# NITRITE TOXICOSIS IN THE ASCORBIC ACID DEFICIENT GUINEA PIG

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ABSTRACT

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ACID DEFICIENT GUINEA PIG

by Richard J. Kociba

Subcutaneous administration of 50 mg./kg. $NaNO_2$ consistently produced higher levels of methemoglobin in ascorbic acid deficient guinea pigs than in controls. This dosage was fatal to a high percentage of the deficient group, whereas all control guinea pigs survived. Pretreatment of deficient guinea pigs with 10 mg./kg. methylene blue protected against high levels of methemoglobin formation following the administration of 50 mg./kg. $NaNO_2$.

Abortions were caused in a high percentage of ascorbic acid deficient pregnant female guinea pigs following the subcutaneous administration of 45 mg./kg. $NaNO_2$. This same dosage caused no abortions in the control females. Ascorbic acid deficient females not given $NaNO_2$ had normal litters.

Maternal blood levels of methemoglobin were consistently higher in ascorbic acid deficient pregnant females following the subcutaneous administration of 40 mg./kg. $NaNO_2$. The accompanying levels of methemoglobin in fetuses derived by laparohysterotomy were similar in both the deficient and control groups. These data indicated that the fetal deaths were related to higher levels of methemoglobin in maternal blood of deficient females and not to increased placental passage of nitrite.

# NITRITE TOXICOSIS IN THE ASCORBIC ACID DEFICIENT GUINEA PIG

By

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TABLE OF CONTENTS

LIST OF FIGURES

## LIST OF TABLES

# INTRODUCTION

The use of nitrite compounds has continued to increase in all phases of food production and preservation. Interest in the toxic properties of nitrites has correspondingly increased with the accumulation of these compounds in the environment.

Nitrite compounds have been incriminated in cases of human infantile deaths as well as in cases of abortion in animals.

To properly evaluate the health hazards of nitrite compounds, various factors which may modify the susceptibility to nitrite toxicosis must be considered.

The population at risk consists of a wide latitude of individuals on various types of diets, some of which may not be providing the nutritional requirements of the body. Thus certain segments of both the human and animal populations may become more susceptible to the toxic effects of nitrite during periods of nutritional deficiencies.

This investigation was designed to furnish suitable data regarding the susceptibility to nitrite during a nutritional deficiency of ascorbic acid. Special emphasis has been placed on the pregnant animal, when nutritional deficiencies are more common.

The guinea pig was selected as the test animal because of its similarity to the human in that both are dependent on a dietary source of ascorbic acid.

Subcutaneous injections of sodium nitrite were used in all cases to eliminate experimental variables which would have been introduced by other methods.

Levels of methemoglobin formed as a result of oxidation of hemoglobin by nitrite were determined. The reproductive performance of ascorbic acid deficient guinea pigs following nitrite administration was also studied.

# REVIEW OF LITERATURE

## Nitrate and Nitrite Toxicosis

Acute nitrite and nitrate poisoning is apparently due to the formation of methemoglobin and the excessive dilatation of vascular channels caused by absorbed nitrites (Winter, 1962). Although nitrate has been considered relatively nontoxic, Lewis (1948) showed that nitrate in the rumen was reduced to nitrite followed by its absorption and the development of nitrite toxicosis. McIlwain and Schipper (1963) demonstrated that bacterial reduction of nitrate to nitrite can occur in the feed prior to ingestion. Nitrate levels as high as 5000 ppm were found in heavily fertilized vegetables by Wilson (1943). Hölscher and Natzschka (1964) reported cases of methemoglobin formation in infants fed spinach puree containing high levels of nitrite and lower levels of nitrate. It was assumed the nitrate content had been reduced to nitrite during storage.

Sodium nitrate and sodium nitrite are permitted in certain meat and fish products at levels of 500 and 200 ppm, respectively, as preservatives (Fassett, 1966). Orgeron et al. (1957) described several outbreaks of nitrite poisoning following the accidental incorporation of excessive amounts of nitrite-nitrate mixtures in meat products. One meat sample contained over 5000 ppm nitrite. Lehman (1958) considered the permitted levels in meat products acceptable for adults but stressed the lower margin of safety in children due to the lower hemoglobin content of their blood.

