingesta was determined by the kind of food and its interaction with the digestive secretions and gastrointestinal microflora. A well balanced ration protected sheep against an oral dose of 30 Gm. KNO_3/cwt (Holtenius, 1957), but this dosage killed sheep fed low-quality grass hay. Sokolowski et al. (1960) stated that feeding poor quality alfalfa did not increase nitrate toxicosis in lambs orally dosed with nitrate, whereas feeding of bluegrass pasture increased nitrate toxicosis. Garner (1961) indicated that fasting increased the susceptibility to both nitrate and nitrite poisoning. Drinking after consuming nitrate-containing food increased fatalities among cattle.

Experimental work and case reports on nitrate toxicosis:

An attempt will be made to emphasize signs and lesions induced by nitrate intoxication in various species. Consumption of nitrate-containing food or water resulted in listlessness, progressive cyanosis, methemoglobinemia and chocolate-colored blood in swine (Tollett et al., 1960; Case, 1957, 1963). infants (Case, 1957), sheep (Campbell et al., 1954), cattle (Newsom et al., 1937; Thorp, 1938; Fincher, 1939; Campbell et al., 1954; Kendrick et al., 1955, Muhrer et al., 1956; Case, 1957; Holtenius, 1957; Ebert, 1959; Bjornson et al., 1961; Garner, 1961; Pfander, 1961; Egyed and Silberman, 1961), dogs (Whitehead, 1953), and mice (Gleason, Gosselin and Hodge, 1963). Signs included increased jugular pulsation, accelerated heart beat, rapid soft pulse and dyspnea (Fincher, 1939). Other signs and complications in different animals included anoxia, chronic diarrhea, abdominal pain, reduced weight gain, laminitis, apparent blindness and a drop in milk production (Davidson et al. 1941; Muhrer et al., 1956). A dog was reported to have had frequent prolonged urination of colorless urine, polydipsia and vomiting (Whitehead, 1953). Other animals had a normal to subnormal rectal temperature (Case, 1957). They trembled and had an incoordinated gait, particularly if exercised and would finally collapse, gasp for breath and have froth at the mouth. Animals usually died with little or no struggle (Newsom et al., 1937; Davidson et al., 1941 and Holtenius,

1957). The general gross findings were dark-brown blood (Kendrick et al., 1955; Hymas and Mesler, 1960), which became brighter on exposure to air (Newsom et al., 1937; Davidson et al., 1941). The blood was poorly coagulated except in the large vessels near the heart (Newsom et al., 1937). The parenchymatous organs and the gastrointestinal, respiratory and urogenital tracts were hemorrhagic and congested. The cut surface of the lymph nodes had a turbid exudate and cortical petechial hemorrhages. The urine contained shreds and flakes of a white precipitate (Davidson et al., 1941).

Lesions of nitrate intoxication in ruminants included congested lungs with greyish-brown streaks throughout. The endocardium and the papillary muscles had petechial and ecchymotic hemorrhages. The myocardium had focal accumulations of lymphocytes and plasma cells. The medial layer of the coronary arteries was vacuolated. The spleen had excessive hemosiderin and the liver had congested portal triads and slight degeneration and necrosis. Lesions in the kidneys included necrosis of glomerular tufts, minor tubular degeneration, hemorrhagic foci, capillary distention and transudation of proteinaceous material (Davidson et al., 1941; Case, 1957; Holtenius, 1957). Winter (1962) reported that the serum nitrate level was the same in cattle given nitrate orally or by intraperitoneal injection. The route of nitrate administration (orally, intraperitoneally or subcutaneously) affected the time of occurrence of the maximum methemoglobinemia.

Diven et al. (1962) suggested that blood nitrate levels were a better indication of the amount of nitrate consumed by sheep than were methemoglobin levels. The blood nitrate level was maximum at 4 to 6 hours after treatment and then declined within 2 hours prior to the period of crisis.

Bodansky (1951) observed that the subcutaneous injection of nitrite into mice produced methemoglobinemia, motor incoordination, prostration, coma and death. Rats with chronic nitrate toxicosis had degenerative changes and reactivity of vascular tissues of the brain, heart, lungs, kidneys and testes, according to Case (1957). Welsch et al. (1961, 1962) reported that rats fed a diet containing nitrate had enlarged thyroid and adrenal glands with decreased gains in body weight particularly when rats were kept at 2 C. Supplementation of iodine prevented the thyroid lesions but the rate of gain in body weight was less than the controls.

Hydroxylamine:

The oral or intravenous injection of hydroxylamine and certain oximes in small amounts caused a severe hemolytic anemia in sheep (Jamieson, 1958), cattle (Winter and Hokanson, 1964) and rabbits as reported by Rieman (1950). The anemia in rabbits could not be cured by liver preparations. The effects of the drug were reversible in animals which were given daily injections over a long period. Hematologic values returned to normal after 16 to 20 days, although bone marrow was hyperplastic until about 2 months later.

Signs of hydroxylamine toxicosis included hemoglobinemia which started as early as the 3rd day of treatment (Jamieson, 1958) and hemolytic and aplastic anemia that was aggravated by lack of yeast in the diet (Jamieson, 1958; Winter and Hokanson, 1964). There were cyanosis, methemoglobinemia, convulsions and coma according to Gleason et al. (1963). Locally, hydroxylamine caused severe skin irritation and sensitization. Other signs included reduced resistance to infection and urobilinuria (Rieman, 1950).

The LD 50 of hydroxylamine hydrochloride was 419 and 408 mg./kg. body weight for female and male rats, respectively. The lethal dose of hydroxylamine for rats was about twice that of sodium nitrite. Rats which were given sublethal doses of hydroxylamine hydrochloride in food for 178 days had normal weight gain, enlarged spleens and reduced size of the thyroid glands. Pigeons which were given only 10 to 50 mg. of hydroxylamine intravenously died within a few minutes (Rieman, 1950).

MATERIALS AND METHODS

Two hundred thirty three guinea pigs were used for 7 experiments. Animals from each experiment were in turn divided into several groups. Each group was given a specific concentration of one of the following chemicals in its drinking water for a known number of days:

Expt.	Chemical		No. of animals	Days of expt.
	Name	%		
I	KNO_3	0.01 to 4	43	11 to 310
II	KNO_2	0.01 to 1.2	62	14 to 300
III	$NaNO_3$	0.5 to 3	19	5 to 152
IV	$NaNO_2$	0.5 to 1.2	19	5 to 207
V- Part 1	$Mg(NO_3)_2$	3	5	22 to 149
Part 2	NH_4NO_3	1	7	16 to 210
VI	$NH_2OH.HCl$	0.02 to 0.3	60	5 to 235
VII	0.00		18	23 to 298

Most of the guinea pigs were 1 to 2 months old when started on their respective treatments. In certain experiments newborn guinea pigs were used. Guinea pigs were housed in topless sheet metal cages 1 foot high, 1.5 feet wide and 2.5 feet long. Basically, 4 females and 1 male (1 group) were housed in each cage. Wood shavings were used for bedding. Appropriately treated drinking water was available for guinea pigs throughout

the period of the experiment in inverted pint bottles closed with stainless steel tube waterers fixed in rubber stoppers. Pelleted guinea pig diet* was fed free choice. The feed was formulated to contain adequate vitamin C.

Stock solutions of 20% KNO_3,(1) 60% KNO_2,(2) 40% $NaNO_3$,(3) 60% $NaNO_2$,(3) 20% $Mg(NO_3)_2$,(3) 20% NH_4NO_3(4) or 10% $NH_2OH.HCl$(2) were prepared as needed in distilled water and kept in tightly closed glass flasks.

The body weight of guinea pigs was recorded at intervals, mostly weekly. Food and water consumption were recorded. The daily gain per 100 Gm. body weight was determined on the basis of the first 50 days of treatment as follows:

$$\text{Daily gain/100 Gm. body weight} = \frac{2 \times \text{Gain}}{\text{Initial wgt.} + \text{Final wgt.}} \times \frac{100}{50}$$

Laboratory investigations:

To obtain a blood sample, an incision was made by a sharp blade between the 1st and the 2nd toes of the front foot. Blood droplets were collected in the appropriate

*Rockland Laboratory Animal Diets; Tekland, Inc., Monmouth, Illinois.

(1)Allied chemical, General chemical division, Morristown, N.J., U.S.A.

(2)Matheson, Coleman and Bell, Division of Matheson Company. Norwood (Cincinnati), Ohio: East Rutherford, N.J., U.S.A.

(3)J. T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.

(4)Fisher Scientific Company, Manufacturing Chemists-Fair Lawn, N.J., U.S.A.

diluents for determination of hemoglobin, leukocyte count and methemoglobin. Erythrocyte counts were performed for some of the guinea pigs. Blood smears were made for differential leukocyte counts and capillary tubes were used for packed cell volume determinations. About 3 to 5 ml. of blood were collected in a clean test tube. Blood was allowed to clot and serum was separated by centrifugation. Sera from all guinea pigs given the same treatment were pooled and kept at -20 C. for later analysis. Bleeding was stopped by applying tannic acid powder to the wound which healed normally within a week. The next bleeding for the same guinea pig (mostly at 10- to 30-day intervals) was from the front foot not used in the preceding collection. A particular number of guinea pigs from each of a selected chemical level were bled each time. Standard procedures (Benjamin, 1961) were employed for the determination of hemoglobin (cyanmethemoglobin method), packed cell volume (capillary tube) and leukocyte count (Turk's diluting fluid and a hemocytometer). The blood smears were stained with Wright's stain and leukocyte differential counts were done. Methemoglobin levels were determined by the method of Evelyn and Malloy (Hawk, Oser and Summerson, 1965). Serum bilirubin levels were determined according to Diven (1962). The levels of blood urea nitrogen were determined with an Auto-Analyser[1] (Technicon Auto-Analyser Manual, 1958), and total serum protein was determined with a refractometer (Benjamin, 1961). Serum sodium and potassium levels were

[1]Technicon Instrument Corporation, Chauncey , New York.

measured by flame photometry.[1] Lab-trol and Patho-trol[2] were the control and Harleco[3] was the standard for the serum electrolytes. Serum electrophoresis was run on a Spinco,[4] Model R, paper electrophoresis unit at room temperature. Spinco-prepared buffer, dye and fixatives were used and the relative intensities of the separated protein fractions were scanned with an analytrol.[5] Serum nitrite levels were measured according to Diven, Pistor and Reed (1962).

Guinea pigs were killed by a blow on the head, by bleeding from the heart or by ether at the end of the experiment. Some animals were found dead. Terminal blood samples were collected from killed guinea pigs. Serum was separated and stored at -20 C. The body weights of the dead and killed guinea pigs were recorded. Guinea pigs were routinely necropsied and gross lesions were recorded. The weights of liver, kidneys, spleen, heart, testes, small and large intestines together, and cecum alone with contents were determined in most guinea pigs. The relative organ weights were calculated on the basis of percentage of total body weight. Tissue sections were preserved in 10% buffered formalin. Collected

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(1) Model 21, Coleman Flame Photometer, 1957. Operating directions D-248 A.

(2) Dade Reagents, Inc., Miami, Florida

(3) Hartman-Leddon Company (Harleco), Philadelphia, Pennsylvania.

(4) Beckman Instrument, Inc. Spinco Division. Stanford Industrial Park, Palo Alto, California

(5) Spinco Model RB.

tissues included samples of tongue, eyes, brain, trachea, esophagus, thyroid glands, thymus, cervical lymph nodes, lungs, heart, diaphragm, liver, spleen, pancreas, stomach, small intestine, large intestine, kidneys, adrenals, ovaries, testes, urinary bladder, uterus, anal glands, skeletal muscle, skin and bone. These tissues were processed by a routine paraffin technic and stained with hematoxylin and eosin. Spleen, liver, kidneys, adrenal glands and lungs from selected guinea pigs were stained with Prussian blue for iron. Sections of liver and kidneys from particular animals were stained with oil-red-O for fat. Selected samples of bone (sternum and femur) were decalcified, washed, processed and stained with hematoxylin and eosin or Giemsa's stain for examination of bone marrow. Specimens from liver, kidneys and adrenals from about half of the guinea pigs were placed in Carnoy's fixative, processed by routine paraffin technic and stained with Best's carmine or the Periodic acid-Schiff method for glycogen. Some of the testicular samples were placed in Bouin's fixative, processed by routine paraffin technic and stained with hematoxylin and eosin. The histologic procedures were done according to the methods described in the Armed Forces Institute of Pathology Manual of Histologic and Special Staining Technique (1960).

The guinea pigs' sex, initial age, period of treatment, number, initial body weights and level of chemicals in drinking water are shown (Table 1 to 5). Whether the guinea pigs died or were killed is indicated. The various levels of chemicals in drinking water were prepared daily by adding the appropriate volume of stock solution to tapwater.

Table 1 - Chemical and its concentration in the drinking water; animal number, sex, initial age, days of the experiment and initial weight of guinea pigs in Experiment I.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
KNO_3	0.01	1	F	60	127	510
		2	M	40	210	420
		3	M	1**	14	80
		4	F	40	275	280
	0.03	5	F	30	99	255
		6	F	40	102	330
		7	F	40	160	375
		8	M	40	225	380
		9	F*	50	233	405
		10	M	1**	11	80
	0.10	11	M	40	102	340
		12	M	30	160	295
		13	M	30	252	240
		14	M	30	310	370
		15	M	30	212	300
	0.25	16	M	30	156	250
		17	F	30	197	230
		18	F	1**	78	80
		19	M	1**	83	80
		20	F	40	306	315

Table 1 - Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in	Days of expt.	Initial weight in Gm.
KNO_3	1.00	21	F*	30	59	220
		22	M	30	94	195
		23	F	30	150	270
		24	F	1**	53	80
		25	F	30	220	210
	2.00	26	M	20	93	180
		27	M	60	114	440
		28	M	20	153	160
		29	M	30	210	250
	2.50	30	M	20	28	205
		31	F	1**	45	80
		32	M	30	57	278
		33	F*	20	211	200
		34	F	1**	17	80
	3.00	35	F	20	50	210
		36	F	18	200	175
		37	F	20	200	215
		38	M	20	200	220

Table 1 - Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
KNO_3	4.00	39	M*	18	16	185
		40	M*	20	28	205
		41	M*	18	37	175
		42	M*	20	136	195
		43	F	18	137	170

* Found dead.
** Dam was given same concentration of chemical during pregnancy and throughout lactation.

Table 2 - Chemical and its concentration in the drinking water; animal number, sex, initial age, days of the experiment and initial weight of guinea pigs in Experiment II.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
KNO_2	0.01	1	M	40	90	355
		2	M	40	126	340
		3	M	40	214	370
		4	M	50	270	435
		5	M	40	300	335
	0.03	6	F	30	161	255
		7	M	1**	45	75
		8	M	40	218	370
		9	F	20	270	260
		10	F	20	290	235
	0.10	11	F	40	117	380
		12	F	40	161	375
		13	M	40	214	400
		14	M	1**	22	70
		15	F	50	250	465

Table 2 Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
KNO_2	0.20	16	M	20	91	215
		17	M	1**	14	80
		18	M	20	152	190
		19	F	20	170	190
		20	F	18	173	185
	0.30	21	F	30	96	110
		22	F*	40	110	425
		23	F	1**	14	75
		24	M	30	150	305
	0.35	25	F	50	99	450
		26	M	1**	18	85
		27	M	50	184	425
		28	F	40	162	390
		29	F	40	150	400
	0.40	30	F	30	63	350
		31	F	20	97	235
		32	M	50	150	490
		33	F	50	87	470
		34	F	40	87	345

Table 2 - Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
KNO_2	0.45	35	F	30	100	475
		36	M	1**	14	80
		37	M	30	154	275
		38	F	30	160	290
	0.50	39	F*	20	119	240
		40	F	20	90	200
		41	F	20	122	220
		42	F	20	209	235
		43	M	20	250	200
	1.00	44	F*	20	52	210
		45	F*	15	71	140
		46	F	25	94	255
		47	F	25	153	260
		48	M	25	210	270
	1.05	49	F*	20	31	205
		50	F*	20	33	200
		51	M*	20	40	235
		52	M*	20	54	185
		53	F*	25	106	255

Table 2 - Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
KNO_2	1.10	54	M*	20	26	200
		55	M*	25	42	275
		56	F*	20	52	205
		57	F*	25	56	275
		58	M*	15	63	150
	1.20	59	F*	30	30	190
		60	F*	35	35	235
		61	M*	41	41	235
		62	M*	58	58	290

* Found dead.
** Dam was given same concentration of chemical during pregnancy and throughout lactation.

Table 3 - Chemical and its concentration in the drinking water; animal number, sex, initial age, days of the experiment and initial weight of guinea pigs in Experiments III, IV and V.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
$NaNO_3$ (Expt. III)	0.5	1	M	35	97	360
		2	M	1**	14	80
		3	F	35	149	360
		4	F	20	152	204
	1.0	5	M	30	97	330
		6	M	1**	18	80
		7	F	40	144	375
		8	F	35	44	325
	1.5	9	M	45	98	350
		10	F	20	98	220
		11	F	20	94	350
	2.0	12	F*	15	5	155
		13	M	20	8	185
		14	F	20	10	195
		15	M*	20	21	220

Table 3 - Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
$NaNO_3$	3.0	16	F*	20	19	220
		17	M	20	213	225
		18	M	20	13	200
		19	F	30	21	275
$NaNO_2$ (Expt. IV)	0.5	1	M	25	95	235
		2	F	25	150	250
		3	M	30	188	335
		4	F	25	207	260
		5	F*	20	20	215
	0.7	6	F	30	84	310
		7	F*	35	103	355
		8	M	40	99	280
	0.9	9	F*	40	5	130
		10	F	30	65	330
		11	M	40	74	400
		12	F*	40	34	435

Table 3 - Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
$NaNO_2$	1.0	13	M*	40	28	315
		14	F	35	62	350
		15	M	50	75	525
		16	F*	40	140	475
	1.2	17	F*	40	10	380
		18	M*	50	31	440
$Mg(NO_3)_2$ (Expt. V)	3	1	M	50	90	515
		2	F	1**	22	80
		3	M	50	149	470
		4	F	20	120	215
		5	F	20	120	205
NH_4NO_3 (Expt. V)	1	1	M	20	156	195
		2	F	1**	16	80
		3	M	20	97	200
		4	F	20	210	205
		5	M	20	204	180
		6	F	1**	18	80
		7	M	20	106	180

* Found dead.
** Dam was given same concentration of chemical during pregnancy and throughout lactation.

Table 4 - Chemical and its concentration in the drinking water; animal number, sex, initial age, days of the experiment and initial weight of guinea pigs in Experiment VI.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
$NH_2OH.HCl$	0.02	1	M	20	92	185
		2	F	20	25	195
		3	F	1**	15	80
		4	F	20	128	225
	0.03	5	M	25	83	245
		6	F	1**	14	80
		7	F	27	120	255
		8	F	30	120	300
	0.05	9	F*	20	13	190
		10	M	30	92	280
		11	M	1**	17	80
		12	F	12	117	210
		13	M	25	150	260
		14	F	20	195	200
		15	M	1**	16	80

Table 4 - Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
$NH_2OH.HCl$	0.10	16	M	30	96	300
		17	M	30	116	325
		18	M	20	205	205
		19	F	20	220	220
		20	M	25	235	235
	0.15	21	F*	30	43	280
		22	F*	30	60	315
		23	M*	25	60	240
		24	M*	20	32	210
		25	F	20	96	200
		26	M	25	90	250
		27	M	30	101	280
		28	M*	20	17	15
	0.20	29	F*	20	33	235
		30	M*	20	44	210
		31	F*	20	86	210
		32	F	25	12	240
		33	M	20	19	235
		34	M	25	31	240

Table 4 - Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
$NH_2OH \cdot HCl$	0.25	35	F*	20	9	220
		36	F*	25	11	245
		37	M*	25	10	260
		38	F	20	10 (13) 14	205
		39	F*	20	10 (13) 20	215
$NH_2OH.HCl$	0.30	40	M*	20	12	195
		41	M*	20	14	200
		42	M*	20	14	95
		43	M*	20	19	215
		44	M*	20	7	200
		45	F	20	7 (10) 8	225
		46	M	20	7 (10)	215
		47	M	50	8 (8) 7	505
		48	M	50	7	530
		49	M	50	7	510
		50	M*	55	10	575

Table 4 - Continued.