The hazards of excess nitrates in water supplies has been generally recognized since Comly (1945) described cyanosis in infants after ingestion of well waters containing nitrate. Metcalf (1962) has reported the increased sensitivity of fetal and infant hemoglobin to the action of methemoglobin-forming agents such as nitrites. The higher gastric pH and greater relative fluid intake in normal infants facilitate the bacterial reduction of nitrate to nitrite. Water containing 10 ppm nitrate-nitrogen has been considered hazardous for use in feeding infants, according to the United States Public Health Service Water Standards (1962).

Methemoglobin Formation and Reduction Mechanisms

Nitrite oxidizes the ferrous porphyrin complex of hemoglobin to the ferric form of methemoglobin, which is incapable of combining with molecular oxygen in the respiratory process. The mechanism of reaction between nitrite and hemoglobin may possibly be explained by the work of Cohen and Hochstein (1964) in which the generation of hydrogen peroxide was demonstrated during the reaction. The role of hydrogen peroxide as an essential intermediary product in the oxidation process has not been elucidated (Kiese, 1966). Winter (1962) reported that hydroxylamine was formed from nitrite in the blood and was itself capable of converting hemoglobin to methemoglobin.

Spicer (1950) noted differences in the reaction rate between nitrite and the hemoglobin of various species. Martin and Huisman (1963) isolated various types of human hemoglobins and noted substantial differences in their rates of reaction with nitrite. Ross (1963) reported that premature infants are more susceptible to methemoglobin formation than infants born at term.

Sinha (1968) found blood of fetal guinea pigs more susceptible to methemoglobin formation as compared to adults. After parturition this difference was reduced.

Methemoglobin has a characteristic light absorption at 635 mμ, which is abolished by the addition of cyanide. This is the basis for the measurement of methemoglobin as described by Evelyn and Malloy (1938).

Studies of the reduction of methemoglobin back to hemoglobin (Kiese, 1944; Gibson, 1948) have shown two electron-transporting systems which can donate electrons to methemoglobin: (1) The Embden-Meyerof glycolytic pathway forms reduced diphosphopyridine nucleotide (DPNH). Methemoglobin-reductase, a diaphorase which Scott and McGraw (1962) have purified and studied, transfers electrons from DPNH to methemoglobin. (2) Reduced triphosphopyridine nucleotide (TPNH) generated by the pentose phosphate pathway can reduce another diaphorase called methemoglobin-reductase TPN (Huennekens et al., 1957a, 1957b) which in turn passes the electron to an unknown cofactor or an artificial electron carrier such as methylene blue. The TPN dependent system reduces methemoglobin rapidly in contrast to the slower reduction by the DPN system (Stolk and Smith, 1966).

Stolk and Smith (1966) also demonstrated greater methemoglobin-reductase activity in red blood cells of the mouse and rabbit, whereas the cat, dog and human red blood cells possessed lesser amounts of reductase activity.

## Role of Ascorbic Acid as a Reducing Agent in the Blood

Smith (1954) listed the most obvious property of ascorbic acid as the ability to undergo oxidation to dehydroascorbic acid. Thus it may serve as a strong reducing agent in the biological systems of the body.

The salts of copper and iron have been shown to catalyze this oxidation (Mapson, 1945). Coudonis (1955) showed that ascorbic acid may function to reduce methemoglobin to hemoglobin. Baike and Valtis (1954) described a decreased plasma ascorbic acid level (despite adequate diet) to be associated with a decreased oxygen carrying capacity of hemoglobin. Bancroft (1948) recommended the therapeutic use of ascorbic acid to reduce the level of methemoglobin in the blood. Kleihauer (1965) demonstrated the ability of ascorbic acid to increase the reduction rate of methemoglobin to hemoglobin in vitro.

Role of Ascorbic Acid in Maintenance of Blood Vessels

Lee and Lee (1947) noted a weakening of the collagen bundles surrounding atonic dilated venules in the peripheral vascular system of scorbutic guinea pigs. They also noted a hyporeactivity of the blood vessels subsequent to treatment with epinephrine. Scorbutic guinea pigs failed to produce renal vasohumoral agents following severe hemorrhage (Lee and Holze, 1951).

Stolman, Goldman and Gould (1961) reported degenerative changes in the perivascular connective tissue and blood vessel walls following ascorbic acid deficiency in the guinea pig. Gore, Fujinami and Shirahama (1965) found that a depletion of subendothelial collagen in the aortas of scorbutic guinea pigs was accompanied by widening of the intercellular spaces. In a recent study, Gore, Wada and Goodman (1968) utilized electron microscopy to detect capillary defects which permitted the abnormal passage of erythrocytes and other blood constituents. The weakening of pericapillary supporting tissue and blood vessel walls may reflect the generalized deficit of collagen synthesis characteristic of ascorbic acid deficiency (Robertson, 1961).

## Role of Ascorbic Acid in Maintenance of Blood Constituents

Meyer and McCormick (1928) reported decreased erythrocytic and hemoglobin values in ascorbic acid deficient guinea pigs. Both leukocytic and leukopenic responses have been reported during ascorbic acid deficiency. Reid (1954) reviewed these results and suggested that concomitant infections may have played a role in these studies. Nungester and Ames (1948) demonstrated decreased phagocytic activity of leukocytes and lessened migration from the blood stream in scorbutic guinea pigs. Reid (1954) concluded that all clotting factors were normal in ascorbic acid deficiency.

## Role of Ascorbic Acid in Female Reproductive Activity

Ingier (1915) described impeded growth of fetuses from scorbutic guinea pigs. Goettsch (1930) reported the necessity of a minimal intake of ascorbic acid for normal estrual cycles in the guinea pig. Harman and Warren (1951) observed smaller embryos resulting from ascorbic acid deficient guinea pigs.

Day (1947) has shown the importance of ascorbic acid in the maternal diet during lactation in order to prevent marked loss of weight in the dam and retarded growth in the young.

The storage of ascorbic acid in the placenta has been demonstrated by Neuweiler (1935). Wahren and Rundqvist (1937) found fetal blood levels of ascorbic acid to be normally higher than maternal blood values. Goldsmith (1961) showed that the fetus may build up a substantial reserve of ascorbic acid at the expense of the maternal reserve.

## MATERIALS AND METHODS

General Plan. A series of 3 experiments was conducted to investigate the toxicity of $NaNO_2$ in ascorbic acid deficient guinea pigs. Experiment A was done to determine the levels of methemoglobin formation following the administration of $NaNO_2$ to ascorbic acid deficient guinea pigs. Experiment B was a study of the fetotoxic properties of $NaNO_2$ in pregnant guinea pigs deficient in ascorbic acid. Experiment C compared the maternal and fetal levels of methemoglobin formation following $NaNO_2$ administration to pregnant guinea pigs deficient in ascorbic acid.

Maintenance of Animals. The English Shorthair variety of guinea pig was utilized in this study. All were housed in groups of 3 to 6 guinea pigs per pen. Control guinea pigs were fed Rockland guinea pig diet* supplemented with fresh cabbage daily. Commercial test diets deficient in ascorbic acid** were used to create an ascorbic acid deficient condition in the test groups. Cabbage was not fed to the deficient animals. In all cases food and water were provided ad libitum.

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* Taklad, Inc., Monmouth, Ill.

** Nutritional Biochemical Corp., Cleveland, Ohio

** Hallwood Diet Foods, Muskegon, Michigan

Collection of Blood Samples. Ether anesthesia was used to facilitate the collection of blood by cardiac puncture from the mature guinea pigs. A 1.5 inch, 20 gauge needle was inserted caudolateral to the xiphoid cartilage toward the location of the heart. Fetal blood samples were obtained from the umbilical vessels after exposure by laparohysterotomy.