Treatment		Guinea pigs				
Chemical	Percent in water	No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
$NH_2OH.HCl$	0.30	51	F*	55	11	575
		52	M	50	8	625
		53	M*	70	5	650
		54	M*	80	7 (5)	775
		55	M*	70	7	655
		56	M	60	6	775
		57	M	50	7 (20)	545
		58	M*	65	6	655
		59	M	60	7 (20)	625
		60	M	70	7 (20)	670

* Found dead.

** Dam was given same concentration of chemical during pregnancy and throughout lactation.

NB. Number between parentheses is the period of untreated water following the indicated days of treatment. At the end of this time, the guinea pig was either killed or treatment was repeated for the number of days indicated.

Table 5 - Animal number, sex, initial age, days of the experiment and initial weight of control guinea pigs given untreated water in Experiment VII.

Guinea pigs				
No.	Sex	Initial age in days	Days of expt.	Initial weight in Gm.
1	F	25	92	255
2	F	20	163	200
3	F	150	145	880
4	F	150	145	880
5	F	20	211	205
6	M	1*	20	80
7	M	20	270	185
8	F	25	298	240
9	U**	1*	30	80
10	U	1*	30	80
11	U	1*	30	80
12	U	1*	30	80
13	U	1*	30	80
14	U	1*	30	80
15	U	1*	30	80
16	M	150	23	810
17	M	140	23	770
18	F	1*	26	80

* Dam was given untreated water during pregnancy and throughout lactation.

** U = Undetermined.

EXPERIMENTAL RESULTS

Experiment I (KNO_3)

Growth. A great decrease in the rate of gain was seen in guinea pigs given 4% KNO_3 (Table 6).

Food and water consumption was normal. The maximum daily consumption of KNO_3 was 1660 mg./100 Gm. body weight by guinea pigs given 4% KNO_3 in the drinking water (Table 6).

Signs. There were no significant signs in guinea pigs given 0.01 to 1% KNO_3 in water. Some of the animals given higher levels of KNO_3 had fluctuations in body weight. Two females (2, 3%)* lost weight steadily after parturition until they were killed. Guinea pigs given 2% KNO_3 had fluctuations in body weight from the 80th day of treatment until they were killed. Most guinea pigs given 3% KNO_3 lost weight steadily from the 190th day of the experiment until they were killed. Animals receiving 4% KNO_3 had fluctuations in body weight within the first 90 days of the experiment after which they steadily lost weight until they were killed.

Reproductive performance. The only reproductive disturbance observed in guinea pigs given 0.01 to 1% KNO_3

*Here and hereafter, the percentage written between parentheses is the employed level of chemical.

was an increased mortality rate among the newborn. One female (2.5%) died during the last third of pregnancy. She had 4 dead and dehydrated fetuses in the left uterine horn and the placental fluids had been absorbed. One female (3%) gave birth to a first litter of 2 weak guinea pigs after which she lost weight steadily until she was killed. Three other females (3%) did not reproduce (Table 7, Fig. 119).

Analyses

Hematologic determinations. The hemoglobin and methemoglobin levels and the differential and the total leukocyte counts were normal. Some erythrocytes had basophilic stippling (Fig. 70)*, polychromasia and nucleation (Fig. 71). The changes were not significantly increased by raising the drug concentration or extending the period of the experiment.

Serum electrolyte and blood urea nitrogen concentrations were normal (Table 8) as were values for total protein, nitrite and bilirubin.

Serum electrophoresis. Only guinea pigs receiving 4% KNO_3 had an increased albumin/globulin ratio (72/28) in comparison to the control level of 58/42 (Table 8).

Lesions

Hearts from 36 guinea pigs were examined. Isolated findings were small areas of vacuolar necrosis in the

*Here and hereafter, the illustration represents a typical lesion. The specific chemical is included in the caption.

myocardium of 2 guinea pigs, increased numbers of sarcolemmal nuclei (Fig. 8) in 11, and albuminous degeneration in 10.

Testes from 18 guinea pigs were examined. Microscopically, there was interference with spermatogenesis at the stage of the primary spermatocyte or spermatid in 4 guinea pigs (2 to 3%, Fig. 13, 14).

Seminal vesicles from 11 guinea pigs were studied and most of them were normal. Isolated findings included a lighter-staining secretion in the tubules of 2 seminal vesicles (2, 2.5%), and a more eosinophilic cytoplasm of the epithelial lining in 1 (3%).

Epididymides from 15 guinea pigs were studied. Microscopically, the tubular epithelium in a few animals was atrophied and some tubules had degenerated spermatozoa and round cells of 15 to 20μ in diameter (Fig. 17). One lobule had scattered interstitial neutrophilic infiltration and some proliferation of fibroblasts. The smooth muscle wall of the epididymal duct was atrophied in 1 male (3%). The epididymis of 1 of the guinea pigs which had an interference with spermatogenesis had either degenerate or no spermatozoa in its ducts.

Ovaries from 17 guinea pigs were studied and most of them were normal. Isolated lesions were hemorrhagic corpora lutea in 2 females (1, 3%, Fig. 43), cystic degeneration of the ovaries with basophilia of stromal cells in 1 guinea

pig (3%), or fibrotic foci replacing the ovarian stroma in another female (4%).

Uteri from 13 females were studied. The lumina of 3 uteri had numerous neutrophils (Fig. 29), and 2 uterine walls were congested. The epithelial nuclei of 1 endometrium were darkly basophilic and another endometrial lining was vacuolated. The vagina of 1 female contained dark brown viscid fluid and Proteus monosani was isolated.

Tracheas from 26 guinea pigs were studied. Microscopically, the findings included an eosinophilic infiltration in the mucosa and submucosa in 11 guinea pigs. One trachea (1%) was ulcerated with eosinophilic infiltration around the ulcers. The lumen contained cellular debris and eosinophils were prominent. There was a slight hyperplasia of the epithelial lining in 4 tracheas and the cytoplasm was vacuolated in 2.

Lungs from 36 guinea pigs were examined. Microscopically, 14 lungs were congested, 13 had hyperplastic septal cells (Fig. 75, 79), and in 7 the media of the pulmonary arterioles was thickened and vacuolated. There was edematous peribronchiolar smooth muscle in the lungs of 3 guinea pigs, edema of the interalveolar septa in 3 (2 to 3%), (Fig. 80), atelectasis and compensatory emphysema in 8 (Fig. 83) and fibrotic foci in the lungs of 2 (4%).

Livers from 42 guinea pigs were examined. The relative weights were normal (Table 9). Two guinea pigs

(1, 3%) had livers with an appearance of centrolobular fatty change (Fig. 84). One animal (2.5%) had a diffusely yellow liver, and 3 (4%) had brown livers. Microscopically, 9 livers had hydropic degeneration of hepatic cells particularly centrolobularly. Hepatic arterioles in 7 guinea pigs had hyalinized media (Fig. 88). There were congestion in 6 livers, cloudy swelling in 12 and centrolobular fatty change in livers of a few (Fig. 85). Incidental findings included some bile pigments and interstitial infiltration with eosinophils and neutrophils (2.5%), and some necrotic foci (3%). Six liver sections were tested for glycogen and 4 were stained for iron (0.1 to 0.3%) with negative results.

Stomachs from 36 guinea pigs were studied. Grossly, there were petechial hemorrhages in the gastric mucosas of 3 animals (1 to 2.5%). Two stomachs were filled with fluid (1, 2.5%). One (3%), and 3 (4%) were empty. There were no outstanding lesions microscopically.

Tongues from 24 guinea pigs were studied, and most of them were normal. Isolated lesions included cells resembling Anitschkow's myocytes among the skeletal muscles of 4 tongues (Fig. 109).

Kidneys from 31 guinea pigs were studied. The relative weights were increased in guinea pigs given 3% or more KNO_3 (Table 9). Microscopically, there were cloudy swelling in 4, congestion in 13, a few scattered foci of interstitial lymphocytic infiltration in 4 (0.01%), and interstitial

fibroblastic proliferation in kidneys from 4 animals (0.1%). Some renal tubules in 3 animals (2.5, 3%) had pyknotic nuclei. The pelvic epithelial lining was hyperplastic in 2 guinea pigs (2.5%, Fig. 95). Other findings included dilated Bowman's capsules in 3 and more cellular and ischemic glomerular tufts in 5 animals (0.1 to 2.5%).

Ureters from 1 female (0.03%) had a diameter of 3 mm. caused by an obstructive pressure of the gravid uterus.

Urinary bladders from 28 guinea pigs were studied and most of them were normal. Gross findings included distention with urine in 2 guinea pigs and greyish-white fleshy material which had occluded the neck of 1 bladder (3%, Fig. 98). In the female which had obstructed ureters, there was papillary hyperplasia of the epithelium of the urinary bladder (Fig. 96), vacuolated cytoplasm and large vesicular nuclei. The fleshy mass which was in 1 urinary bladder appeared microscopically as eosinophilic granular debris (Fig. 97).

Spleens from 34 animals were studied. The relative weights were normal (Table 9). Microscopically, there were numerous brown pigments in the red pulp of 9 spleens, numerous Prussian blue-positive pigments in 8, numerous macrophages with intensely eosinophilic cytoplasm in 3, and in 2 others a decreased number of lymphocytes in the white pulp (2.5 or 4%, Fig. 51, 52).

Cervical lymph nodes from 18 guinea pigs were studied. Microscopically 2 guinea pigs (1%) and 3 guinea pigs (3%) had hyperplastic lymphocytes (Fig. 56, 57), and 1 (4%) had hypoplastic cortical lymphocytes (Fig. 58, 59). Lymph nodes from 3 (4%) were edematous and congested.

Thymus glands from 25 guinea pigs were examined. Microscopically, the cortex was atrophied in 9 animals. (Fig. 53, 54) and lobules were atrophied in 7 (0.1 to 4%) with increased interlobular adipose tissue (Fig. 55). Three thymuses were congested (4%).

Bone marrow sections from 17 guinea pigs were studied. The histopathologic findings included hyperplasia of erythroblasts in 2 animals (0.1 or 2%, Fig. 64), and depleted erythroid elements in 3 animals (2.5 or 3%) which were replaced by fat and fibrillar material (Fig. 67). There were numerous focal eosinophilic droplets (3%, Fig. 68) and numerous brown pigments (0.1%). Megakaryocytes had filamentous borders and several vesicular nuclei (0.1 or 1%). Some megakaryocytes had more eosinophilic cytoplasm (1%), indistinct nuclei and mesh-like cytoplasm (2%, Fig. 66), or had cytoplasm with dark granules (3%).

Skin sections from 41 guinea pigs were studied. Microscopically, the epidermis from 22 guinea pigs was thin, and this was observed for the most part in animals given high levels of KNO_3 (Fig. 101, 102). Five guinea pigs had

excessively vacuolar nuclei in the epidermis (0.1 to 3%, Fig. 99, 100), and increased numbers of fibroblasts under the epidermis.

Skeletal muscles from 17 guinea pigs were examined. The only lesion was a focus of necrosis and calcification in 1 animal.

Thyroid glands from 17 guinea pigs were studied. Microscopically, 6 guinea pigs (0.1 to 2.5%) had congested glands, and 2 animals had pale or no colloid in the follicles (2.5 or 4%, Fig. 116).

Adrenal glands from 32 guinea pigs were studied. Gross lesions included ecchymotic hemorrhages in 2 guinea pigs. Microscopically, there were congestion and hemorrhage of the zona reticularis in 3, and pyknotic individualized cells of the zona reticularis in 15 guinea pigs. The cytoplasm of most of the cells of the zona reticularis in 6 animals had fine brown pigments, and 3 of these 6 had Prussian blue-positive pigments. The zona glomerulosa was particularly atrophied in 3 animals (Fig. 112, 113).

Eyes from 22 guinea pigs were studied. Microscopically, there were vacuolar degeneration of the corneal epithelium in 2, and focal eosinophilic infiltration in the corneas of 1 animal.

Sections from cerebellum, midbrain, salivary glands, esophagus, small and large intestines, anal glands and prostate glands were normal.

Table 6 - Average daily gain in body weight and consumption of food, water and KNO_3 by guinea pigs in Experiment I.

KNO_3%	Daily gain* Gm./100 Gm. body weight	Daily feed consumption Gm./100 Gm. body weight	Daily water consumption ml./100 Gm. body weight	Daily nitrate intake mg./100 Gm. body weight
0.01	1.13	7.2	36.4	3.6
0.03	1.16	7.2	29.7	8.9
0.10	1.48	8.1	35.5	35.5
0.25	1.31	7.1	29.7	65.3
1.00	1.45	8.0	36.7	367.8
2.00	1.42	7.8	33.8	845.0
2.50	1.56	11.4	41.8	1045.0
3.00	1.15	7.5	27.3	819.0
4.00	0.43	8.2	41.5	1660.0
0.00	1.53	8.9	32.5	0.0

* Daily gain was calculated for the 1st 50 days of treatment.

Table 7 - Reproductive performance for female guinea pigs in Experiment I.

KNO_3%	Number of females	Number of litters	Total alive	Total dead	Average size of live litter	Average number of litters per female
0.01	3	6	15	4	2.5	2.0
0.03	3	6	18	7	3.0	2.0
0.25	3	6	19	3	3.15	2.0
1.00	3	5	15	1	3.00	1.66
2.50	1	1	0	4	0.0	1.0
3.00	3	1	2	0	2.0	1.0
4.00	1	0	0	0	0.0	0.0
0.00	4	8	31	1	3.9	2.0

Table 8 - Average levels of serum electrolytes, albumin/globulin ratio (A/G) and blood urea nitrogen (BUN) in guinea pigs of Experiment I.

KNO_3%	Na	K	A/G	BUN
	m Eq./L serum			mg./100 ml. serum
0.01	135.7	4.9	60/40	24.2
0.03	136.0	6.0	53/47	27.2
0.10	135.7	4.9	54/46	26.7
0.25	146.0	6.1	60/40	20.7
1.00	136.2	5.3	60/40	23.0
2.00	135.0	5.5	65/35	26.3
2.50	137.0	6.0	58/42	23.5
3.00	140.0	5.6	60/40	23.3
4.00	--	--	72/28	27.5
0.00	126 to 140	4.6 to 5.4	58/42	23 to 30

Table 9 - Average relative weights (x) of selected number (n) of organs of guinea pigs in Experiment I.

KNO_3%		Liver	Spleen	Kidneys	Heart	Intestine*	Cecum**	Testes
0.01	x	3.31	0.1	0.9	0.31	12.95	5.13	--
	n	5	4	3	1	5	5	--
	st.d.	0.9	0.0	0.17	0.0	2.5	1.8	--
0.03	x	3.64	0.12	0.73	--	10.37	4.57	--
	n	5	3	3	--	5	5	--
	st.d.	1.4	0.04	0.2	--	4.74	2.45	--
0.10	x	3.0	0.1	0.67	0.3	11.57	4.0	0.8
	n	5	4	3	1	5	5	2
	st.d.	0.3	0.03	0.03	0.0	1.54	1.81	0.3
0.25	x	4.43	0.1	0.84	0.28	13.13	6.6	--
	n	5	5	4	1	5	5	--
	st.d.	0.95	0.0	0.07	0.0	1.73	1.21	--
1.00	x	3.84	0.13	0.88	--	15.0	8.2	--
	n	6	3	3	--	5	5	--
	st.d.	0.6	0.08	0.07	--	1.82	3.64	--
2.00	x	2.97	0.12	0.8	--	11.8	5.23	0.8
	n	4	2	2	--	4	4	1
	st.d.	0.5	0.07	0.19	--	0.8	1.0	0.0
2.5	x	3.24	0.15	0.88	--	10.78	4.23	--
	n	5	5	4	--	5	5	--
	st.d.	0.6	0.03	0.2	--	3.9	2.2	--
3.00	x	3.73	0.15	1.4	0.36	14.3	7.8	1.02
	n	2	4	3	3	4	4	1
	st.d.	0.18	0.06	0.27	0.05	0.05	3.2	0.0
4.00	x	4.31	0.5	1.3	--	--	6.97	--
	n	5	2	2	--	--	5	--
	st.d.	0.8	0.0	0.41	--	--	2.0	--
0.00	x	4.1	0.17	0.74	0.42	14.7	6.15	0.65
	n	5	10	8	4	11	11	3
	st.d.	0.8	0.01	0.17	0.17	1.77	0.08	0.13

* Intestine was weighed with food contents.

** Cecum alone was weighed with food contents.

st.d. Standard deviation.

Weights were in grams.

Experiment II (KNO_2)

Growth. There was no marked depression of growth related to KNO_2 administration at levels as high as 0.5% (Table 10). At 1.0% and higher levels growth was impaired and at the highest levels (1.2%), there was loss of weight (Table 10).

Food and water consumption. Food consumption was not appreciably affected (Table 10). Water consumption was variable for guinea pigs given 1% or less KNO_2, but decreased markedly at levels higher than 1% KNO_2. The maximum daily KNO_2 intake was 352 mg./100 Gm. body weight and this was observed in animals given 1% KNO_2 in drinking water (Table 10).

Signs. Guinea pigs given 0.01 to 0.5% KNO_2 had no outstanding abnormal signs. Four guinea pigs (0.01 to 0.03%) had a loss of muscle tone particularly in the abdominal muscles. The interval between the initial administration and the start of weight loss was decreased by increasing the concentration of KNO_2 in the drinking water. This period was 4 days in 3 guinea pigs (1.2%), 24 days in 3 animals (1.1%), and 39 days for 5 guinea pigs (1%). Other signs, which started around the 40th day of the experiment in animals given higher levels, included emaciation and ruffled cheek hair. One guinea pig was comatose and 2 animals were dull and had yellowish spots on their white hair.

Reproductive performance. The size of live litters was normal in guinea pigs given 0.2% or less KNO_2, slightly decreased for females receiving 0.3 to 0.45% KNO_2, and was zero for females given 0.5% or more KNO_2 (Fig. 119, Table 11). The mortality rate among the newborn was generally increased and reached the maximum in guinea pigs given 0.5% KNO_2. Some females (0.5% KNO_2) apparently had a toxemia induced by the dead fetuses in their uteri. Those dead fetuses were near full term. Some females survived after abortion or absorption of dead fetuses. Yellowish placental masses attached to the endometrium was considered evidence of fetal absorption. Other females died and their uteri contained nearly dehydrated fetuses.

Analyses

Hematologic determinations are summarized (Table 12). Guinea pigs receiving 0.35% or more KNO_2 had some depression of hemoglobin levels. The methemoglobin values were slightly elevated in animals given 1% or more KNO_2. The relative and absolute monocyte counts were increased for animals given 0.2 to 0.45% KNO_2 and was decreased in those given 1.05% or more KNO_2. Erythrocytes from many of the guinea pigs had basophilic stippling and slight polychromasia. A few of the animals given 0.03% or more KNO_2 had a slight anisocytosis. Some receiving 0.1% or more KNO_2 had a variable number of nucleated erythrocytes (Fig. 71). A few (0.5% or more KNO_2) had

hypochromatic erythrocytes, and guinea pigs given 1.2% KNO_2 had macrocytic erythrocytes. Levels of serum electrolytes, total protein and blood urea nitrogen were apparently normal (Table 13). Some guinea pigs (1% or more KNO_2) had slightly increased levels of serum nitrite and normal serum bilirubin values. Serum electrophoresis, generally, showed a normal albumin/globulin ratio. However, animals given 1.05 and 1.1% KNO_2 had an albumin/globulin ratio of 67/33 and 64/36 respectively, compared to the control ratio of 58/42 (Table 13).

Lesions

Hearts from 55 guinea pigs were studied. There were 2 hearts with increased pericardial fluid, 1 with whitish streaks in the wall of the right ventricle (1.2%), and 2 were flabby. Many hearts had albuminous degeneration and hyperplastic sarcolemmal nuclei (Fig. 8). The media of the arterioles in the interventricular septa was vacuolated in 1 heart, and thickened in the newborn of animals given 0.2 to 0.35% KNO_2. Some Anitschkow's myocytes replaced the smooth muscles of arterioles in the interventricular septa of 3 guinea pigs (0.1 to 0.3% KNO_2, Fig. 5, 6), and were focally distributed in the ventricular wall of 2 (0.35%, Fig. 7). There were foci of vacuolar necrosis in the wall of the left ventricle in 10 guinea pigs, in the right ventricular wall of 5 and in the interventricular septum of 1 (Fig. 1, 2, 3, 4).

Incidental observations included eosinophilic and lymphocytic infiltration in the myocardium (0.4%), lymphocytic infiltration in the endocardium (0.35%), and foci of eosinophilic infiltration in the epicardium around the coronary arteries (1% KNO_2).

Testes from 27 guinea pigs were examined and 5 were from immature guinea pigs. There was an apparent partial interference of spermatogenesis at the primary spermatocyte or spermatid stages and atrophied Leydig cells in guinea pigs given 0.35 to 1.1% KNO_2 (Fig. 11).

Seminal vesicles from 15 guinea pigs were examined and all were normal except for an apparent atrophy of 4 (0.35 to 1.1%, Fig. 20).

Epididymides from 23 males were studied. Four of the males which had testicular lesions had degenerated spermatozoa inside the ducts (0.35 to 1.05%), and 1 (0.45%) had a few round cells of 15 to 20 μ in diameter and fine eosinophilic droplets in the ducts (Fig. 17).

Ovaries from 19 guinea pigs were examined. Microscopic findings included a proliferated granulosa cell layer of the Graafian follicles which had formed a corpus luteum-like body with cells having bluish cytoplasm in 2 animals (0.5 and 1%, Fig. 41, 42). Ovaries from females given 1% KNO_2 in drinking water had extremely numerous Graafian follicles in the cortex. Follicles were hemorrhagic (Fig. 38). Syncytial giant cells, macrophages and intracellular yellowish green granules were observed.

Uteri from 16 guinea pigs were examined. The only gross finding was a prolapsed uterus with a hemorrhagic wall in 1 animal (0.3%). Microscopically, there was endometritis in 7 guinea pigs (0.03 to 0.5%) with papillary hyperplasia of the endometrium (Fig. 28). There was retarded involution and erosion in the endometrium of 3 guinea pigs (0.4%). The prolapsed uterine wall was necrotic. The wall of a uterus which contained a mummified fetus (0.5%) was congested but had no bacterial growth on culture. One uterus (0.4%) had 2 discoidal placental masses and fetuses. Remnants of a fetal eye were present. There was subacute cervicitis in several females (0.5%, Fig. 22, 23, 24, 25).

Placentas from 13 guinea pigs were examined. Microscopically, there were ischemia (Fig. 35), pinkish necrotic foci with pyknosis and karyorrhexis in 6 (0.2 to 0.5%), and bluish foci resembling calcium in 3 placentas (0.35 to 0.5%, Fig. 33). One placenta was hemorrhagic (0.5%).

Skin sections from 56 guinea pigs were studied. Microscopically, the epidermis was thin in 27 guinea pigs particularly in those animals receiving 0.3% or more KNO_2 (Fig. 101, 102). Fourteen guinea pigs had excessively vacuolated nuclei in the epidermis (0.01 to 0.5%, Fig. 99, 100). The dermal connective tissue of 9 animals was hyalinized (0.2 to 1.2%).

Lungs from 54 guinea pigs were studied. Microscopically, 24 were congested particularly in animals given 0.5% or more KNO_2. Thickened mediae of pulmonary arterioles were noted particularly in animals given 1% or more KNO_2 (Fig. 75, 76). The mediae of the pulmonary arterioles in 7 lungs were vacuolated (0.01 to 1%). Atelectasis with compensatory emphysema were seen in 15 animals, particularly those given 0.3% KNO_2 (Fig. 83). The interalveolar septa were hyperplastic in 22 animals (0.01 to 0.45%, Fig. 75, 79), and edematous in 4 (0.5 to 1%, Fig. 80). Lungs of 7 animals had interstitial lymphocytic infiltrations (0.03 to 0.4%), and 3 (1.05 to 1.2% KNO_2) had perivascular eosinophilic infiltrations. Eight lungs had hyperplastic peribronchiolar smooth muscle (0.2 to 1%).

Stomachs from 57 guinea pigs were studied. There were 4 stomachs distended with gas bubbles and fluid (0.35 to 1.1%, Fig. 45), and 6 stomachs were empty (0.4 to 1.1%). Microscopically, there were vesicular nuclei of the superficial layer of the gastric mucosa of 4 stomachs.

Livers from 60 guinea pigs were studied. The relative weights were variable for animals given 0.5% or less KNO_2 and increased at levels of 1% or more KNO_2 (Table 14). Four livers (0.4 to 1.05%) were yellow and 12 had an appearance of slight centrolobular fatty change (0.5 to 1.2%, Fig. 84). One liver (1.1%) had patchy yellowish foci 5 mm. in diameter. Microscopic findings included vacuolar degeneration

of hepatic cells in 11 guinea pigs (0.03 to 0.5%) which was mostly centrolobular. This change was more frequent in guinea pigs given 0.03 to 0.1% KNO_2. Two livers had periportal vacuolar degeneration (0.4 to 0.5%), 7 had diffuse round vacuoles of irregular size and shape (0.1 to 0.2%), and 13 livers had centrolobular fatty change (0.4 to 1.2% but mostly 1.1% or more KNO_2, Fig. 85). There was cloudy swelling in 13 livers (0.1 to 1.05%), and congestion in 9 (0.5 to 1.2%). Only 2 livers had traces of Prussian blue-stained pigments in the cytoplasm of the hepatic cells particularly around the portal triads (Fig. 90, 91). The results of Best's carmine stain indicated that there was a lack of positive material in 14 livers, either completely, centrolobularly or both centro- and peripherolobularly (Fig. 86).

Kidneys from 59 guinea pigs were studied. The relative weights were increased for guinea pigs given 1% or more KNO_2 (Table 14). One animal (1.05%) had yellow kidneys. Microscopically, there were scattered foci of interstitial fibroblastic proliferation in kidneys from 9 animals (0.01 to 0.5%), interstitial lymphocytic infiltration in 6 (0.01 to 0.1%), cloudy swelling in 10 (1 to 1.2%), and congestion in 14 (mostly 0.5% or more).

Urinary bladders from 41 guinea pigs were examined. Gross findings included white precipitates in 4 urinary bladders (0.3 to 1.1%), and distention with clear yellow

urine in 5 others (1 to 1.2%). There were no outstanding microscopic lesions.

Spleens from 56 guinea pigs were studied. The relative weights were nearly doubled in guinea pigs given 1% KNO_2 (Table 14). Microscopically, there were numerous hematopoietic centers in 15 guinea pigs (0.01 to 1.1%), particularly in females given 0.4 to 0.5% KNO_2. There were a few scattered megakaryocytes in 6 spleens (0.03 to 0.5%), hyperplasia of lymphocytes in the white pulp in 5 (0.03 to 0.3%, Fig. 49, 50), and a hypoplasia of lymphocytes in the white pulp of 6 spleens (0.35 to 1.1%, Fig. 51,52). There were numerous macrophages filled with brown pigments in spleens of 15 guinea pigs, particularly those given 0.5% or more KNO_2 in drinking water. Spleens of 5 females had macrophages with intensely eosinophilic cytoplasm (0.1 to 0.45%). Thirteen spleens, mostly in animals given 0.3% or more KNO_2 were congested and spleens of 4 males had increased reticular cells in the red pulp (0.2 to 0.45%). There were a few Prussian blue-positive pigments in 13 spleens, particularly in those given 0.2 to 0.35% KNO_2. Spleens of 4 newborn (0.2, 0.45%) and spleens of 2 pregnant females (0.4, 0.45%) had no Prussian blue-positive granules.

Cervical lymph nodes from 24 guinea pigs were studied. There were some lymph follicles, in 2 females, which were surrounded by a few prominent rows of lymphocytes (0.03%). The cortical lymphocytes were hyperplastic in 6

guinea pigs (0.03 to 0.4%, Fig. 56, 57), or decreased (Fig. 58, 59), particularly in the germinal centers in 3 females (0.5 to 1.2%). Lymph nodes from 5 animals (0.5 to 1.2%) were congested.

Thymus from 46 guinea pigs were examined. Many thymuses had cortical and lobular atrophy which was difficult to differentiate from physiologic atrophy. There was increased interlobular adipose tissue, congestion and excessive hyalinization of Hassall's corpuscles especially in animals given 0.5% or more KNO_2 (Fig. 53, 54).

Bone marrow sections from 15 guinea pigs were studied. There were hyperplastic erythroblasts in 9 guinea pigs (Fig. 64). The nuclei of megakaryocytes were vesicular in 2 guinea pigs and were pyknotic in 1 animal. Some megakaryocytes had indistinct nuclei. The cytoplasm of many megakaryocytes was light and had a vacuolar or reticular appearance (Fig. 66). Bone marrow had excessive fibrillar deposits (Fig. 67), numerous brown pigments and sickle-shaped erythrocytes (0.3 to 0.45%).

Cerebellums from 43 guinea pigs were studied. The only microscopic lesion was congestion in 11 animals, mostly in those given 0.3 to 1.2% KNO_2 in drinking water.

Midbrains from 49 guinea pigs were examined. Eight were congested, mostly in animals given 0.5% or more KNO_2. The choroid plexuses in 2 guinea pigs had fine brown pigments.

Skeletal muscles from 36 guinea pigs were examined. The only gross finding was greyish-white muscles in 1 (0.4%). Microscopically, there was degeneration and necrosis of some muscle bundles in 5 animals (0.3 to 1.1%, Fig. 105), with foci of fibrosis in 2. Glycogen could not be demonstrated in muscle from 6 females (0.2 to 0.4%).

Thyroid glands from 34 animals were studied. The only histopathologic findings were more numerous vacuoles in the colloid adjacent to the follicular epithelium in 9 guinea pigs (0.01 to 1%, Fig. 117), and congested thyroid glands from 6 (0.01 to 1.2%).

Adrenal glands from 54 guinea pigs were examined. Grossly, the adrenals of 2 guinea pigs were hemorrhagic. Microscopically, the adrenals from 5 animals had a hemorrhagic zona reticularis (particularly males given 0.2% KNO_2). Nine animals had congested adrenals (mostly those given 1.05% or more KNO_2), and 12 had many individualized pyknotic cells of the zona reticularis (0.03 to 1%). There were a few Prussian blue-positive granules in the cytoplasm of cells of the zona reticularis in 8 guinea pigs (0.01 to 0.5%). There was a mild interstitial lymphocytic infiltration in the medulla of 3, and in the zona reticularis of another 3 guinea pigs (Fig. 110, 111). There was a partially atrophied zona glomerulosa in 10 guinea pigs given 1% or more KNO_2 (Fig. 112, 113).

Sections from eyes, prostate glands, small and large intestines, pancreas, esophagus, salivary glands, trachea and anal glands were normal.

Table 10 - Average daily gain in body weight and consumption of food, water and KNO_2 by guinea pigs in Experiment II.

KNO_2%	Daily gain* Gm./100 Gm. body weight	Daily food consumption Gm./100 Gm. body weight	Daily water consumption ml./100 Gm. body weight	Daily nitrite intake mg./100 Gm. body weight
0.01	1.17	--	54.1	5.41
0.03	1.39	8.1	36.4	10.91
0.10	0.92	6.6	27.2	27.2
0.20	2.20	12.1	47.1	94.2
0.30	1.15	9.1	37.1	111.3
0.35	0.63	7.2	25.0	87.5
0.40	1.15	8.7	29.0	119.2
0.45	1.25	7.1	17.8	80.1
0.50	1.60	8.4	29.8	149.0
1.00	0.19	6.1	35.2	352.0
1.05	0.35	8.3	19.3	202.7
1.10	0.07	6.4	11.6	127.6
1.20	(-0.40)	6.1	16.9	202.8
0.00	1.53	8.9	32.5	0.0

* Daily gain was calculated for the 1st 50 days of treatment.

Table 11 - Reproductive performance for female guinea pigs in Experiment II.

KNO_2%	Number of females	Number of litters	Total alive	Total dead	Average size of live litter	Average number of litters per female
0.03	3	8	21	3	3.9	2.7
0.10	3	6	17	3	2.8	2.0
0.20	3	4	12	1	3.0	1.3
0.30	3	2	5	1	2.5	0.7
0.35	4	6	15	3	2.5	1.5
0.40	3	2	5	1	2.5	0.7
0.45	2	2	5	1	2.5	1.0
0.50	4	4	0	10	0.0	1.0
1.00	4	4	0	2	0.0	0.25
0.00	4	8	31	1	3.9	2.0

Table 12 - Partial hemogram of guinea pigs in Experiment II.

KNO_2%	Hemoglobin Gm./100 ml. blood	Methemoglobin Gm./100 ml. blood	Monocyte %	Monocytes per cmm. blood
0.01	14.3	0.0	2.3	117
0.03	13.0	0.0	2.0	116
0.10	13.5	0.0	2.8	126
0.20	12.5	0.1	5.6	258
0.30	13.4	0.0	4.7	248
0.35	11.7	0.1	6.7	366
0.40	10.8	0.1	7.6	431
0.45	11.4	0.3	4.5	279
0.50	12.0	0.12	2.9	116
1.00	11.9	1.66	2.9	154
1.05	11.3	2.13	0.2	8
1.10	11.2	0.82	1.6	64
1.20	9.5	1.85	0.37	23
0.00	14.24	0.02	2.64	143

Table 13 - Average values of serum electrolytes, total protein, blood urea nitrogen (BUN) and albumin/globulin ratios (A/G) for guinea pigs in Experiment II.

KNO_2%	Na / K m Eq./L serum		Serum protein Gm./100 ml. serum	BUN mg./100 ml. serum	A/G
0.01	140	5.8	4.6	23.5	60/40
0.02	140	5.0	5.0	24.7	59/41
0.10	140	6.3	4.4	27.2	59/41
0.20	139	6.5	4.1	27.0	63/37
0.30	--	--	--	25.3	61/39
0.35	137	6.0	3.4	--	60/40
0.40	140	6.1	4.0	27.2	57/43
0.45	123	5.0	3.0	31.5	65/35
0.50	146.2	5.6	4.0	26.0	56/44
1.00	143.0	6.1	4.0	30.7	58/42
1.05	170	8.3	4.3	--	67/33
1.10	137	4.7	3.2	30.5	64/36
0.00	126 to 140	4.6 to 5.4	3.7 to 4.5	23 to 30	58/42

Table 14 - Average relative weights (x) of selected number (n) of organs of guinea pigs in Experiment II.

KNO_2%		Liver	Spleen	Kidneys	Heart	Intestine*	Cecum**	Testes
0.01	x	3.5	0.15	0.7	0.3	12.0	4.5	0.7
to	n	10	8	7	4	10	10	3
0.03	st.d.	0.9	0.04	0.07	0.03	1.6	1.2	0.1
0.10	x	4.25	0.13	0.75	0.33	12.5	5.7	0.5
to	n	10	8	8	4	10	10	3
0.20	st.d.	0.9	0.08	0.10	0.13	1.0	1.2	0.3
0.30	x	4	0.16	0.7	0.3	12.0	6.0	0.9
to	n	14	13	12	8	14	14	3
0.40	st.d.	1.0	0.06	0.2	0.1	4.0	2.2	0.01
0.45	x	3.4	0.17	0.8	0.33	11.7	6.0	0.80
to	n	9	6	6	3	9	9	1
0.50	st.d.	0.5	0.04	0.15	0.07	3.0	1.5	0.0
1.00	x	5.6	0.33	0.90	--	13.5	5.2	0.9
to	n	13	4	2	--	13	14	1
1.10	st.d.	1.7	0.15	0.0	--	3.0	1.5	0.0
1.20	x	6.4	--	--	--	13.2	6.6	--
	n	3	--	--	--	3	3	--
	st.d.	1.4	--	---	---	1.13	1.0	--
0.00	x	4.1	0.17	0.74	0.42	14.70	6.15	0.7
	n	11	10	8	4	11	11	3
	st.d.	0.9	0.07	0.17	0.24	1.77	0.08	0.13

* Intestine was weighed with food contents.

** Cecum alone was weighed with food contents.

st.d. Standard deviation.

Weights were in grams.

Experiment III ($NaNO_3$)

Growth. The daily gain was normal for guinea pigs given 0.5 to 1.5% $NaNO_3$ in drinking water. Animals given 2% or more $NaNO_3$ lost weight (Table 15).

Food and water consumption. Guinea pigs given 3% $NaNO_3$ consumed about 50% of the normal amount of food. Water consumption was about normal in guinea pigs given 0.5 to 1.5% $NaNO_3$ and decreased for animals at levels of 2 and 3% $NaNO_3$. The highest calculated daily $NaNO_3$ consumption was 636.3 mg./100 Gm. body weight. This amount was consumed by guinea pigs given 3% $NaNO_3$. Animals which consumed 356 mg./100 Gm. body weight lost weight while those which consumed 457.5 mg./100 Gm. body weight daily gained weight. The former consumed less water and more food than the latter (Table 15).

Signs. Two of the animals given 0.5% $NaNO_3$ lost weight. One male started losing weight from the 93rd day of the experiment, and 1 female lost weight continuously for 20 days after parturition. The only sign observed in animals given 1% $NaNO_3$ was increased excitability. Animals receiving 2% $NaNO_3$ became recumbent or died. Animals given 3% $NaNO_3$ started to lose weight as early as the 5th day of experiment and died as early as the 13th day. Severe weakness was noted before death and there was a loss of hair in the region of the nostrils.

Reproductive performance (Fig. 119, Table 16). Guinea pigs given 0.5 to 1% $NaNO_3$ had a mild disturbance in reproductive performance. One female (0.5%) gave birth to a normal 1st litter of 3 guinea pigs. A 2nd litter of 6 was born 15 days before term, 3 were dead and 3 were alive. The live newborns averaged only 50 Gm. They were weak and died within 24 hours. One female (1%) had a litter of 5 which averaged 50 Gm. All of them were weak, 2 died within 7 days and the rest survived. Animals which were bred while given 1.5% $NaNO_3$ in drinking water became pregnant, but later were unable to walk and were killed near the middle of the gestation period. Their uteri contained dead fetuses averaging 27 Gm. Females given 2% $NaNO_3$ or more died before becoming pregnant.

Analyses

Hematologic determinations. Guinea pigs given $NaNO_3$ in the drinking water had normal values for hemoglobin, methemoglobin, and total and differential leukocytes, except for monocytes. The percentage of monocytes was increased in animals given 0.5 to 1% $NaNO_3$, and was normal or decreased in those given 1.5% or more $NaNO_3$ (Table 17). Slight polychromasia, hypochromasia and anisocytosis were observed in the erythrocytes of some animals.

Serum electrolytes and total protein. The potassium levels were increased in guinea pigs given 1% $NaNO_3$

and serum protein levels were increased in animals given 1.5% $NaNO_3$ (Table 17).

Serum nitrite, bilirubin and blood urea nitrogen (BUN). The serum nitrite and bilirubin levels were normal for all treatments. The BUN values were normal for the levels tested.

Serum electrophoresis. The albumin/globulin ratio was increased in guinea pigs given 1.5% $NaNO_3$ (Table 17).

Lesions

Hearts from 16 guinea pigs were studied. Two guinea pigs had a flabby heart which contained chocolate-brown blood (1.5%). Microscopically, the myocardium of 5 guinea pigs was edematous (0.5 to 3%), and the media of the arterioles in the interventricular septa of the newborns from animals given 1% $NaNO_3$ was thickened and vacuolar. Foci of Anitschkow's myocytes (Fig. 7) and vacuolar necrosis were noted in the myocardium especially in the papillary muscles of 2 guinea pigs (1.5%, Fig. 4). Three animals had congested myocardiums (1.5 to 3%), and 1 guinea pig had a few foci of lymphocytes in the endocardium.

Uteri from 6 guinea pigs were studied. Microscopic findings included abnormally proliferated uterine glands in 4 animals, papillary hyperplasia of the endometrium in 3 (Fig. 8), and retarded involution of 1 endometrium.

Placentas from 2 guinea pigs were examined. There were necrotic changes at the utero-placental junction (1.5%, Fig. 33).

Lungs from 13 animals were studied. There were follicular lymphocytic accumulations in lungs from 3 animals (1 to 1.5%), eosinophilic infiltrations in 2 (3%), and eosinophils in the wall of pulmonary arterioles of 1 (3%). Lungs from 8 guinea pigs were congested (1.5 to 3%). There were hyperplastic septal cells (Fig. 79), and thickened mediae of the pulmonary arterioles in 6 guinea pigs (0.5 to 1.5%, Fig. 75, 76).