Determination of Methemoglobin (MetHb.). The method of Evelyn and Malloy (1938) was utilized for the determination of methemoglobin. A M/60 phosphate buffer solution (Sleight and Sinha, 1968) was used to prevent reduction of methemoglobin. Determinations were made on a Coleman Junior Spectrophotometer.*

Determination of Hemoglobin (Hb.) and Packed Cell Volume (PCV). Hemoglobin values were determined by the cyanmethemoglobin method (Benjamin, 1961). The microhematocrit method (Benjamin, 1961) was used for the estimation of the PCV.

Determination of Plasma Ascorbic Acid. The plasma levels of ascorbic acid were determined according to the Caraway modification (Caraway, 1968) of the method of Roe and Keuther (1943). Powdered L-ascorbic acid** (M.W. 176.12) was used in the preparation of a reference standard.

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* Coleman model 6D Junior Spectrophotometer - Coleman Instruments, Inc., 42 Madison Street, Maywood, Ill.

** Eastman Organic Chemicals, Distillation Products Industries, Rochester 3, New York.

Experiment A. Levels of Methemoglobin Formation Following $NaNO_2$ Treatment of Ascorbic Acid Deficient Guinea Pigs. Twelve mature guinea pigs with subnormal plasma ascorbic acid levels were treated with 50 mg./kg. $NaNO_2$ subcutaneously. Six guinea pigs with normal plasma ascorbic acid levels were also given 50 mg./kg. $NaNO_2$. Blood samples from all guinea pigs were collected by cardiac puncture at 60, 90 and 120 minutes after treatment. Hemoglobin, PCV, and methemoglobin values were determined in addition to plasma levels of ascorbic acid.

Three additional guinea pigs with subnormal plasma ascorbic acid levels were pretreated with 10 mg./kg. methylene blue intraperitoneally 30 minutes prior to injection of 50 mg./kg. $NaNO_2$. Three control guinea pigs were treated in an identical manner. Blood samples were collected by cardiac puncture from both groups of guinea pigs at 60, 90 and 120 minutes after injection of $NaNO_2$. Blood values were determined in the manner outlined previously.

Experiment B. Fetotoxic Properties of $NaNO_2$ in Pregnant Guinea Pigs Deficient in Ascorbic Acid. Eleven pregnant guinea pigs were fed a diet deficient in ascorbic acid the latter part of the gestation period. During the final week of gestation, 8 of these guinea pigs were given 45 mg./kg. $NaNO_2$ subcutaneously. The remaining 3 deficient guinea pigs were allowed to carry their fetuses through normal parturition with no additional treatment.

Three additional pregnant guinea pigs were maintained on a diet supplemented with ascorbic acid during the entire gestation period. During the last week of gestation these control guinea pigs were also treated with 45 mg./kg. $NaNO_2$.

Blood samples were collected from all guinea pigs during the last week of pregnancy by cardiac puncture. Hemoglobin, PCV, and plasma ascorbic acid levels were determined. Reproductive performance was recorded at time of abortion or parturition.

Experiment C. Maternal and Fetal Levels of Methemoglobin Formation in Ascorbic Acid Deficient Guinea Pigs. Five pregnant guinea pigs were maintained on a diet deficient in ascorbic acid the latter part of the gestation period. Three additional pregnant guinea pigs were maintained on a diet supplemented with ascorbic acid.

During the last week of gestation all guinea pigs were injected with 40 mg./kg. $NaNO_2$, subcutaneously. Fifty-five minutes after injection of $NaNO_2$, ether anesthesia was induced and laparohysterotomy was performed. Fetal and maternal blood samples were collected at 60 minutes for determination of hemoglobin, PCV, and methemoglobin. Terminal blood samples were collected for plasma ascorbic acid determinations.

## RESULTS

Experiment A. Methemoglobin Formation Induced by $NaNO_2$ in Ascorbic Acid Deficient Guinea Pigs. Guinea pigs maintained on the ascorbic acid deficient diet possessed subnormal plasma levels of ascorbic acid (Table 1). Control guinea pigs maintained on the ascorbic acid supplemented diet had plasma ascorbic acid levels in the normal range (Schow, 1966). Hemoglobin and PCV values were lower in the deficient group.