Stomachs from 15 guinea pigs were examined. The gastric contents were liquid in 3 (0.5 to 1.5%), and absent in 2 (1.5%). No outstanding lesions were found microscopically.

Small intestines from 15 guinea pigs were studied. The relative weights were decreased in guinea pigs given 1.5% $NaNO_3$. There were no outstanding lesions microscopically.

Large intestines from 8 guinea pigs were examined. The only observation was the reduced size of 1 cecum. Microscopically, this cecum had papillary cystic hyperplasia of the epithelial lining with brown casts inside the cysts.

Livers from 19 guinea pigs were studied. The relative weights were increased at 2 and 3% levels of $NaNO_3$ (Table 18). The liver of 1 male (1.5%) had uniformly distributed yellow foci of 1 to 2 mm. in diameter (Fig. 84)

which microscopically was centrolobular fatty change. Histopathologic findings included cloudy swelling in 6 livers (0.5 to 1.5%) and congestion in another 6 (2 to 3%). There was a complete absence of PAS-positive material from 2 livers (0.5%), and a periportal absence in 4 (1 to 1.5%, Fig. 86). Cytoplasm of the hepatic cells had iron granules in 2 livers (1, 1.5%), particularly periportal. The cytoplasm of the hepatic cells in 3 livers (1.5 to 3%) had round vacuoles of irregular size distributed diffusely, but particularly periportal. Numerous hematopoietic centers were noticed in sinusoids of 3 livers (0.5 to 3%, Fig. 92, 93).

Kidneys from 18 guinea pigs were studied. The relative weights were slightly increased (Table 18). Microscopically, all animals receiving 2% or more $NaNO_3$ had congested kidneys. Numerous renal tubules were dilated in 2 animals (1, 1.5%, Fig. 94). The cytoplasm of the renal tubules was vacuolated in 1 animal (1.5%) and a few cortical renal tubules had Prussian blue-positive granules, and hematin or hyalin casts in the lumina.

Spleens from 16 guinea pigs were examined. Two spleens were enlarged and another 2 were black. Histopathologic findings included congestion of 6 spleens (1.5 to 3%), Prussian blue-positive reticulum in 1 spleen (1.5%), and a decreased number of lymphocytes in the white pulp of 2 (1, 3%, Fig. 51, 52). Other observations included numerous

hematopoietic centers, a few scattered megakaryocytes, and fairly numerous macrophages filled with brown pigments.

Cervical lymph nodes from 4 guinea pigs were studied. The only lesions, observed microscopically, were increased (0.5%, Fig. 56, 57), or decreased (1.5, 2%) numbers of lymphocytes in the cortex with fibrillar eosinophilic material replacing the centers of lymph follicles (Fig. 58, 59).

Thymuses from 14 guinea pigs were studied. Microscopically, there was lobular (0.5 to 1.5%) and cortical (1.5 to 3%) atrophy in 7 animals. Hassall's corpuscles were excessively hyalinized (Fig. 54) with increased interlobular adipose tissue in 2 thymuses (1.5%). There was cortical edema in 2 (3%) and congestion in 5 (2, 3%).

Cerebellums from 12 guinea pigs were examined. The only histopathologic finding was congestion in animals given 2% or more $NaNO_3$ in drinking water.

Midbrains from 17 guinea pigs were examined. Seven were congested (2, 3%) and 1 was hemorrhagic (3%).

Skin sections from 17 guinea pigs were examined. Microscopically, the epidermis was thin in 8 animals (1.5 to 3%, Fig. 101, 102), and had vacuolar nuclei in 7 (1 to 3%, Fig. 99, 100). The dermis had an increased number of fibroblasts, mostly under the epidermis, and hyalinized dermal collagen in the newborns.

Thyroid glands from 12 guinea pigs were studied. Congestion was the only lesion seen microscopically.

Adrenal glands from 16 animals were examined. Grossly, 1 animal had hemorrhagic adrenals (3%). Microscopically, there was congestion (mostly in animals given 2% or more $NaNO_3$), hemorrhage of the zona reticularis in 3 animals (3%), and numerous fine brown pigments in the cytoplasm of most cells of the zona reticularis (1.5%).

Sections from testes, epididymides, seminal vesicles, prostate gland, ovaries, urinary bladder, trachea, esophagus, tongue, salivary glands, pancreas, large intestine, skeletal muscles and eyes were normal.

Table 15 - Average daily gain in body weight and consumption of food, water and nitrate by guinea pigs in Experiment III.

$NaNO_3$%	Daily gain* Gm./100 Gm. body weight	Daily food consumption Gm./100 Gm. body weight	Daily water consumption ml./100 Gm. body weight	Daily nitrate consumption mg./100 Gm. body weight
0.5	1.08	7.0	38.4	192
1.0	1.11	7.2	35.0	350
1.5	1.08	7.5	30.0	457
2.0	(-2.89)	9.5	17.0	356
3.0	(-1.88)	4.6	21.0	635
0.0	1.50	8.9	33.0	000

* Daily gain was calculated for the 1st 50 days of treatment.

Table 16 - Reproductive performance for female guinea pigs in Experiment III.

$NaNO_3$%	Number of females	Number of litters	Total alive	Total dead	Average size of live litter	Average number of litters per female
0.5	2	3	6	6*	2.0	1.5
1.0	2	2	6	2	3.0	1.0
1.5	2	2	0	5	0.0	1.0
2.0	2	0	0	0	0.0	0.0
3.0	2	0	0	0	0.0	0.0
0.0	4	8	31	1	3.9	2.0

* Three died within 24 hours after birth and 3 were born dead.

Table 17 - Monocyte count, serum electrolytes, total protein, blood urea nitrogen (BUN), and albumin/globulin ratio (A/G) for guinea pigs of Experiment III.

$NaNO_3$%	Monocytes per cmm. blood	Na (m Eq./L serum)	K (m Eq./L serum)	Total serum protein Gm./100 ml.	BUN mg./100 ml. serum	A/G
0.5	261	137	5.2	4.2	21.2	60/40
1.0	399	135	6.6	4.2	21.7	60/40
1.5	133	140	5.0	6.4	26.7	73/27
2.0	000	--	--	--	--	--
3.0	000	--	--	--	--	--
0.0	143	126 to 140	4.6 to 5.4	3.7 to 4.5	23 to 30	58/42

Table 18 - Average relative weights (x) of a selected number (n) of organs in guinea pigs of Experiment III.

$NaNO_3$%		Liver	Spleen	Kidneys	Heart	Intestine*	Cecum**	Testes
0.5	x	4.0	0.24	0.8	0.37	15.56	7.36	--
	n	4	4	4	4	4	4	--
	st.d.	1.6	0.03	0.07	0.06	2.28	2.24	--
1.0	x	3.98	0.15	0.81	0.31	12.84	5.86	--
	n	4	4	4	4	4	4	--
	st.d.	0.56	0.04	0.11	0.08	1.86	0.7	--
1.5	x	2.96	0.29	0.88	0.36	5.59	1.94	0.9
	n	3	2	3	1	3	3	1
	st.d.	0.9	0.03	0.25	0.0	1.14	0.8	0.0
2.0	x	6.0	--	--	--	16.33	6.68	--
	n	4	--	--	--	4	4	--
	st.d.	1.44	--	--	--	1.36	0.9	--
3.0	x	4.94	--	--	--	14.2	7.0	--
	n	3	--	--	--	3	3	--
	st.d.	0.9	--	--	--	1.59	2.0	--
0.0	x	4.08	0.17	0.74	0.42	14.70	6.15	0.65
	n	11	10	8	4	11	11	3
	st.d.	0.9	0.07	0.17	0.24	1.77	0.08	0.13

* Intestine was weighed with food contents.

** Cecum alone was weighed with food contents.

st.d. Standard deviation.

Weights were in grams.

Experiment IV ($NaNO_2$)

Growth. The rate of gain was greatly decreased in guinea pigs given 0.5 to 0.7% $NaNO_2$ in the drinking water. Animals given 0.9% or more $NaNO_2$ lost weight (Table 19).

Food and water consumption. Food consumption was normal or slightly decreased in guinea pigs given 0.5 to 1% $NaNO_2$. Guinea pigs given 1.2% $NaNO_2$ consumed a very small amount of food. Water consumption was decreased at all levels and most markedly for guinea pigs given 1.2% $NaNO_2$. The calculated maximum daily $NaNO_2$ consumption was 197.1 mg./100 Gm. body weight. This amount was consumed by guinea pigs given 0.9% $NaNO_2$. Guinea pigs which were given 1.2% $NaNO_2$ and which consumed 109 mg./100 Gm. body weight daily lost weight while animals which were given 0.5 and 0.7% $NaNO_2$ and consumed nearly the same amount of chemical daily gained weight. However, water and food consumption was much lower for guinea pigs given 1.2% $NaNO_2$ (Table 19).

Signs. Most of the guinea pigs receiving 0.5% $NaNO_2$ had periods of fluctuation in body weight. One animal died on the 20th day of the experiment. Two animals lost weight steadily from the 160th day of the experiment. Guinea pigs which were given 0.7% $NaNO_2$ had successive periods of slight loss and gain in body weight and 1 animal lost weight steadily from the 50th day of the experiment until it was killed. This animal was comatose and had labored breathing on the 8th day of the experiment and, although it recovered,

it acted similarly before it was killed on the 84th day. Two female guinea pigs which were given 0.9% $NaNO_2$ started losing weight from the 2nd day of the experiment. One died 3 and the other 32 days later. Another female continued to lose weight after the 40th day until she was killed 25 days later. A male guinea pig (0.9%) kept gaining weight until it was killed on the 74th day of the experiment. Animals which were given 1% $NaNO_2$ started losing weight from the 2nd day of treatment and 1 died 28 days later. Body weight fluctuated until they were killed or died after periods as long as 140 days. Two animals had a partial hair loss from the face after 20 days of the experiment. Two of the guinea pigs given 1.2% $NaNO_2$ died after 10 and 31 days. They started losing weight from the 2nd day. Two white hair-coated guinea pigs had focally yellowish stained hair starting from the 13th day.

Reproductive performance. Only 2 females out of 11 became pregnant. One of the 2 had 3 embryos of less than 1 cm. in length. The other female died and endometritis was noted. Its uterus contained 1 embryo that was less than 1 cm. long.

Analyses

Hematologic determinations. The hemoglobin levels were slightly decreased. Methemoglobin levels and the monocyte percentages were slightly increased. The total leukocyte

counts and the differential counts of neutrophils, lymphocytes, basophils and eosinophils were normal (Table 22). Guinea pigs given 0.5% or more $NaNO_2$ had clear water-like serum with a highly contractile clot. Abnormalities which were observed in erythrocytes included hypochromasia, polychromasia, basophilic stippling, anisocytosis, poikilocytosis and macrocytosis. There were variable numbers of nucleated erythrocytes and only a few animals had normal erythrocytes.

Serum electrolytes and total protein. The serum sodium and potassium levels were slightly raised and the total serum proteins were in the upper normal range (Table 20).

Serum nitrite and bilirubin. Animals given 0.7% or more $NaNO_2$ had increased levels of serum nitrite and normal levels of serum bilirubin.

Blood urea nitrogen (BUN) and serum electrophoresis. Data indicated a normal range for BUN (Table 20) and albumin/globulin ratios were normal.

Lesions

Hearts from 16 guinea pigs were examined. The blood was chocolate brown in several animals. The relative weight of the heart was increased for guinea pigs given 0.7% or more $NaNO_2$ (Table 23). Histopathologic lesions included edema of the myocardium particularly of the papillary muscles, congestion (4/16) and hyperplasia with hypertrophy of the

sarcolemmal nuclei (5/16, Fig. 8). Many of the guinea pigs given 0.9 to 1.2% $NaNO_2$ had foci of vacuolar necrosis in the ventricular wall especially in the papillary muscles (6/11, Fig. 1, 2, 3, 4). Other isolated observations, seen in animals given 1% or more $NaNO_2$ included lymphocytic infiltration around the coronary arteries (2/7), foci of lymphocytic and eosinophilic infiltration in the myocardium and Anitschkow's myocytes in the wall of the coronary arteries.

Seminal vesicles from 4 guinea pigs were studied. Two were grossly atrophied (Fig. 20, 21), and microscopically, there was atrophy of the epithelial lining (Fig. 19).

Epididymides from 8 guinea pigs were examined. The epithelial lining was atrophied in 1 animal. (0.5%). The spermatozoa in the ducts were reduced in number and mostly disintegrated. A few foci of interstitial lymphocytic infiltration were seen in 2 animals (1, 1.2%).

Ovaries from 8 guinea pigs were examined. Histopathologic findings were noted at levels of 0.9% or more $NaNO_2$. They included a large eosinophilic mass with a focus of calcification in ovaries from 1 animal. Ovaries of 1 female were congested and had disintegrated ova with necrosis of the zona granulosa (Fig. 37). There were some foci of interstitial lymphocytic infiltration (1.2%).

Uteri from 8 animals were examined. One uterus had a dark-reddish wall and the vaginal lumen was distended with viscid blood. Microscopically, there were foci of pyknotic

nuclei at the utero-placental junction in 2 guinea pigs given 0.5 or 0.7% $NaNO_2$ (Fig. 33). The wall of 1 uterus was necrotic (0.5% $NaNO_2$), and the uterine glands were abnormally proliferated in 5 (0.5 to 0.9%). Some uteri had necrotic foci in the endometrium with congestion and edema of the deeper layers (1.2%).

Lungs from 18 animals were studied, and 3 were emphysematous. Microscopically, there was hyperplastic septal cells in 9 animals, congestion in 11, and atelectasis and compensatory emphysema in lungs from 5 (Fig. 83). Other incidental observations were peribronchiolar, or perivascular eosinophilic infiltration. The mediae of the pulmonary arterioles were vacuolated in 4 animals (0.7 to 1.2%). There was perivascular lymphocytic infiltration around the veins in the lungs from 1 animal (1.2%).

Tongues from 15 guinea pigs were examined. Microscopic findings included hyperplasia and hypertrophy of sarcolemmal nuclei in 7 tongues (0.7 to 1%). There were numerous cells resembling Anitschkow's myocytes among skeletal muscles of tongues in 2 guinea pigs (0.9%, Fig. 109), and myofibrillar separation and loss of striation of many muscle fibers in 5 (0.9, 1%). There were foci of necrosis and calcification in 1 tongue (1%). Squamous metaplasia of the epithelial lining of the mucous glandular ducts was noted in 1 animal (1.2%).

Stomachs from 19 guinea pigs were studied. The consistency of the ingesta was more fluid and more greenish or yellowish than normal in 5 stomachs (0.5 to 1%). Single observations included an empty stomach (1%), a stomach distended with gas and greenish fluid (0.5%), and a few petechial hemorrhages in the gastric mucosa (0.9%).

Small intestines from 13 guinea pigs were studied. The relative weights were slightly less than normal at all levels of treatment (Table 23). Microscopically, there were no outstanding lesions.

Livers from 19 guinea pigs were examined. Gross lesions included 3 diffusely yellowish livers (0.9, 1%) and in 1 animal, the liver had regularly distributed yellowish spots 1 mm. in diameter (Fig. 84). The relative weights did not deviate widely from normal (Table 23). Microscopically, there were cloudy swelling in 5 livers (1%) and fatty change either centrolobular (1 animal, 0.9%, Fig. 85) or in the region of the portal triads (4/19). The mediae of the hepatic arterioles were vacuolated in 1 animal (1%), and hyalinized in 7 (0.5 to 1%, Fig. 87, 88). The absence of PAS-positive material was noted in several animals and was variable in degree and location. Complete absence of PAS-positive material was seen in 1 animal (0.7%), and in several, the depletion was least in the mid-zonal hepatic cells, and was greatest centrolobularly (0.9%), or in the periportal hepatic cells in 2 guinea pigs (0.5%, Fig. 86). Brown pigments were in the

cytoplasm of some hepatic cells of 2 animals (0.7, 1.2%), or in the macrophages among hepatic cells.

Pancreases from 15 guinea pigs were studied and all were normal except for incidental findings. Islets of Langerhans had a few pyknotic nuclei in 4 animals, and an increased number of alpha cells in 3.

Kidneys from 18 guinea pigs were studied. One animal (1%) had yellowish kidneys. The relative weights were increased at all levels of treatment (Table 23). Microscopically, lesions were inconsistent and mild, and were not considered related to level of chemical.

Urinary bladders from 15 guinea pigs were examined. Grossly, there were white precipitates in the urinary bladder of 1 animal (0.9%). The epithelium was thinner than normal and contained many vacuoles in 3 guinea pigs (0.9 to 1.2%).

Spleens from 14 guinea pigs were studied. The relative weights were slightly increased for guinea pigs given 0.7% or more $NaNO_2$ (Table 23). Grossly, 2 spleens (0.9%) were enlarged to an estimated 2 or 3 times normal size (Fig. 44), and 1 was black (1.2%). Microscopically, 8 spleens were markedly congested. The amount of Prussian blue-positive pigments varied from none in 5 to little in 3 and much in another 3. Hematopoietic centers were numerous in 4 spleens (0.7 to 1%). There were small foci of necrotic lymphocytes in the white

pulp of 1 spleen. Lymphocytes were hypoplastic in the white pulp of 3 (0.9%, Fig. 51, 52).

Cervical lymph nodes from 7 guinea pigs were examined. The only histopathologic findings were congestion in 3 (0.7 to 1%), and hyperplasia of the cortical lymphocytes in 2 guinea pigs (0.9%, Fig. 56, 57).

Thymuses from 14 animals were examined. There was cortical and lobular atrophy with excessively hyalinized Hassall's corpuscles, particularly in those given higher levels of $NaNO_2$.

Skin sections from 19 guinea pigs were studied. Microscopically, the epidermis was thin (6/19, Fig. 101, 102), or had excessively vacuolar nuclei (7/19, Fig. 99, 100). The dermal connective tissue was hyalinized (7/19), or had an increased number of fibroblasts in the papillary layer (7/19). Two animals (1.2%) had necrotic foci with increased numbers of lymphocytes in the dermis.

Skeletal muscles from 14 guinea pigs were examined. Two animals had pale skeletal muscles (0.9, 1.2%). Microscopically, the sarcolemmal nuclei were hyperplastic in 8 guinea pigs (Fig. 106). A few muscle fibers in 1 animal were necrotic and were replaced by eosinophilic debris (Fig. 105), and 1 section had a focus of necrosis and calcification.

Thyroid glands from 11 guinea pigs were examined, and the glands from 2 animals were congested. The colloid contained more vacuoles than normal (0.7, 0.9%, Fig. 117),

and many follicles were devoid of colloid (0.7 to 1%, Fig. 116). The thyroid gland of 1 animal had interstitial lymphocytic infiltration (0.9%).

Adrenal glands from 16 guinea pigs were studied. Microscopically, there was extensive interstitial lymphocytic infiltration in the medulla and among the innermost cells of the zona reticularis in 4 animals (Fig. 110, 111). The zona glomerulosa of 4 guinea pigs was partially atrophied (Fig. 112, 113). Cells of the zona reticularis were individualized and pyknotic in 8 animals. The zona reticularis was congested in 2 (0.9, 1%) and hemorrhagic in 1 (1.2%).

Sections from eyes, testes, prostate gland, esophagus, large intestine, anal glands, trachea, cerebellum and midbrain were normal.

Table 19 - Average daily gain in body weight and consumption of food, water and $NaNO_2$ by guinea pigs in Experiment IV.

$NaNO_2$%	Daily gain* Gm./100 Gm. body weight	Daily feed consumption Gm./100 Gm. body weight	Daily water consumption ml./100 Gm. body weight	Daily $NaNO_2$ intake mg./100 Gm. body weight
0.5	0.75	8.3	22.1	110.5
0.7	0.35	5.7	16.1	112.7
0.9	(-0.20)	8.8	21.9	197.1
1.0	(-0.14)	5.2	16.9	169.0
1.2	(-3.4)	2.4	9.1	109.0
0.0	1.53	8.9	32.5	0.0

* Daily gain was calculated for the 1st 50 days of treatment.

Table 20 - Average levels of serum electrolytes, total protein and blood urea nitrogen (BUN) in guinea pigs of Experiment IV.

$NaNO_2$%	Na m Eq./L. serum	K	Serum protein Gm./100 ml.	BUN mg./100 ml. serum
0.5	140	7.0	4.5	28
0.7	--	--	--	32
0.9	143	7.0	4.6	29
1.0	149	6.2	4.6	29
1.2	151	6.4	4.4	--
0.0	126 to 140	4.7 to 5.4	3.7 to 4.5	23 to 30

Table 21 - Reproductive performance for female guinea pigs in Experiment IV.

$NaNO_2$%	Number of females	Number of litters	Number of litters per guinea pig
0.5	3	1*	0.33
0.7	2	1**	0.50
0.9	3	0	0.00
1.0	1	0	0.00
1.2	2	0	0.00
0.0	4	8	2.00

* Dam was killed during pregnancy and 3 fetuses were found.

** Dam died and 1 fetus was found.

Table 22 - Partial hemogram for guinea pigs in Experiment IV.

$NaNO_2$%	Hemoglobin Gm./100 ml. blood	Methemoglobin Gm./100 ml. blood	Total leukocyte count thousands/ cmm.	Monocytes per cmm. blood
0.5	10.30	0.45	5.5	220
0.7	12.00	0.85	5.4	254
0.9	12.25	1.37	4.1	164
1.0	11.70	0.99	4.1	281
0.0	14.24	0.02	5.4	143

Table 23 - Average relative weights (x) of a selected number (n) of organs of animals in Experiment IV.

$NaNO_2$%		Liver	Spleen	Kidneys	Heart	Intestine*	Cecum**	Testes
0.5	x	3.3	0.17	0.93	0.35	12.0	4.8	0.48
	n	4	4	4	2	4	4	1
	st.d.	0.9	0.05	0.05	0.04	2.38	1.31	0.0
0.7	x	4.2	0.28	1.22	0.49	10.6	3.53	--
	n	3	3	3	3	3	3	--
	st.d.	1.3	0.11	0.35	0.1	1.83	0.6	--
0.9	x	4.54	0.34	1.3	0.6	12.62	5.0	0.94
	n	4	4	3	2	4	4	1
	st.d.	1.56	0.28	0.45	0.18	1.76	1.34	0.0
1.0	x	4.23	0.24	1.22	0.53	12.74	5.13	0.79
	n	4	4	3	2	4	4	2
	st.d.	0.8	0.04	0.31	-.1	1.09	0.9	0.13
1.2	x	5.32	0.22	1.28	0.71	12.43	4.58	0.79
	n	3	3	3	1	3	2	1
	st.d.	1.25	0.08	0.04	0.0	1.17	1.11	0.0
0.0	x	4.1	0.17	0.74	0.42	14.07	6.15	0.65
	n	11	10	8	4	11	11	3
	st.d.	0.19	0.07	0.17	0.24	1.77	0.88	0.13

* Intestine was weighed with food contents.

** Cecum alone was weighed with food contents.

st.d. Standard deviation.

Weights were in grams.

Experiment V (3% $Mg(NO_3)_2$ and 1% NH_4NO_3)

Growth. The rate of gain was low for guinea pigs given $Mg(NO_3)_2$ in the drinking water. Animals given NH_4NO_3 gained normally (Table 24).

Food and water consumption. Food consumption was normal and water consumption was slightly decreased ($Mg(NO_3)_2$). The calculated $Mg(NO_3)_2$ consumption reached 600 mg./100 Gm. body weight, and NH_4NO_3 consumption was 296 mg./100 Gm. body weight (Table 24).

Signs. Guinea pigs given $Mg(NO_3)_2$ or NH_4NO_3 did not have abnormal signs.

Reproductive performance. Reproduction was not adversely affected (Table 25).

Analyses

Hematologic determinations. The levels of hemoglobin and methemoglobin and the total and differential leukocyte counts were normal except for the monocyte count. Guinea pigs given $Mg(NO_3)_2$ or NH_4NO_3 had increased monocyte counts (Table 26). Some guinea pigs given NH_4NO_3 had erythrocytes with slight polychromasia, hypochromasia and anisocytosis. Animals given $Mg(NO_3)_2$ had apparently normal erythrocytes.

Serum electrolytes, total protein, nitrite, bilirubin and blood urea nitrogen values were normal (Table 26).

Serum electrophoresis. The albumin/globulin ratio was 66/34 in guinea pigs given $Mg(NO_3)_2$, and 67/33 in animals given NH_4NO_3 in comparison to 58/42 in control animals.

Lesions

Part I $Mg(NO_3)_2$

Hearts from 5 guinea pigs were examined. The relative weight was decreased more in the $Mg(NO_3)_2$ group than in the NH_4NO_3 group (Table 27). Three hearts had edematous myocardiums and many shrunken sarcolemmal nuclei (Fig. 9).

Uteri from 2 guinea pigs were examined. The only histopathologic findings were atrophied and hyperchromatic uterine glands (Fig. 31, 32) and a more cellular endometrium.

Lungs from 5 guinea pigs were examined. Septal cells were hyperplastic in 4 animals (Fig. 79). Other incidental observations included atelectasis and compensatory emphysema (Fig. 83), vacuolar and necrotic mediae of pulmonary arterioles, and numerous interstitial rounded foci of lymphocytes similar to lymph follicles with no germinal centers. There was peribronchiolar and perivascular lymphocytic infiltration.

Livers from 5 guinea pigs were examined. The relative weights were decreased (Table 27). Microscopically, there was cloudy swelling and hyalinized or vacuolar hepatic arterioles (Fig. 87, 88). A few Kupffer cells had brown pigments.

Spleen sections from 5 guinea pigs were examined. Three spleens had numerous hematopoietic centers and

numerous iron pigments. The spleens of the newborn guinea pigs had no Prussian blue-positive material.

Cervical lymph nodes from 3 guinea pigs were examined. The only histopathologic finding was hyperplastic cortical lymphocytes in 2 guinea pigs (Fig. 56, 57).

Thyroid glands from 4 guinea pigs were examined. Microscopically, there were fairly numerous round vacuoles in the colloid, mostly adjacent to the follicular epithelium (Fig. 117). Thyroid glands from 2 animals had interstitial edema and congestion.

Sections from tongue, trachea, salivary glands, esophagus, thymus, stomach, small and large intestines, anal glands, seminal vesicles, prostate gland, epididymides, testes, kidneys, urinary bladder, placenta, midbrain, cerebellum, eyes and adrenal glands were normal.

Part II (NH_4NO_3)

Hearts from 7 guinea pigs were examined. The myocardium was edematous in 3 guinea pigs. There was vacuolar necrosis particularly in the papillary muscles of the right ventricle in 2 guinea pigs (Fig. 4) and congestion and foci of Anitschkow's myocytes were noted. The media of the arterioles in the interventricular septa had Anitschkow's myocytes (Fig. 5, 6).

Lungs from 7 guinea pigs were examined. An interesting gross observation was a greyish-white fleshy mass

replacing the left lung and in adhesion with the surrounding surfaces. Its cut surface had a sinusoidal appearance and was covered with blood and pus. Microscopically, this lung had small encapsulated abscesses and foci of fetalized or adenomatous alveolar lining. Lungs from another 3 animals had congestion, atelectasis and compensatory emphysema, thickened vacuolated mediae of pulmonary arterioles and hyperplastic septal cells.

Livers from 7 guinea pigs were examined. The relative weights were slightly less than the controls (Table 27). Microscopically, there were many vacuoles in the periportal hepatic cells. These periportal cells were PAS-negative in 3 guinea pigs and had Prussian blue-positive pigments in 1 female.

Kidneys from 6 guinea pigs were examined. The relative weights were slightly increased (Table 27). In 1 male, there were calcium-like deposits in the wall of some arcuate and interlobular arterioles, and in some glomerular tufts and Bowman's capsules. The same animal had a hyperplastic epithelial lining of the renal pelvis (Fig. 95) and vacuolar mediae of the renal arterioles.

Spleens from 7 guinea pigs were studied. The relative weights were normal (Table 27). Microscopically, there were congestion in 2 spleens, numerous hematopoietic centers in spleens of 2 females, hyperplastic lymphocytes in the white pulp of 2 (Fig. 49, 50), and increased reticular

cells in the spleen of 1 newborn in which there were no Prussian blue-positive granules.

Skin sections from 7 guinea pigs were examined. Three animals had thin epidermis (Fig. 101, 102) and 2 had excessively vacuolar nuclei in the epidermis (Fig. 99, 100). One newborn had numerous histiocytes in the dermis.

Sections from tongue, salivary glands, cervical lymph nodes, thymus, thyroid gland, trachea, stomach, small and large intestines, pancreas, anal glands, eyes, midbrain, cerebellum, ovaries, uterus, testes, epididymides, seminal vesicles, prostate gland, urinary bladder, adrenal glands and skeletal muscles were normal.

Table 24 - Average daily gain in body weight and consumption of food, water and nitrate by guinea pigs in Experiment V.

Chemical and %	Daily gain* Gm./100 Gm. body weight	Daily feed consumption Gm./100 Gm. body weight	Daily water consumption ml./100 Gm. body weight	Daily nitrate consumption mg./100 Gm. body weight
$Mg(NO_3)_2$ 3	0.45	9.6	20.0	600.0
NH_4NO_3 1	1.66	10.6	29.0	295.0
0	1.53	8.9	32.0	0.0

* Daily gain was calculated for the 1st 50 days of treatment.

Table 25 - Reproductive performance for female guinea pigs in Experiment V.

Chemical and %	Number of females	Number of litters	Total alive	Total dead	Average size of live litter	Average number of litters per female
$Mg(NO_3)_2$ 3	2	3	6	0	2.0	1.5
NH_4NO_3 1	1	3	13	0	4.3	3.0
0	4	8	31	1	3.9	2.0

Table 26 - Average values of serum electrolytes, total protein, blood urea nitrogen (BUN) and monocyte count for guinea pigs in Experiment V.

Chemical and %	Monocytes per cmm. blood	Na m Eq./L. serum	K m Eq./L. serum	Serum protein Gm./100 ml.	BUN mg./100 ml.
$Mg(NO_3)_2$ 3	518	136	5.6	4.0	29.7
NH_4NO_3 1	265	137	6.0	4.1	31.6
0	143	126 to 140	4.7 to 5.4	3.7 to 4.5	23 to 30

Table 27 - Average relative weights (x) of a selected number (n) of organs in guinea pigs of Experiment V.

Chemical and %		Liver	Spleen	Kidneys	Heart	Intestine*	Cecum**	Testes
$Mg(NO_3)_2$								
3	x	3.0	0.12	0.77	0.27	13.6	5.75	0.83
	n	5	5	5	4	5	5	2
	st.d.	0.5	0.07	0.39	0.11	2.67	1.17	0.16
NH_4NO_3								
1	x	3.53	0.15	0.81	0.37	14.66	6.0	0.76
	n	7	7	7	3	7	7	3
	st.d.	0.8	0.07	0.23	0.12	4.0	1.6	0.18
0	x	4.08	0.17	0.74	0.42	14.7	6.15	0.65
	n	11	10	8	4	11	11	3
	st.d.	0.9	0.07	0.17	0.14	1.77	0.08	0.13

* Intestine was weighed with food contents.

** Cecum alone was weighed with food contents.

st.d. Standard deviation.

Weights were in grams.

Experiment VI ($NH_2OH.HCl$)

(Hydroxylamine hydrochloride will be referred to as hydroxylamine.)

Growth. The daily gain was normal in guinea pigs given 0.02 to 0.05% hydroxylamine. At the 0.1% level, the weight gain was much less than normal and animals given 0.15% or more hydroxylamine lost weight (Table 28).

Feed and water consumption. Feed consumption was not adversely affected at levels from 0.02 to 0.2%, but was greatly reduced in animals given 0.25% or more hydroxylamine (Table 28). Water consumption decreased as the level of hydroxylamine increased (Table 28). The calculated maximum hydroxylamine consumed per day reached 53 mg./100 Gm. body weight. This amount was consumed by animals given 0.2% hydroxylamine.

Signs. Guinea pigs given 0.02 to 0.1% hydroxylamine had no abnormal signs, except for 1 female (0.1%) which aborted near full term and continued losing weight until she was killed 45 days later. Five guinea pigs (0.15%) started losing weight from the 6th day of the experiment. The weight loss was continuous in 1 animal which was killed on the 32nd day of the experiment. Two of these 5 guinea pigs died, 1 on the 17th day of treatment and the other 26 days later, after depression and emaciation. The other 2 died on the

60th day. All of these animals had ruffled hair, palpable ribs and vertebrae and an enlarged abdomen which was apparently caused by an enlarged cecum (Fig. 47). The loss in weight was delayed until the 44th day of the experiment in 1 guinea pig which was killed 46 days later. Two animals lived until they were killed on the 96th and 101st days of the experiment. Animals which were given 0.2% hydroxylamine started losing weight within the first 30 days of the experiment. Three were killed early, and the rest except 1 died within 14 days from the start of the weight loss. Three of 5 animals given 0.25% hydroxylamine died within the first 11 days of the experiment. The 2 surviving guinea pigs were given untreated water starting from the 10th day of the experiment. Thirteen days later, they were returned to the original treatment after regaining weight. One animal became inactive and was killed after 14 days of the 2nd treatment The other animal lost weight and died after 20 days of the 2nd experimental period. Guinea pigs given 0.3% hydroxylamine started losing weight from the 2nd day of treatment. Weanlings were dull and huddled together. They had ruffled hair and closed eyes. They died between the 12th and the 19th days except those which were returned to untreated water. Nine mature guinea pigs out of 14 were moribund and killed or died between the 5th and the 12th days of the experiment in spite of replacing the hydroxylamine-treated

water with untreated water on the 8th day. The other 5 survived and regained weight until they were killed.

Reproductive performance. One guinea pig receiving 0.02% hydroxylamine gave birth to 5 healthy guinea pigs and another female aborted 6 dead guinea pigs averaging 48 Gm. Guinea pigs given 0.03% hydroxylamine had normal-sized live litters. Their newborn averaged 95 Gm. body weight. Two females given 0.05% hydroxylamine in drinking water either died early in the experiment or within 5 days of parturition. Another female had a small-sized litter. Only 1 female was given 0.1% hydroxylamine in the drinking water. This female aborted near full term. Guinea pigs given 0.15% or more hydroxylamine did not reproduce (Fig. 119, Table 29) and most of them died early in the experiment.

Analyses

Hematologic determinations. Guinea pigs given 0.02% or more hydroxylamine had a decreased hemoglobin level. This level returned towards normal within 20 days after treated water (0.3%) was replaced by untreated water. Most animals had normal methemoglobin levels, and the differential leukocyte counts were normal except for an increased number of monocytes. Erythrocyte counts were made on blood from guinea pigs given 0.3% hydroxylamine, and the numbers were greatly decreased. The number of erythrocytes gradually returned towards normal after the chemical was withdrawn.

Erythrocytes of guinea pigs given 0.3% hydroxylamine had slight polychromasia, anisocytosis (Fig. 73) or hypochromasia (Fig. 72). The sera were yellowish-green and the clots contracted. Erythrocytes from guinea pigs given 0.1% hydroxylamine were more resistant to hemolysis in M/60 phosphate buffer at pH 6.6 than were controls. Some of the guinea pigs given 0.05% hydroxylamine had nucleated erythrocytes (Fig. 71), Howell-Jolly bodies, basophilic stippling (Fig. 70) and macrocytosis.

Serum electrolytes and total protein. The sodium levels were slightly increased for guinea pigs given 0.05% or more hydroxylamine in the drinking water. The increase was in positive correlation with the drug concentration. Sodium levels returned to normal a few days after hydroxylamine was withdrawn. The potassium levels were slightly increased in guinea pigs given 0.02 to 0.15% hydroxylamine. Potassium levels were higher for guinea pigs given 0.3% hydroxylamine followed by 20 days of untreated water. The serum protein was in the normal range (Table 30).

Serum nitrite and bilirubin. Animals given 0.1% or more hydroxylamine had no elevation in levels of serum nitrite and slightly increased serum bilirubin.

Blood urea nitrogen was increased in guinea pigs given 0.3% hydroxylamine (Table 30).

Serum electrophoresis. The albumin/globulin (A/G) ratios are shown (Table 31). The greatest increase recorded was at the 0.25% level of hydroxylamine.

Lesions

Hearts from 52 guinea pigs were examined. Grossly, there were neither consistent lesions nor demonstrable effect of hydroxylamine upon relative weight (Table 32). Microscopically, 14 hearts had foci of vacuolar necrosis in the ventricular wall (Fig. 1, 2). Sarcolemmal nuclei were increased in size and number in several hearts. The myocardiums of 2 animals had foci of Anitschkow's myocytes (Fig. 7).

Testes from 35 guinea pigs were studied. Most of the seminiferous tubules (0.25%) were lined by spermatogonia and primary spermatocytes. The Leydig cells were atrophied and had hyperchromatic nuclei (0.3%, Fig. 11).

Seminal vesicles from 25 guinea pigs were examined. Grossly, there was atrophy of seminal vesicles from 10 guinea pigs, particularly those given 0.3% hydroxylamine (Fig. 20, 21). Microscopically, there was atrophied and degenerated epithelial lining in 11 guinea pigs (Fig. 19). Two animals had papillary hyperplasia of the epithelial lining (0.15, 0.3%).

Epididymides from 31 animals were examined. Two epididymides had no spermatozoa (0.15, 0.3%). Most males given 0.3% hydroxylamine had disintegrated spermatozoa in the

epididymal ducts. Some ducts in 2 guinea pigs (0.15, 0.3%) had a few round cells of 15 to 20 μ in diameter (Fig. 17). One epididymis had a hyperplastic epithelial lining, which almost occluded the ductile lumen, in addition to the presence of eosinophilic droplets (0.15%).

Ovaries from 9 guinea pigs were examined. Atresia of almost all the Graafian follicles was noted in 2 females (0.02, 0.3%, Fig. 37).

Uteri from 9 guinea pigs were examined. Grossly, 1 uterus was hemorrhagic and edematous with involvement of the urinary bladder and abdominal wall. Microscopically, there were proliferated uterine glands in 3 females (0.02 to 0.05%), and a vacuolated and hyperplastic papillary endometrium of 1 uterus (0.02%, Fig. 28). One female (0.03%) had dilated uterine glands and vesicular nuclei of the endometrium (0.03%). There was vacuolar degeneration in the wall of the spiral arterioles, and the lumen of 1 horn was filled with eosinophilic homogeneous material, some calcium-like deposits, neutrophils, brown pigments and an embryo (0.05%).

Lungs from 49 guinea pigs were examined. Grossly, 3 animals had hydrothorax (0.02 to 0.1%), and 6 had emphysema (0.2 to 0.3%). Histopathologic lesions were hyperplastic septal cells and thickened mediae of the pulmonary arterioles in 20 animals, mostly those given 0.2% or more hydroxylamine (Fig. 75). Eight of these animals had vacuoles in the medial

portion of the pulmonary arterioles (Fig. 76). Eighteen guinea pigs had congested lungs (0.15 to 0.3%) and, in 7, there were atelectasis and compensatory emphysema (Fig. 83).

Stomachs from 46 guinea pigs were examined and many of the animals given higher levels of hydroxylamine had empty stomachs. In some, the contents were more fluid than normal. Microscopically, there were no significant lesions but numerous protozoa were observed on the gastric mucosa of 4 animals.

Small intestines from 42 guinea pigs were examined. The relative intestinal weights were normal (0.02%), slightly decreased (0.03 to 0.2%) or greatly decreased (0.25% or more). Guinea pigs which were given 0.3% hydroxylamine for 7 days followed by untreated water for 20 days still had lesser intestinal weights. There were no outstanding lesions microscopically.

Large intestines from 38 guinea pigs were studied. The relative cecal weights were variable (0.02 to 0.15%), or lower than normal (0.2% or more). Guinea pigs which were given 0.3% hydroxylamine for 7 days followed by 5 to 49 days of untreated water still had lower cecal weights (Table 32). Microscopically, there were no significant lesions.