Administration of 50 mg./kg. $NaNO_2$ produced higher levels of methemoglobin in ascorbic acid deficient guinea pigs when compared to the controls (Figure 1). These higher levels of methemoglobin were noted in the blood samples collected at 60, 90 and 120 minutes after injection of $NaNO_2$.

Pretreatment with 10 mg./kg. methylene blue prevented the formation of high levels of methemoglobin subsequent to the injection of 50 mg./kg. $NaNO_2$ in deficient guinea pigs (Figure 2). The guinea pigs deficient in ascorbic acid had higher levels of methemoglobin at 60, 90 and 120 minutes than the controls.

A high mortality rate (83.3%) occurred in the ascorbic acid deficient guinea pigs injected with 50 mg./kg. $NaNO_2$ alone. No deaths occurred in the ascorbic acid deficient guinea pigs pretreated with 10 mg./kg. methylene blue before injection of 50 mg./kg. $NaNO_2$ (Table 2).

Table 1. Hematologic values of guinea pigs used in Experiment A

| Guinea Pig No. | Dietary Ascorbic Acid | Plasma Ascorbic Acid (mg./100 ml.) | Hb (Gm./100 ml.) | PCV (%) |
|---|---|---|---|---|
| 1 | - | 0.42 | 10.9 | 37.0 |
| 2 | - | 0.40 | 12.9 | 45.5 |
| 3 | - | 0.25 | 10.1 | 34.0 |
| 4 | - | 0.41 | 4.6 | 13.5 |
| 5 | - | 0.36 | 9.3 | 30.0 |
| 6 | - | 0.24 | 9.2 | 31.0 |
| 7 | - | 0.44 | 13.5 | 44.0 |
| 8 | - | - | 13.7 | 42.5 |
| 9 | - | 0.37 | 13.9 | 44.5 |
| 10 | - | 0.32 | 13.1 | 42.0 |
| 11 | - | 0.34 | 11.6 | 38.5 |
| 12 | - | 0.24 | 9.9 | 31.5 |
| 13 | - | 0.45 | 9.0 | 33.0 |
| 14 | - | 0.30 | 13.1 | 42.5 |
| 15 | - | 0.36 | 8.3 | 28.5 |
| 16 | + | 0.82 | 12.8 | 41.0 |
| 17 | + | 0.95 | 12.9 | 43.0 |
| 18 | + | 0.86 | 13.7 | 45.0 |
| 19 | + | 0.90 | 12.2 | 38.0 |
| 20 | + | 0.90 | 8.8 | 24.0 |
| 21 | + | 0.90 | 10.3 | 33.0 |
| 22 | + | 0.66 | 13.2 | 45.0 |
| 23 | + | 0.52 | 12.4 | 43.0 |
| 24 | + | 1.72 | 11.9 | 35.0 |

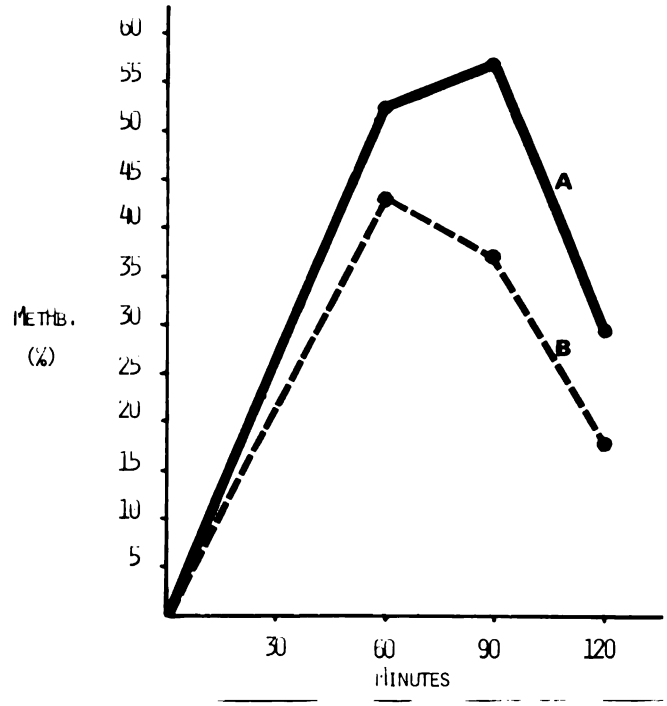


**Figure 1. Methemoglobin formation induced by subcutaneous administration of 50 mg./kg. $NaNO_2$ to 12 ascorbic acid deficient guinea pigs (A) and 6 control guinea pigs (B).**