Livers from 60 guinea pigs were examined. The relative weights were normal (0.02 to 0.03%), or increased (0.05% or more, Table 32). Guinea pigs which were given

0.3% hydroxylamine for 7 days followed by 20 days of untreated water had normal liver weights (Table 32). Four livers were black (0.1 to 0.2%) and 10 were brown (0.15 to 0.3%). One liver had yellowish foci at its borders and many had regularly distributed yellow foci 1 to 2 mm. in diameter (Fig. 84). Microscopically, there was centrolobular fatty change (0.2 to 0.3%, Fig. 85). Prussian blue-positive pigments were noted in Kupffer cells, macrophages, hepatic cells or free in sinusoids in the majority of examined sections. These pigments were most numerous in the region of the portal triads. Hematopoietic centers were noted in animals given 0.1% or more hydroxylamine (Fig. 92, 93) and 12 livers were congested.

Kidneys from 59 guinea pigs were studied. The relative weights were increased (0.02% or more, Table 32). Thirteen animals had dark brown kidneys. Microscopically, lesions included numerous dilated renal tubules in 10 animals (Fig. 94). There was hyperplasia or vacuolization of the epithelial lining of the pelvis in 7 (Fig. 95). Kidneys of 7 of the animals given 0.03% or more hydroxylamine had numerous fine Prussian blue-positive pigments in the cytoplasm of the renal tubules, particularly in the proximal convoluted tubules. These iron granules appeared as brown pigments in sections stained with hematoxylin and eosin. An interesting observation was the presence of

Prussian blue-positive granules in the wall of the arcuate and renal arteries in a single animal (0.03%).

Urinary bladders from 42 guinea pigs were examined. Seven were distended with yellow or brown urine (0.15 to 0.3%). The necks of 2 urinary bladders were occluded with greyish-white fleshy material (0.3%, Fig. 98). In 6 there were white (0.05%) or brown (0.3%) precipitates which appeared as pale eosinophilic granular material microscopically along with neutrophils and exfoliated cells. One interesting observation was focal hyperplasia of smooth muscles in the wall of 1 urinary bladder (0.1%).

Spleens from 59 animals were studied. Marked enlargment was a consistent finding. In many instances, the spleen was 2 to 10 times the normal size (Fig. 44, 45). The size was normal at necropsy in guinea pigs given 0.3% hydroxylamine for 7 days followed by 20 days of untreated water. Many spleens were black. Microscopically, the lesions included congestion, megakaryocytes (Fig. 48) and extensive hematopoiesis at all levels of hydroxylamine. Most spleens had numerous macrophages filled with brown pigments. In Prussian blue-stained sections, the positive granules were most numerous at the 0.02 to 0.1% levels of hydroxylamine, and less numerous at the 0.3% level. There were no pigments in spleens from newborn animals.

Cervical lymph nodes from 30 guinea pigs were examined. Hyperplastic cortical lymphocytes were seen in 12 animals, and numerous eosinophils were noted near the corticomedullary junction in lymph nodes from 3 animals (0.3%)

Thymuses from 38 guinea pigs were examined. Lobular (Fig. 55) and cortical (Fig. 53, 54) atrophy was noted in thymuses from 14 animals. The change was difficult to differentiate from atrophy caused by aging.

Bone marrow sections from 15 guinea pigs were studied. There was fibrillar material in areas where bone marrow was lacking (0.3%, Fig. 67, 68), and hyperplasia of the erythroid elements in 6 guinea pigs (0.15 to 0.3%, Fig. 64). The nuclei of megakaryocytes were often pyknotic or vesicular. Their cytoplasm was light, reticular or had many vacuoles (0.3%, Fig. 66).

Cerebellums from 18 guinea pigs were examined. Microscopically, there was congestion in 11.

Skin sections from 55 guinea pigs were studied. Microscopically, there was a thin epidermis in 35 guinea pigs (Fig. 101, 102), and atrophied nuclei of the epidermis in 10 (mostly 0.2% or more). Seventeen animals (mostly 0.2% or more) had hyalinized dermal collagen, and 7 had increased numbers of fibroblasts in the papillary layer of the dermis.

Skeletal muscles from 30 guinea pigs were examined. Lesions were confined to 3 animals given 0.3% hydroxylamine. These lesions included focal disappearance of the sarcoplasm (Fig. 107), necrosis and calcification.

Thyroid glands from 37 guinea pigs were examined. The colloid of many follicles had a mesh-like appearance or numerous round vacuoles, mostly adjacent to the follicular epithelium (Fig. 117). The female which was given 0.1% hydroxylamine and aborted had foci of interstitial lymphocytic infiltration and extensive interstitial fibrosis.

Adrenal glands from 46 guinea pigs were examined. Grossly, adrenal glands from 7 guinea pigs were hemorrhagic (0.15 to 0.3%). Microscopically, the zona glomerulosa was partially atrophied in 11 guinea pigs (Fig. 112, 113). There were congestion and individualized pyknotic cells of the zona reticularis, mostly in animals given 0.2% or more hydroxylamine. The grossly observed hemorrhage was confirmed microscopically and involved the cortex and medulla.

Eyes from 36 guinea pigs were examined. Isolated observations included focal vacuolation of the epithelial layer of the cornea in 1 animal, and fibrinous eosinophilic material on the outer surface of corneas from 2 guinea pigs. Lacrimal glands had cystic degeneration in 1 animal, and a few foci of lymphocytes infiltrating the interstitium in 2.

Examination of sections from pancreas, salivary glands, tongue, esophagus, trachea, anal glands, prostate glands and mammary glands revealed no abnormalities.

Table 28 - Average daily gain in body weight and consumption of food, water and hydroxylamine by guinea pigs in Experiment VI.

$NH_2OH.HCl$%	Daily gain* Gm./100 Gm. body weight	Daily food consumption Gm./100 Gm. body weight	Daily water consumption ml./100 Gm. body weight	Daily hydroxylamine consumption mg./100 Gm. body weight
0.02	1.67	9.9	52.0	10.4
0.03	1.72	10.3	47.1	14.1
0.05	1.60	13.0	44.0	22.1
0.10	1.10	10.3	31.0	31.1
0.15	(-0.07)	7.7	29.8	44.7
0.20	(-1.45)	10.6	26.5	53.0
0.25	(-4.25)	1.5	10.0	25.0
0.30	(-3.40)	0.9	9.8	29.0
0.00	1.53	8.9	32.5	00.0

* Daily gain was calculated for the 1st 50 days of treatment.

Table 29 - Reproductive performance for female guinea pigs in Experiment VI.

$NH_2OH.HCl$ %	Number of females	Number of litters	Total alive	Total dead	Average size of live litter	Number of litters per guinea pig
0.02	2	2	5	6	2.5	1.0
0.03	2	2	7	0	3.5	1.0
0.05	3	2	4	1	2.0	0.66
0.10	1	1	0	4	0.0	1.0
0.15	3	0	0	0	0.0	0.0
0.00	4	8	31	1	3.9	2.0

Table 30 - Average levels of serum electrolytes, total protein and blood urea nitrogen (BUN) in guinea pigs of Experiment VI.

$NH_2OH.HCl$%	Na m Eq./L. serum	K	Total serum protein Gm./100 ml.	BUN mg./100 ml. serum
0.02	135.3	6.3	4.2	24.8
0.03	136.0	6.0	4.7	24.0
0.05	140.0	6.0	4.0	24.7
0.10	142.0	6.0	4.0	25.7
0.15	143.0	6.8	4.6	24.0
0.20	--	--	--	26.2
0.30	144.0	5.3	4.6	43.0
0.30*	131.0	10.0	4.1	--
0.00	126 to 140	4.6 to 5.4	3.7 to 4.5	23 to 30

* Animals were given 0.3% hydroxylamine for 5 to 7 days followed by 20 days of untreated water.

Table 31 - Average values of a partial hemogram and albumin/globulin ratio (A/G) of guinea pigs in Experiment VI.

$NH_2OH.HCl$%	Hemoglobin Gm./100 ml.	Monocytes per cmm. blood	Erythrocytes millions/cmm.	A/G
0.02	11.96	298	--	61/39
0.03	10.80	502	--	59/41
0.05	9.00	221	--	58/42
0.10	8.45	242	--	55/45
0.15	7.93	232	--	57/43
0.20	7.70	143	--	60/40
0.25	--	--	--	72/28
0.30	9.00	1403	3.6	--
0.30*	11.30	499	4.4	--
0.00	14.24	143	5.8	58/42

* Animals were given 0.3% hydroxylamine for 5 to 7 days followed by 1 week of fresh water before blood was examined.

Table 32 - Average relative weights (x) of a selected number (n) of organs of guinea pigs in Experiment VI.

$NH_2OH.HCl$%		Liver	Spleen	Kidneys	Heart	Intestine*	Cecum**	Testes
0.02	x	3.97	0.22	0.9	0.37	15.23	6.31	0.65
	n	4	4	4	4	4	4	1
	st.d.	0.35	0.19	0.28	0.05	3.25	1.67	0.0
0.03	x	4.11	0.3	0.85	0.36	12.54	4.9	0.55
	n	4	4	4	4	4	4	1
	st.d.	0.7	0.13	0.55	0.06	1.14	0.8	0.0
0.05	x	4.69	0.8	1.05	0.42	13.0	5.24	0.7
	n	7	6	6	3	7	7	2
	st.d.	1.51	0.38	0.29	0.13	3.63	1.41	0.0
0.10	x	4.36	0.81	0.92	0.44	11.0	5.0	0.69
	n	6	5	4	3	6	5	4
	st.d.	1.3	0.24	0.08	0.12	3.0	0.33	0.09
0.15	x	5.4	1.2	1.37	0.50	13.72	6.32	0.56
	n	8	7	5	2	8	8	2
	st.d.	2.36	0.8	0.6	0.14	5.7	2.0	0.13
0.20	x	6.2	1.0	1.76	--	13.04	5.43	0.67
	n	5	4	4	--	6	6	1
	st.d.	1.3	0.35	0.6	--	1.39	2.36	0.0
0.25	x	6.57	--	--	--	9.8	4.72	--
	n	3	--	--	--	3	3	--
	st.d.	0.39	--	--	--	1.32	0.28	--
0.30	x	5.11	0.48	1.24	0.46	7.9	3.9	0.79
	n	11	10	10	5	10	10	9
	st.d.	1.25	0.24	0.4	0.3	2.19	1.4	0.10
0.30*	x	4.05	0.18	0.85	0.43	11.89	5.06	0.60
	n	6	5	4	4	6	6	4
	st.d.	0.8	0.05	0.34	0.17	3.17	2.0	0.25
0.00	x	4.08	0.17	0.74	0.42	14.7	6.15	0.65
	n	11	10	8	4	11	11	3
	st.d.	0.9	0.07	0.17	0.24	1.77	0.08	0.13

* Intestine was weighed with food contents.
** Cecum alone was weighed with food contents.
st.d. Standard deviation. Weights were in grams.

DISCUSSION

Toxicosis was produced in guinea pigs given nitrate, nitrite or hydroxylamine in their drinking water, but relatively high levels were necessary. This may indicate a resistance in the guinea pig related to its ability to survive hypoxemia for a considerably longer time than other species (Worden and Lane-Petter, 1957). Studies in which nitrate or nitrite have been administered to other species for long periods of time have yielded essentially negative results, but the levels of administration were considerably lower than those used in the present studies (Seerley et al., 1965; Nelson, 1965).

At lower levels of treatment, water and food consumption were normal and growth and reproduction were maintained for long periods. At higher levels, the general signs included a loss of or a lowered rate of gain in body weight. Decreased water consumption was apparently more responsible in this respect than decreased food consumption or even an increased chemical intake. The maximum amount of chemical was not always consumed by animals given the highest concentration. In some instances, the rate of gain was lowered in spite of normal consumption of food and water. This could indicate a disturbance in digestion, absorption or

in metabolic processes at the cellular level. Loss in weight occurred after a variable number of days for different chemicals. This period was 190 days (3% KNO_3), 160 days (0.5% $NaNO_2$), 90 days (4% KNO_3), 5 days (3% $NaNO_3$) or 2 days (1.2% $NaNO_2$ or 0.2 to 0.3% hydroxylamine). The mortality rate was high at these levels. After parturition, weight loss was associated with a subacute suppurative endometritis, retarded involution, erosion of the endometrium or necrosis of the uterine wall.

Reproductive failure or disturbance was dose-related and was particularly evident at higher levels of nitrite and hydroxylamine. Some females aborted and others absorbed their fetuses or had mummified fetuses in their uteri. Simon et al. (1958) and Winter and Hokanson (1964) also reported abortion in cattle given a nitrate-containing ration. Abortion, in the present work, was attributed to hypoxemia due to placental ischemia and methemoglobinemia. Similar ideas were advanced by Simon et al. (1959) and by Winter and Hokanson (1964). The development of the placental lesions may have been related to narrowing of arterioles. Placental lesions were acute or subacute and were characterized by necrotic foci with pyknosis, karyorrhexis and calcification. Similar placental lesions were reported in cattle by Simon et al. (1958). They found intercotyledonary circumscribed necrotic and calcified foci in the fetal membranes. Many

of the blood vessels were either congested, thrombosed or thickened. Winter and Hokanson (1964) however, reported no significant lesions in fetal membranes from $NaNO_3$ poisoned cattle.

Absorption or mummification of the dead fetuses were the alternatives of abortion for survival. The termination of pregnancy may have been due to a local action of the chemicals or caused by an imbalance in the ovarian steroids. Females which failed to abort or whose fetuses did not mummify died during middle or late pregnancy. Death was apparently caused by toxemia induced by the dead fetuses. Some females died within a few days after a normal parturition as a result of metritis and secondary respiratory infection.

In some instances the average body weight was much less in both prematurely and maturely born offspring. They were weak and many died. Some females failed to conceive or absorbed their embryos early in gestation. These findings agreed with several investigators who worked with other species. Muhrer et al. (1956) reported that 75% of rats given 2% KNO_3 in the ration failed to produce litters and the author suggested that the fetuses were absorbed or aborted. Davidson et al. (1964) noted that cattle given 660 mg. nitrate/kg. body weight daily had a low conception rate and some of the pregnant cattle aborted.

Death of the fetuses of the guinea pig has been induced by vitamin E deficiency (Reid, 1958). He reported fetal death in the middle of the gestation period caused by necrosis and premature separation of the placenta. He also observed extensive degeneration of the voluntary muscles in vitamin E-deficient guinea pigs. The same author noted stillbirth or death of young guinea pigs soon after birth as a result of hemorrhage caused by vitamin K deficiency. There were a few signs and lesions which could possibly have been related to vitamins A and K deficiencies in the present experimental animals, but further studies are necessary.

The failure of some guinea pigs, which were given particular levels of chemicals, to become pregnant may have been caused by either ovarian, uterine or cervical lesions. The ovarian lesions included absence of mature Graafian follicles or the presence of cystic or atretic Graafian follicles. The uterine or cervical lesions were papillary hyperplasia of the endometrium (particularly with $NaNO_3$ and $NaNO_2$), vacuolar degeneration and narrowing of the spiral arterioles, subacute suppurative cervicitis or subacute suppurative endometritis.

The lesions in the male genital tract were generally mild and reversible. The interference with spermatogenesis, the atrophied Leydig cells, the decreased size

and atrophied epithelial lining of the seminal vesicles and the absence of spermatozoa or the presence of disintegrated spermatozoa and spermatocytes in the epididymal ducts could have been due to either a local effect of the chemicals or indirectly caused by an imbalance in gonadotrophic hormones, mainly FSH and LH. It is known that FSH maintains the spermatogenic epithelium and spermatogenesis, and LH is trophic to the Leydig cells and controls testosterone output (Ganong, 1963). Perhaps it would be useful to study the pituitary gland and hypothalamus from nitrate-poisoned animals using histochemical techniques and electron microscopy in addition to routine histopathologic procedures. The study of the levels of the anterior pituitary hormones in these animals might be informative.

The cardiovascular lesions were mainly vacuolar necrosis of the myocardium and hyalinization or vacuolization of the wall of many arterioles. Heuper and Landsberg (1940) have also described degenerative changes in the vascular tissue of the hearts, lungs and livers of rats chronically poisoned with $NaNO_3$. Winter and Hokanson (1964) reported vacuolization of the medial layer of the coronary arterioles in cattle poisoned with $NaNO_3$, and Simon *et al.* (1958) found focal vacuolar degeneration of the myocardium in aborted fetuses from nitrate-poisoned cattle. Other changes included Anitschkow's myocytes in the wall of coronary arterioles and myocardium and both hyperplasia and

hypertrophy of the sarcolemmal nuclei. These changes were probably related to hypoxemia. Newberne (1964) found that rats given a potassium-deficient diet had similar vacuolar degeneration of the myocardium.

Respiratory lesions were primarily in the lungs. These alterations were of the reactive type which apparently reduced the rate of gaseous exchange between the blood and alveolar atmosphere. These lesions included variable numbers of hyperplastic septal cells which masked the alveolar arrangement in many lungs and were likely a predisposing factor for the development of atelectasis and compensatory emphysema. Lesions were more prominent in animals treated with potassium salts than sodium salts and were the least in hydroxylamine-treated animals. The congestion and edema in the interalveolar septa and thickened and vacuolated pulmonary arterioles were considered to have increased the resistance in the pulmonary circulation and thereby probably caused hypoxemia and damage to the heart. Similar thickening of the pulmonary arterioles and septa were reported in nitrate-poisoned cattle (Simon et al., 1958). Stevenson and Wilson (1963) reported that generally the interstitial pulmonary edema was caused by endothelial damage, increased capillary permeability or by increased blood pressure.

Hepatic lesions were a reflection of the degree of hypoxemia, cardiac insufficiency and hemolytic anemia. The

lesions were characterized for the most part by fatty change and hyalinization and vacuolization of the hepatic arterioles. The hypoxemia was apparently responsible for the numerous hematopoietic centers and agreed with similar findings in $NaNO_3$-poisoned cattle (Winter and Hokanson, 1964). The brown livers may have been caused by numerous pigments containing iron in Kupffer and hepatic cells particularly at the periphery of the lobules. This was apparently related to the hemolytic anemia. It was reported that loading of the hepatic cells with iron did not injure them under normal conditions except for depressing the metabolic rate, but that hepatic damage occurred under stress (Volini et al., 1965).

Gastric changes were only macroscopic, but they seemed important because of a possible contribution to anemia. Many stomachs had fluid contents mixed with gas bubbles or were empty. This change might be caused by an upset in the secretory mechanism of the gastric mucosa and irregular emptying of the stomach into the intestine rather than by an increased water or a decreased food consumption.

The lesions in the urinary system were mild and did not interfere with renal function. The material which occluded the neck of the urinary bladder and appeared as granular eosinophilic debris, microscopically, and the white precipitate inside the urinary bladder need analysis.

The source and distribution of the stainable iron in the liver and kidneys of guinea pigs, particuarly in those given hydroxylamine, require more study. Hydroxylamine by itself or through another chain of reactions may have disturbed the iron metabolism with the appearance of Prussian blue-positive pigments in the cytoplasm of renal and hepatic cells. If the hypothetical site of action of hydroxylamine can be located in such reactions, it may provide a basis for explaining such seemingly unrelated conditions as idiopathic hemochromatosis in man, copper and cobalt deficiency diseases in animals and many other diseases characterized by Prussian blue-positive pigments in the cytoplasm of the renal tubules.

Quantitative analysis of the urine for iron would have been helpful in determining whether animals which had Prussian blue-positive granules in the renal tubules actually excreted the iron. Histologically, it appeared that certain guinea pigs given $NaNO_2$ had some iron in the lumen of the renal tubules. A decision regarding its significance was not possible without quantitative urine analysis of treated and control animals.

Lesions of the hematopoietic system were mainly a reflection of hypoxemia and hemolytic anemia. The enlargment of spleens which were especially outstanding in hydroxylamine-treated guinea pigs was apparently caused by congestion, numerous hematopoietic centers and many macrophages filled

with Prussian blue-positive granules. Similarly, Rieman (1950) reported enlarged spleens in rats given hydroxylamine, but he did not report histopathologic findings. Erythropoiesis was thought to be stimulated by hypoxemia as stated by Winter and Hokanson (1964). The lymphocytes in the white pulp of the spleen, lymph follicles in the cervical lymph nodes and erythroblasts in the bone marrow were hyperplastic in many animals given lower levels of chemical and were hypoplastic in many animals given higher levels. Fibrillar or granular eosinophilic material replaced the hypoplastic hematopoietic tissue. Aplasia of the bone marrow in the guinea pig has been induced by folic acid deficiency (Reid, 1958). Future studies could include the assay of folic acid in nitrate-poisoned animals.

A decreased hemoglobin level and increased methemoglobin values resulting from certain chemicals reflected hypoxemia. The erythrocytic abnormalities such as hypochromasia, macrocytosis, polychromasia, nucleated erythrocytes, poikilocytosis, anisocytosis, Howell-Jolly bodies and basophilic stippling were suggestive of impaired bone marrow and defective oxygen transport. The increased serum bilirubin, the fatty livers and the low hemoglobin levels indicated both hemolytic and toxic jaundice in the hydroxylamine-treated guinea pigs.

The skin lesions were mostly in the epidermis and could be related to metabolic or endocrine disturbances, particularly of the adrenal and thyroid glands (Hall, 1959). The epidermis was thin or had excessive vacuolization of nuclei

The thyroid gland lesions were generally mild and included absence or vacuolization of the colloid in some lobules. These lesions could have been caused by alteration in the pituitary gland and hypothalamus.

It is apparent that the nitrates or nitrites of different metals have varying degrees of toxicity. The relatively low toxicity of nitrate salts of magnesium or potassium could be due to the fact that magnesium salts are slowly absorbed and potassium is rapidly excreted except in the case of pre-existing renal damage (Gleason et al., 1963). The higher toxicity of $NaNO_3$ than KNO_3 could be due to the apparent involvement of the central nervous system in the former, as the guinea pigs given 1% $NaNO_3$ had excitability.

Some signs of toxicity were reversed when untreated water was given after the first 7 to 10 days of the experiment (0.3% hydroxylamine). Similar reversible effects were reported in rabbits (Rieman, 1950). Repeated administration of hydroxylamine with periods of untreated water progressively increased the survival period of guinea pigs given 0.3% hydroxylamine.

The present work indicates that hydroxylamine is more toxic than either nitrate or nitrite, but Rieman (1950) reported that the lethal dose of hydroxylamine for rats was about twice that of $NaNO_2$. The disagreement could be due to interspecies variability in susceptibility to toxic agents (Newsom et al., 1937; Thorp, 1938; Olson and Moxon, 1942; Riggs, 1945; Case, 1957; Garner, 1961). Rieman (1950) cited Lewin (1889) who stated that the toxic effect of hydroxylamine was through its conversion to nitrite, but Conn and Stumph (1963) and Jamieson (1958) indicated that hydroxylamine is an intermediate in the reduction of nitrate to nitrite to hyponitrite to hydroxylamine to ammonia. The latter concept is in agreement with the present work which suggests that the toxicity of hydroxylamine is not due to its conversion to nitrite since the signs and lesions of hydroxylamine-treated animals differed from the nitrite-treated groups.

SUMMARY AND CONCLUSIONS

An experimental study was conducted to evaluate nitrate, nitrite and hydroxylamine toxicoses. Two hundred thirty-three guinea pigs were used. Animals of each experiment were given either untreated water or KNO_3 (0.01 to 4%), KNO_2 (0.01 to 1.2%), $NaNO_3$ (0.5 to 3%), $NaNO_2$ (0.5 to 1.2%), NH_4NO_3 (1%), $Mg(NO_3)_2$ (3%), or $NH_2OH.HCl$ (0.02 to 0.3%) in the water for periods ranging from 5 to 310 days. Toxicities were evaluated by recording the growth rate, food and water consumption, clinical signs, reproductive performance, and gross and histopathologic changes. In addition, many clinico-pathologic values were determined. The most important findings are summarized as follows:

1) High levels were needed (except for hydroxylamine) to cause death or such effects as retarded growth, decreased hemoglobin or increased methemoglobin.
2) Reproductive disturbances included a high mortality rate among the newborn (0.01 to 1% KNO_3 or 0.5% or more $NaNO_3$), and among pregnant females (0.5% KNO_2 or 1.5% $NaNO_3$). Some females aborted or absorbed their fetuses. This termination of pregnancy was associated with ovarian and placental lesions, and with cervicitis and endometritis. No live litters were produced by females given 4%

KNO_3, 0.5% KNO_2, 1.5% $NaNO_3$, 0.5% $NaNO_2$ or 0.1% $NH_2OH.HCl$

Atrophy of the Leydig cells and seminal vesicles and disintegration of the spermatozoa inside the epididymal ducts were noted in males given 2 to 3% KNO_3, 0.35 to 1.1% KNO_2, $NaNO_2$ or $NH_2OH.HCl$.

3) Vacuolar necrosis of the cardiac muscles, hyperplasia and hypertrophy of the sarcolemmal nuclei and foci of Anitschkow's myocytes occurred in varying numbers of animals of each chemical level except $Mg(NO_3)_2$.

4) There was extensive hyperplasia of the pulmonary interalveolar septal cells in animals given KNO_3 or KNO_2. This change masked the alveolar arrangement and apparently predisposed to atelectasis and compensatory emphysema.

5) The splenic size was 2 to 3 times normal at higher levels of $NaNO_3$ and $NaNO_2$ and 10 times normal in some animals given $NH_2OH.HCl$. Hematopoietic centers and megakaryocytes in varying numbers were noted in most spleens except in those animals given KNO_3. Generally, the low levels of treatment caused hyperplasia, and higher levels caused hypoplasia in most of the hematopoietic tissues. Most of the experimental animals, except those given $Mg(NO_3)_2$ had some or all of the following erythrocytic abnormalities: basophilic stippling, polychromasia, hypochromasia, poikilocytosis, anisocytosis, nucleated erythrocytes and macrocytosis. Erythrocytes from guinea pigs given 0.1%

or more $NH_2OH.HCl$ were more resistant to hemolysis in M/60 buffer at pH 6.6 than were controls. The monocyte count was increased with some levels of the chemicals and decreased with others.

6) The epidermis was considerably thinner in many of the animals which were given various chemical levels except those given $Mg(NO_3)_2$. Vacuolar nuclei of the epidermis and hyalinized dermal collagen were also observed.

7) In the adrenals, hemorrhage, partial atrophy of the zona glomerulosa and individualization and pyknosis of the cells of the zona reticularis were found in many animals of all treatments except $Mg(NO_3)_2$ or NH_4NO_3.

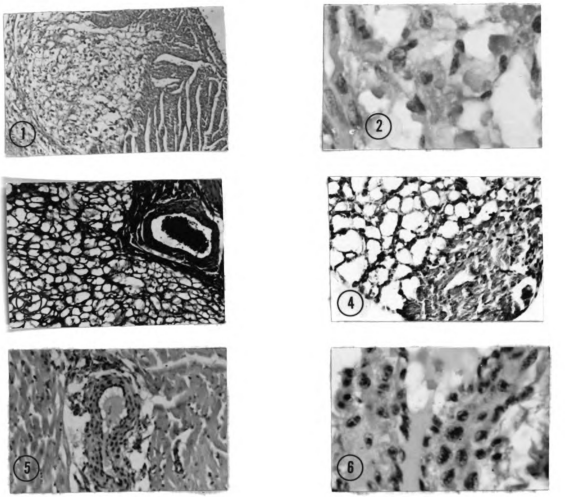


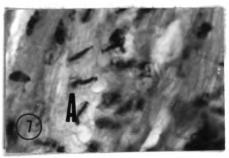
Fig. 1 to 6. Heart. H & E stain.

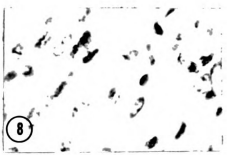
1 & 2. Vacuolar degeneration of the myocardium in the right ventricular wall. x 50 & 500 (0.05% $NH_2OH.HCl$).

3. Vacuolar degeneration of the interventricular septum. x 50 (1% NH_4NO_3).

4. Vacuolar degeneration of the papillary muscles. x 125 (1% NH_4NO_3).

5 & 6. Anitschkow's myocytes in the wall of arterioles in the interventricular septum. x 125 & 500 (0.25% KNO_3).





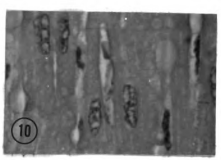
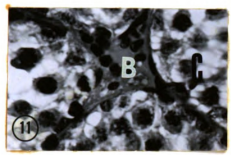
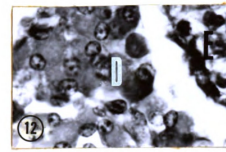
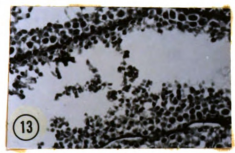


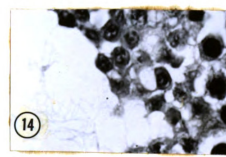
Fig. 7 to 10. Heart. H & E stain.

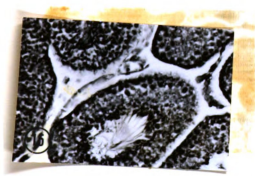
7. Anitschkow's myocytes (A) in the myocardium. x 500 (1.2% $NaNO_2$)
8. Hyperplastic sarcolemmal nuclei in the myocardium x 500 (1.05% $NH_2OH.HCl$).
9. Atrophic sarcolemmal nuclei in the myocardium. x 500 (3% $Mg(NO_3)_2$).
10. Control. x 500 (HOH).











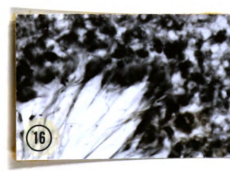


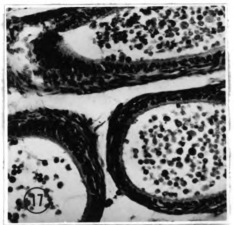
Fig. 11 to 16. Testis. H & E stain.

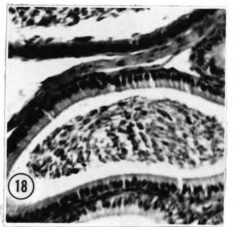
11. Atrophied Leydig cells with hyperchromatic nuclei (B). Seminiferous tubules (C). x 500 (0.3% $NH_2OH.HCl$).

12. Control Leydig cells (D). Seminiferous tubule (E). x 500 (HOH).

13 & 14. Aspermatogenesis. x 125 & 500 (0.3% $NH_2OH.HCl$).

15 & 16. Control. x 125 & 500 (HOH).





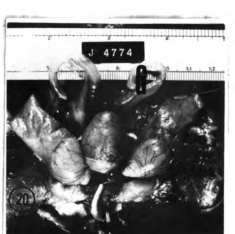
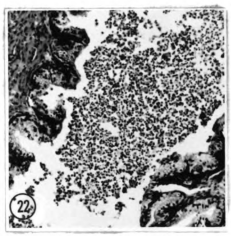
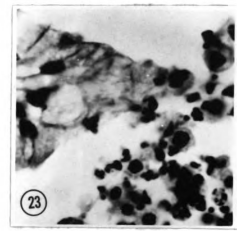
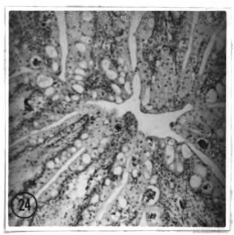


Fig. 17 & 18. Epididymis. H & E stain.
17. Lack of spermatozoa with eosinophilic debris x 185 (0.3% $NH_2OH.HCl$).
18. Control. x 185 (HOH).

Fig. 19 to 21. Seminal vesicles.
19. Thin epithelial lining (F). H & E stain; x 187 (0.3% $NH_2OH.HCl$).
20. Atrophied seminal vesicles (G). (0.25% KNO_3).
21. Control seminal vesicles (H). (HOH).







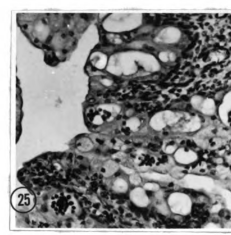
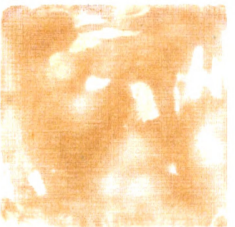
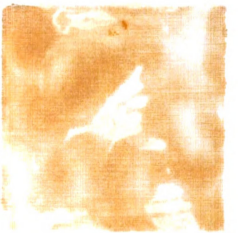
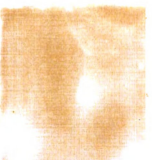
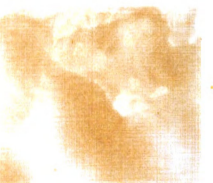


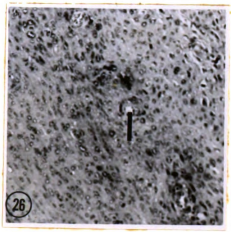
Fig. 22 to 25. Cervix. H & E stain.
22 & 23. Neutrophilic exudate in the lumen. x 62 & 625 (0.45% KNO_2).
24 & 25. Cervicitis with vacuolar degeneration of the epithelial lining. x 62 & 158 (1% KNO_2).

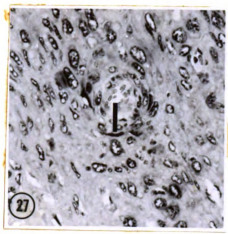


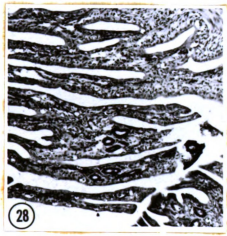


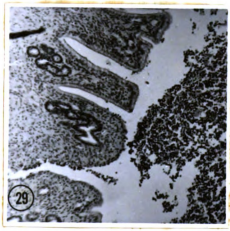












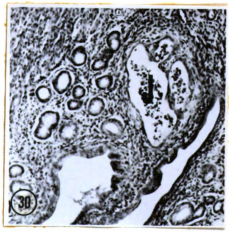
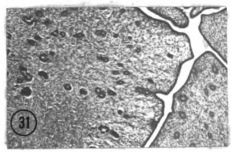


Fig. 26 to 30. Uterus. H & E stain.
26 & 27. Narrowed arterioles (I) in uterine wall indicating ischemia. x 75 & 187 (0.5% KNO_2).
28. Papillary hyperplasia of the endometrium. x 75 (0.5% $NaNO_2$).
29. Neutrophils in the uterine lumen. x 187 (0.4% KNO_2).
30. Dilated uterine gland (J) with neutrophils. x 75 (0.03% KNO_2).



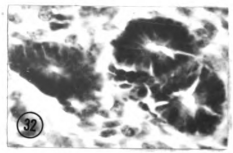
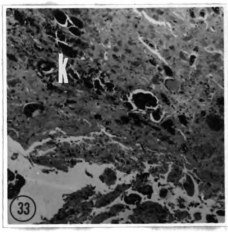
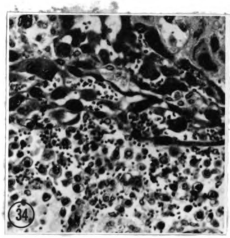
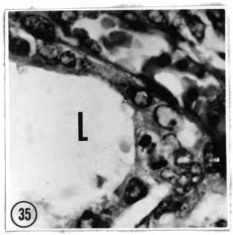


Fig. 31 & 32. Uterus. H & E stain.
Hyperchromatic cells of the uterine glands.
x 50 & 500 (3% $Mg(NO_3)_2$).







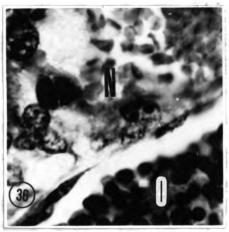


Fig. 33 to 36. Placenta. H & E stain.
33. Necrotic placental-uterine junction (K). x 75 (0.5% KNO_2).
34. Control placental-uterine junction. x 187 (HOH).
35. Ischemic placenta. Maternal side (L). Fetal side (M). x 500 (0.5% KNO_2).
36. Control placenta. Maternal side (N). Fetal side (O). x 1000 (HOH).

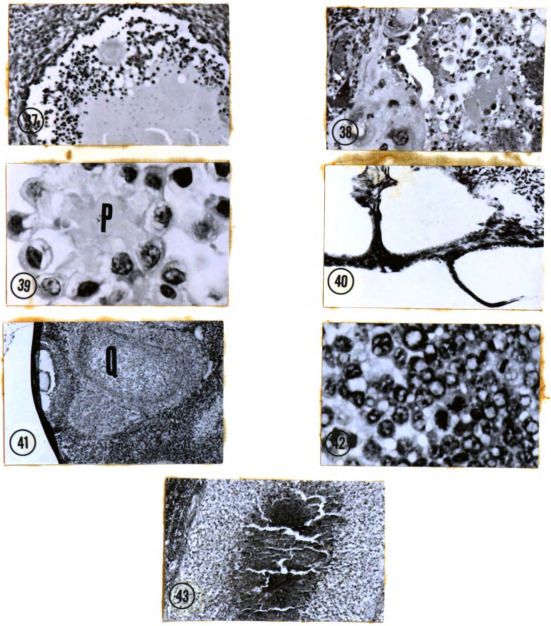


Fig. 37 to 43. Ovary. H & E stain.
37. Atretic Graafian follicle. x 125 (0.03% KNO_2).
38. Hemorrhagic Graafian follicle. x 125 (1% KNO_2).
39. Degeneration of the ovum (P). x 500 (1% KNO_2).
40. Cystic Graafian follicles. x 125 (3% KNO_2).
41 & 42. Proliferated cells of zona granulosa (Q) before ovulation. x 150 (0.5% KNO_2).
43. Hemorrhagic corpus luteum. x 62 (3% KNO_3).

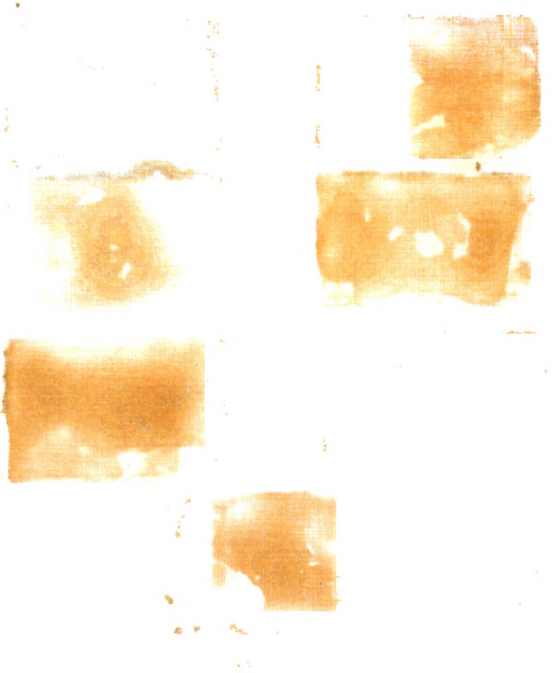
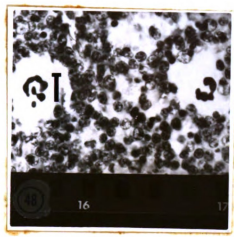
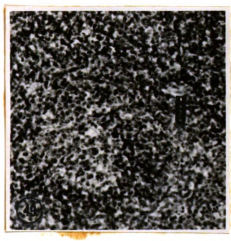
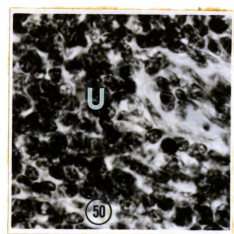


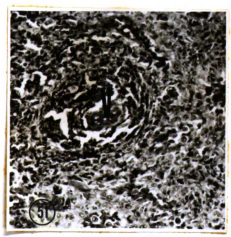
Fig. 44 to 46. Spleen.
44. Moderately enlarged spleens (0.5% $NH_2OH.HCl$).
45. Greatly enlarged spleen (R). Stomach (S) distended with gas bubbles and fluid (0.1% $NH_2OH.HCl$).
46. Control spleens (HOH).

Fig. 47. Enlarged cecum (0.15% $NH_2OH.HCl$).









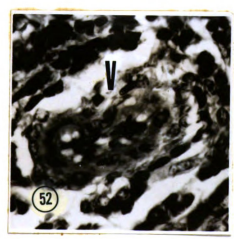
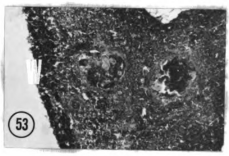
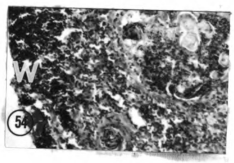


Fig. 48 to 52. Spleen. H & E stain.
48. Megakaryocytes. x 187 (0.15% $NH_2OH.HCl$).
49 & 50. Hyperplasia of lymphocytes in the white pulp (U). x 158 & 625 (0.1% KNO_3).
51 & 52. Hypoplasia of lymphocytes in the white pulp (V). x 158 & 625 (1% KNO_2).