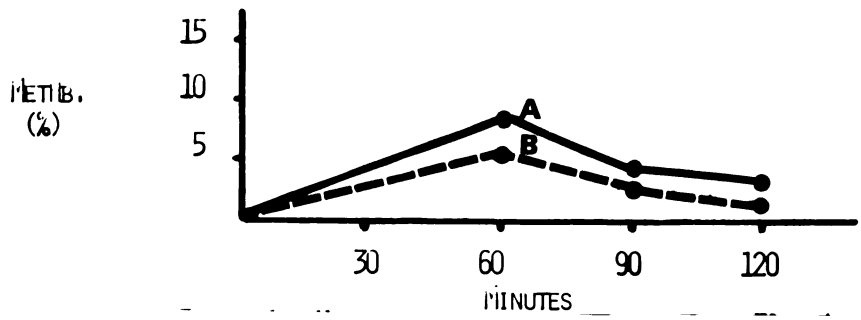


Figure 2. Methemoglobin formation induced by subcutaneous administration of 50 mg./kg. $NaNO_2$ following pretreatment with 10 mg./kg. methylene blue of 3 ascorbic acid deficient guinea pigs (A) and 3 controls (B).

Table 2. Mortality rates in guinea pigs produced by treatment with $NaNO_2$

| Type Diet | Average Plasma Ascorbic Acid Value (mg./100 ml.) | $NaNO_2$ (mg./kg.) | Methylene Blue (mg./kg.) | No. Injected | Mortality Rate (%) |
|---|---|---|---|---|---|
| Ascorbic Acid Deficient | 0.34 | 50 | 0 | 12 | 83.3 |
| Ascorbic Acid Supplemented | 0.88 | 50 | 0 | 6 | 0 |
| Ascorbic Acid Deficient | 0.37 | 50 | 10 | 3 | 0 |
| Ascorbic Acid Supplemented | 0.97 | 50 | 10 | 3 | 0 |

Experiment B. Fetotoxic Effects of $NaNO_2$ in the Ascorbic Acid Deficient Guinea Pig. The injection of 45 mg./kg. $NaNO_2$ caused abortion in the pregnant guinea pigs with plasma ascorbic acid levels below 0.40 mg./100 ml. These abortions occurred within 24 hours after treatment (Table 3). This same dosage of $NaNO_2$ did not cause abortion in guinea pigs with higher levels of plasma ascorbic acid. The ascorbic acid deficient guinea pigs that were allowed to undergo normal parturition with no $NaNO_2$ treatment did not abort, and all gave birth to live fetuses.

Experiment C. Maternal and Fetal Levels of Methemoglobin Formation in Ascorbic Acid Deficient Guinea Pigs. A dietary deficiency of ascorbic acid caused a decrease in the maternal plasma ascorbic acid levels (Table 4). The respective fetuses of these deficient dams showed a smaller decrease in plasma ascorbic acid levels. Maternal hemoglobin and PCV values also decreased to lower values than fetal hemoglobin and PCV values during the deficiency.

Sixty minutes after injection of 40 mg./kg. $NaNO_2$ the deficient dams had an average methemoglobin level of 35.2% (Figure 3). At this same time interval the methemoglobin level was 25.9% in the control dams. The methemoglobin levels in the fetuses of the deficient and control groups were 10.1% and 10.3%, respectively.

Table 3. Hematologic values and reproductive performance of pregnant guinea pigs utilized in Experiment B

| Guinea Pig No. | Dietary Ascorbic Acid | Plasma Ascorbic Acid (mg./100 ml.) | Hb (Gm./100 ml.) | PCV (%) | $NaNO_2$ (mg./kg.) | Fetuses | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Live | Dead | Total |
| 1 | - | 0.26 | 10.4 | 33.5 | 45 | 0 | 4 | 4 |
| 2 | - | 0.20 | 11.9 | 40.5 | 45 | 0 | 4 | 4 |
| 3 | - | 0.12 | 11.0 | 36.0 | 45 | 0 | 3 | 3 |
| 4 | - | 0.32 | 12.2 | 43.0 | 45 | 3 | 2 | 5 |
| 5 | - | 0.40 | 12.0 | 40.0 | 45 | 4 | 0 | 4 |
| 6 | - | 0.64 | 12.2 | 42.0 | 45 | 3 | 0 | 3 |
| 7 | - | 0.64 | 11.6 | 40.0 | 45 | 3 | 0 | 3 |
| 8 | - | 0.72 | 10.4 | 33.0 | 45 | 4 | 0 | 4 |
| 9 | - | 0.40 | 12.2 | 43.0 | 0 | 4 | 0 | 4 |
| 10 | - | 0.26 | 10.4 | 33.0 | 0 | 1 | 0 | 1 |
| 11 | - | 0.36 | 11.3 | 38.0 | 0 | 3 | 0 | 3 |
| 12 | + | 1.10 | 11.9 | 39.0 | 45 | 4 | 0 | 4 |
| 13 | + | 1.00 | 11.2 | 39.0 | 45 | 3 | 0 | 3 |
| 14 | + | 0.74 | 11.8 | 39.0 | 45 | 3 | 0 | 3 |

Table 4. Maternal and fetal hematologic values of guinea pigs utilized in Experiment C

| Guinea Pig No. | Ascorbic Acid Diet | Plasma Ascorbic Acid (mg./100 ml.) | Hb (Gm./100 ml.) | PCV (%) |
|---|---|---|---|---|
| Dam #1 | Deficient | 0.32 | 10.9 | 35 |
| Fetus | | 0.63 | 11.6 | 38 |
| Dam #2 | Deficient | 0.40 | 9.6 | 28 |
| Fetus | | - | 10.7 | 37 |
| Dam #3 | Deficient | 0.26 | 9.0 | - |
| Fetus | | - | 13.7 | 54 |
| Dam #4 | Deficient | 0.20 | 7.8 | 27 |
| Fetus | | - | 11.0 | 44 |
| Dam #5 | Deficient | 0.46 | 9.9 | 32 |
| Fetus | | 0.50 | 8.0 | 29 |
| Dam #6 | Supplemented | 1.82 | 12.6 | 43 |
| Fetus | | 2.00 | 10.7 | 38 |
| Dam #7 | Supplemented | 1.20 | 11.9 | 47 |
| Fetus | | 1.10 | 13.3 | 43 |
| Dam #8 | Supplemented | 0.90 | 11.5 | 40 |
| Fetus | | 0.86 | 11.3 | 41 |

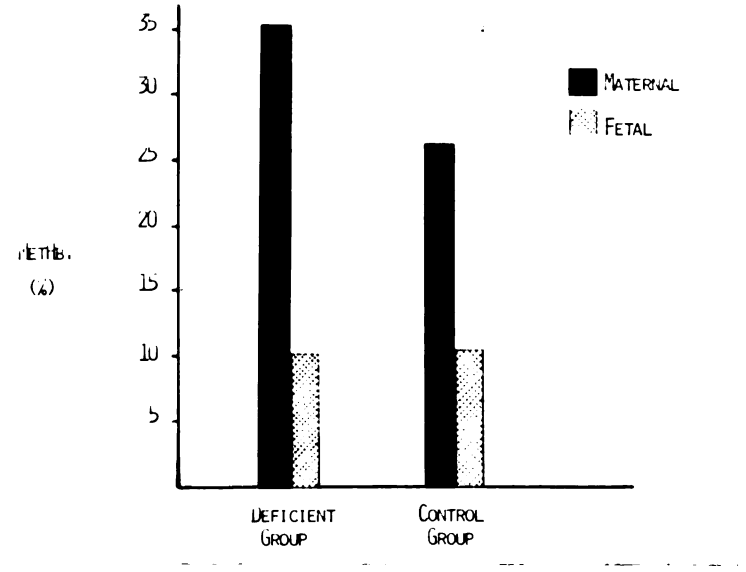


**Figure 3. Methemoglobin levels 60 minutes after subcutaneous administration of 40 mg./kg. $NaNO_2$ to pregnant guinea pigs.**