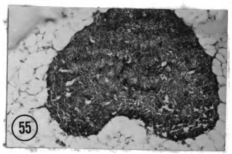


Fig. 53 to 55. Thymus. H & E stain
53 & 54. Cortical atrophy (W). x 50 & 125 (0.3% $NH_2OH.HCl$)
55. Lobular atrophy. x 50 (0.5% KNO_2).

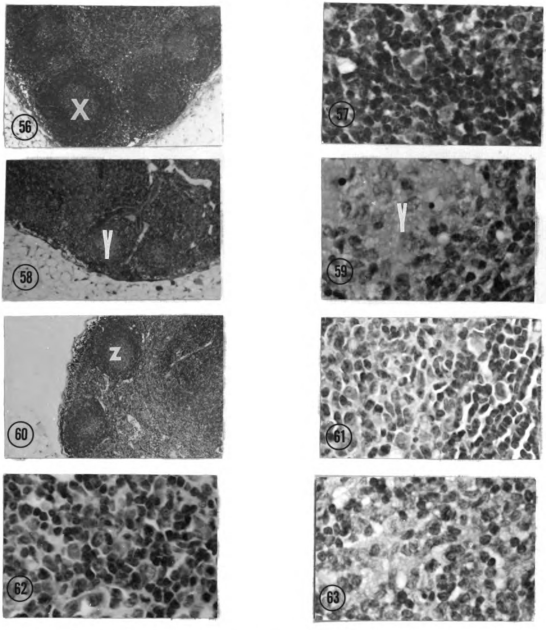
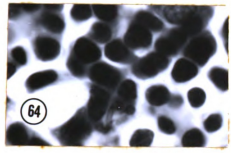
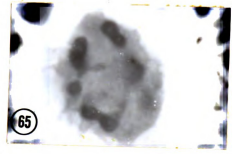
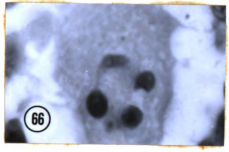
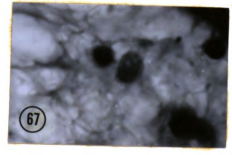


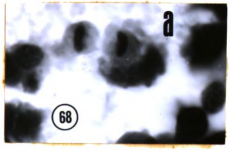
Fig. 56 to 63. Cervical lymph node. H & E stain.
56 & 57. Hyperplastic follicular lymphocytes (X). x 50 & 500 (0.2% $NH_2OH.HCL$).
58 & 59. Hypoplastic follicular lymphocytes (Y). x 50 & 500 (1.5% $NaNO_3$).
60 & 61. Control follicular lymphocytes (Z). x 50 & 500 (HOH)
62. Control cortical lymphocytes. x 500 (HOH).
63. Hypoplastic cortical lymphocytes. x 500 (1.5% $NaNO_3$).











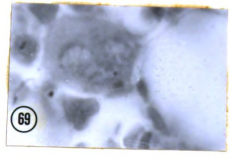


Fig. 64 to 69. **Bone marrow. H & E stain. x 1250.**

64. Hyperplastic bone marrow.
65. Shrunken nuclei of the megakaryocytes (0.3% $NH_2OH.HCl$).
66. Meshlike appearance of cytoplasm of megakaryocytes (0.3% $NH_2OH.HCl$).
67. Fibrillar material which has replaced the bone marrow (3% KNO_3).
68. Droplets (a) which have partially replaced the bone marrow (3% KNO_2).
69. Control bone marrow (HOH).

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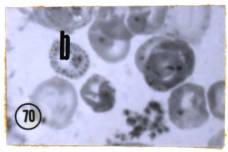

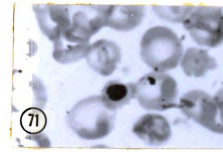

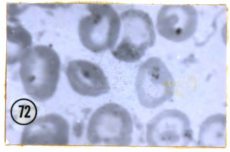

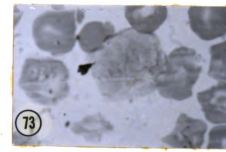

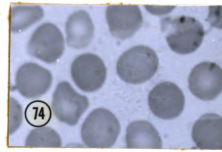

Fig. 70 to 74. Erythrocytes. Wright's stain. x 1250.
70. Basophilic stippling (b) (0.3% $NH_2OH.HCl$).
71. Nucleated erythrocytes (0.3% $NH_2OH.HCl$).
72. Hypochromasia (0.3% $NH_2OH.HCl$).
73. Anisocytosis (0.3% $NH_2OH.HCl$).
74. Control (HOH).

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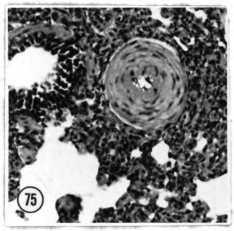
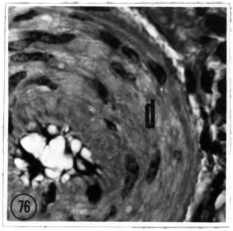
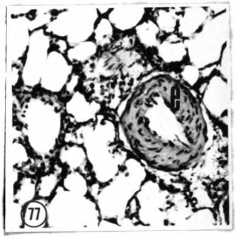






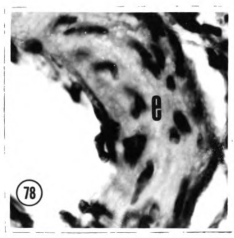
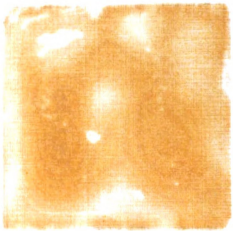
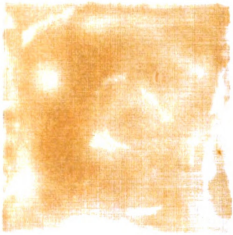
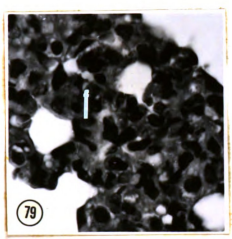
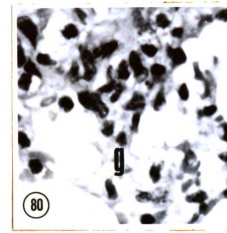


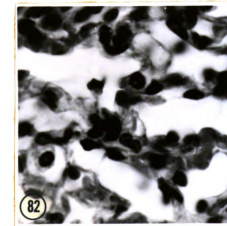
Fig. 75 to 78. Lung. H & E stain.
75. Hyperplastic cells of interalveolar septa (c), and thickened wall of pulmonary arteriole. x 187 (0.25% KNO_3).
76. Thickened wall of pulmonary arteriole (d). x 750 (0.25% KNO_2).
77 & 78. Control pulmonary arteriole (e). x 187 & 750 (HOH).









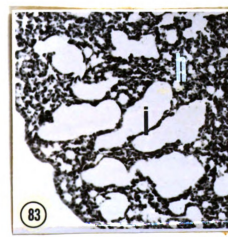
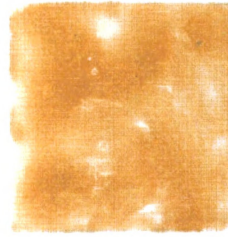
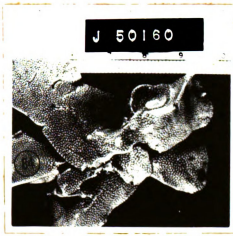
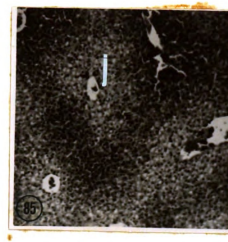
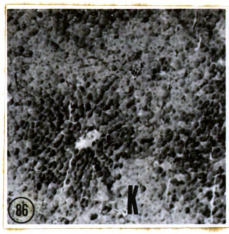


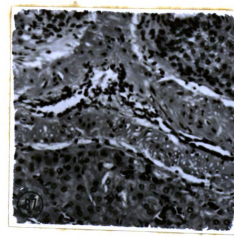
Fig. 79 to 83. Lung. H & E stain.
79. Hyperplastic septal cells (f). x 500 (0.25% KNO_3).
80. Edematous interalveolar septa (g). x 500 (0.5% KNO_2).
81. Control interalveolar septa. x 500 (HOH).
82. Atelectasis. x 500 (2% KNO_3)
83. Atelectasis (h), and compensatory emphysema (i). x 50 (2% KNO_3)

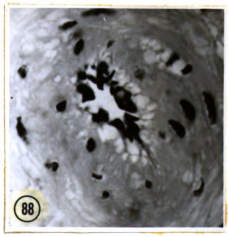












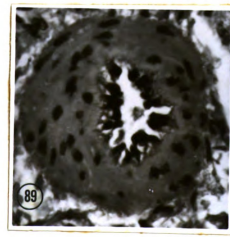


Fig. 84 to 89. Liver.

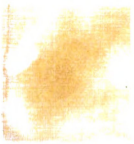
84. Centrolobular fatty change (1% KNO_2).

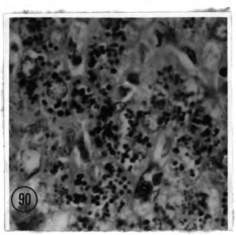
85. Centrolobular fatty change (j) H & E Stain; x 75 (1% KNO_2).

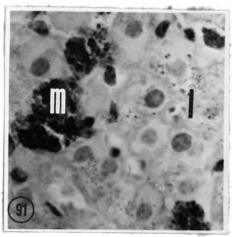
86. Peripherolobular glycogen depletion (k). Periodic acid-Schiff method; x 75 (0.1% $NH_2OH.HCl$).

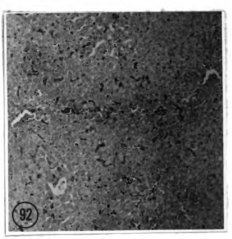
87 & 88. Hyalinization and vacuolar degeneration of a hepatic arteriole. H & E stain; x 750 (0.15%, 0.05% $NH_2OH.HCl$ respectively).

89. Control hepatic arteriole. H & E stain; x 750 (HOH)









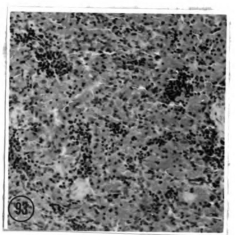
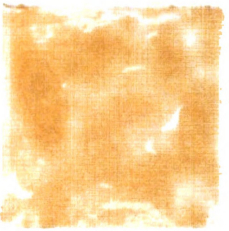


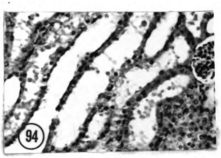
Fig. 90 to 93. Liver.

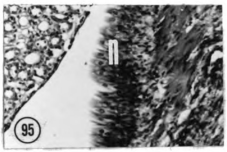
90. Iron in the hepatic and Kupffer cells. Prussian blue stain; x 750 (0.15% $NH_2OH.HCl$).

91. Pigments in the hepatic (i), and Kupffer (m) cells. H & E stain; x 750 (0.15% $NH_2OH.HCl$).

92 & 93. Numerous hematopoietic centers. H & E stain; x 75 & 750 (0.1% $NH_2OH.HCl$).







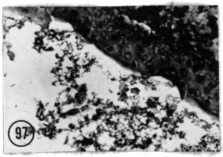
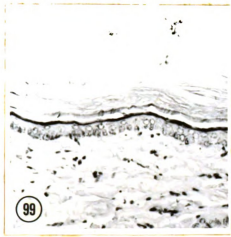
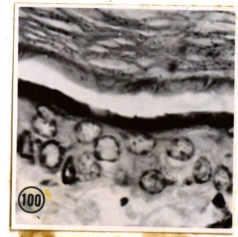
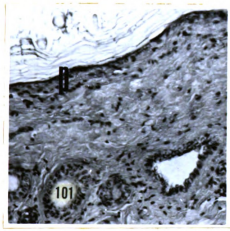
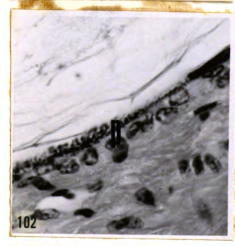


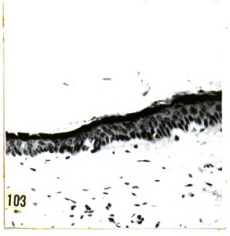
Fig. 94 & 95. Kidney. H & E stain.
94. Dilated renal tubules. x 187 (0.3% $NH_2OH.HCl$).
95. Hyperplastic epithelial lining of the pelvis (n). x 187 (0.5% $NH_2OH.HCl$).
Fig. 96 to 98. Urinary bladder. H & E stain.
96. Papillary hyperplasia of the mucosa. x 75 (0.15% $NH_2OH.HCl$).
97. Granules in lumen. x 187 (0.3% $NH_2OH.HCl$).
98. Fleshy mass (o) which occluded the neck of the bladder (p). Parts of the genital tract (q). (0.3% $NH_2OH.HCl$).











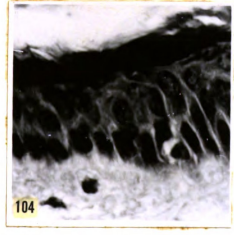
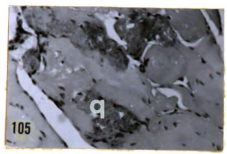
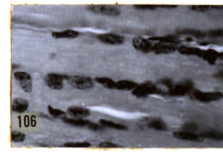
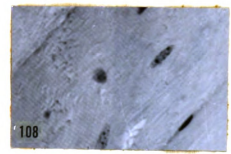


Fig. 99 to 104. Skin. H & E stain.
99 & 100. Vacuolar nuclei of the epidermis. x 187 & 625 (2.5% KNO_3).
101 & 102. Thin epidermis (p). x 187 & 750 (1.05% KNO_2).
103 & 104. Control. x 187 & 750 (HOH).







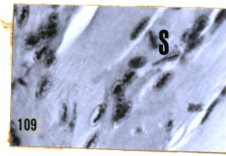
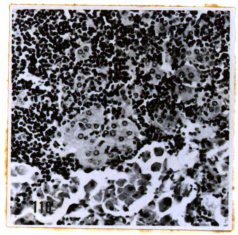
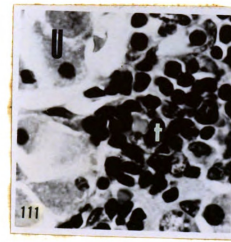
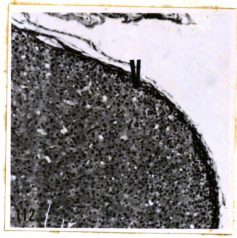


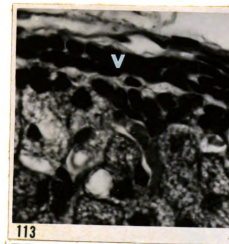
Fig. 105 to 108. Skeletal muscle. H & E stain.
105. Necrotic focus (q). x 125 (0.4% KNO_2).
106. Hyperplastic sarcolemmal nuclei. x 500 (0.3% $NH_2OH.HCl$).
107. Focal disappearance of sarcoplasm (r). x 500 (0.3% $NH_2OH.HCl$).
108. Control. x 500 (HOH).

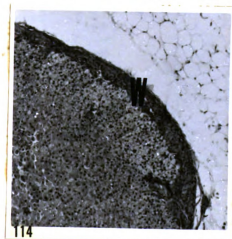
Fig. 109. Tongue. H & E stain.
Cells resembling Anitschkow's myocytes (s). x 625 (0.02% $NH_2OH.HCl$).











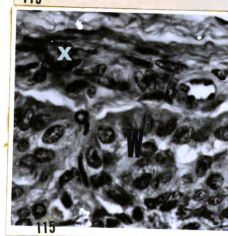
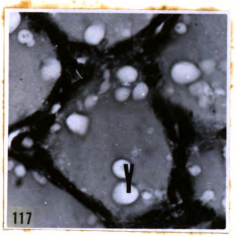


Fig. 110 to 115. Adrenal gland. H & E stain.
110 & 111. Infiltration of lymphocytes (t) in the medulla, with pyknotic nuclei (u). x 187 & 750 (0.01% KNO_3).
112 & 113. Atrophied zona glomerulosa (v). x 75 & 750 (0.1% $NH_2OH.HCl$)
114 & 115. Control zona glomerulosa (w). Capsule (x). x 50 & 500 (HOH).



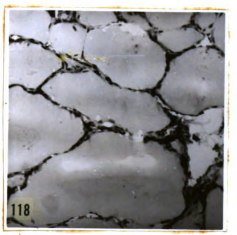
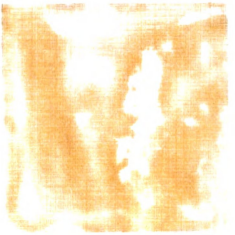


Fig. 116 to 118. Thyroid gland. H & E stain.
116. Lack of colloid (x). x 158 (0.15% $NH_2OH.HCl$).
117. Vacuoles (y) in the colloid. x 158 (0.15% $NH_2OH.HCl$)
118. Control. x 158 (HOH).



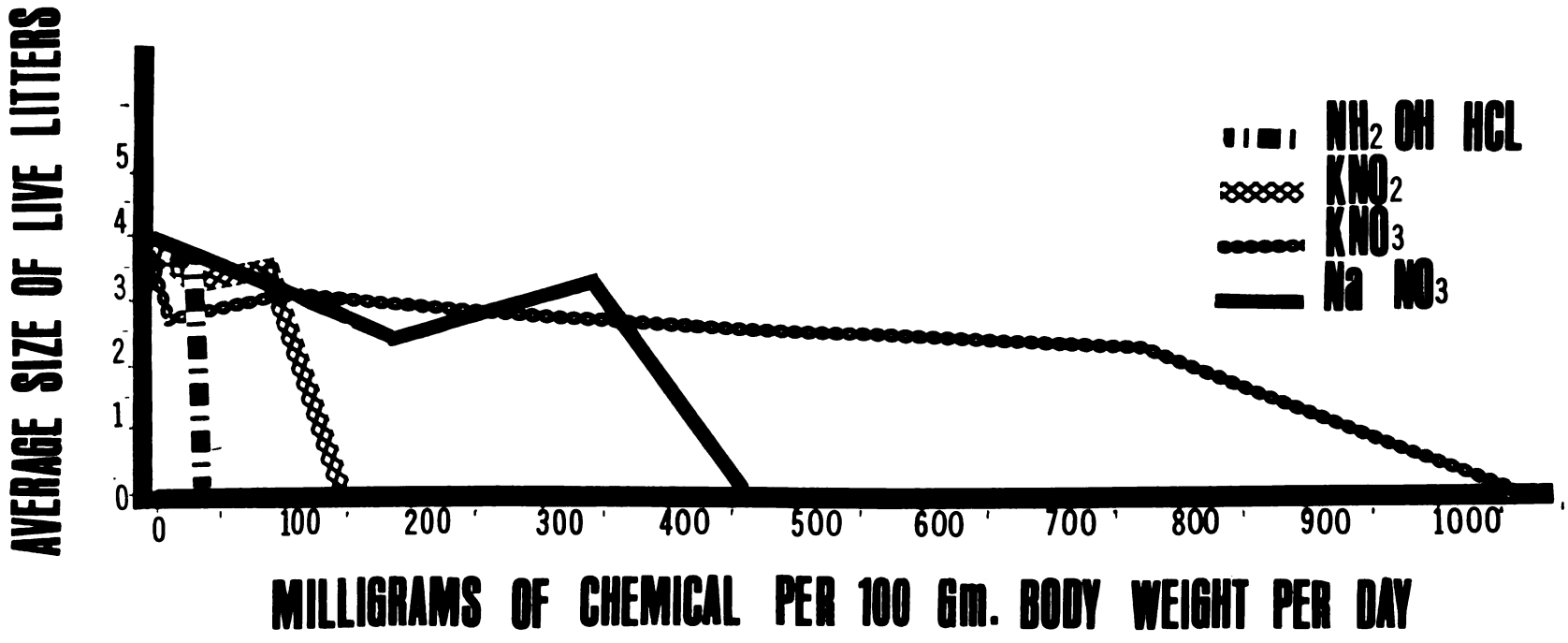


Fig 119. Average size of live litters correlated with administered chemicals.

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