## DISCUSSION

Nitrite toxicity is due to (1) the oxidation of hemoglobin to methemoglobin and (2) the excessive dilatation of vascular channels. The formation of methemoglobin leads to hypoxia, and the vasodilatation causes hypotension.

The properties of ascorbic acid have been shown to play roles in the physiological processes of the body which are directly related to the formation of both of these conditions. Kleihauer (1965) has shown the ability of ascorbic acid to be an effective reducing substance that can reduce methemoglobin back to hemoglobin. Lee and Lee (1947) reported on the hyporeactivity of blood vessels during ascorbic acid deficiency. A failure of scorbutic guinea pigs to produce renal vasohumoral agents following hemorrhage has also been shown by Lee and Holze (1951). The effects of these factors could increase the hypotensive effect of $NaNO_2$.

Meyer and McCormick (1928) demonstrated lower hemoglobin and erythrocytic values during ascorbic acid deficiency. This would increase the ratio of nitrite ions per erythrocyte in the scorbutic animal during nitrite poisoning.

The increased susceptibility of ascorbic acid deficient guinea pigs to nitrite toxicity was demonstrated in Experiment A. Higher levels of methemoglobin were consistently found 60, 90 and 120 minutes after treatment of ascorbic acid deficient guinea pigs with 50 mg./kg. $NaNO_2$. These higher methemoglobin levels were probably the result of (1) a relative decrease in ascorbic acid available as a reducing substance and (2)

decreased hemoglobin and PCV values, which increased the ratio of nitrite per erythrocyte.

A high mortality rate (83.3%) occurred in this deficient group due to (1) hypoxia subsequent to high levels of methemoglobin and (2) inability to compensate for the excessive vasodilatation.

Pretreatment with 10 mg./kg. methylene blue 30 minutes prior to injection of 50 mg./kg. $NaNO_2$ prevented the formation of high levels of methemoglobin in both the deficient and control groups. The methemoglobin levels remained slightly higher in the deficient group when compared to the controls. No mortalities occurred in the methylene blue pretreated groups. Methylene blue facilitates the enzymatic reduction of methemoglobin back to hemoglobin by acting as an electron transporter. Activation of this reduction system apparently prevented the formation of high levels of methemoglobin in the deficient as well as the control groups of guinea pigs. The higher levels of methemoglobin noted in the pretreated deficient group possibly could be explained by the lack of ascorbic acid as a reducing substance and lowered hemoglobin and PCV values.

In Experiment B the increased fetotoxic effects of $NaNO_2$ were demonstrated in the ascorbic acid deficient guinea pig. Previous work (Sinha, 1968) had shown a minimal dose of 60 mg./kg. $NaNO_2$ was capable of causing abortion in the guinea pig. In this study pregnant guinea pigs with plasma ascorbic acid levels below 0.40 mg./100 ml. aborted following the injection of 45 mg./kg. $NaNO_2$. This dose did not cause abortion in any guinea pigs with plasma ascorbic acid levels above 0.40 mg./100 ml.

This indicated a subnormal plasma ascorbic acid level was associated with an increased susceptibility to $NaNO_2$ toxicity in the pregnant guinea

pig. A subnormal plasma level of ascorbic acid alone was not sufficient to cause abortion, as all deficient guinea pigs not treated with $NaNO_2$ gave birth to live fetuses.

In Experiment C the maternal and fetal levels of methemoglobin formation were compared in ascorbic acid deficient guinea pigs and controls. Plasma values of ascorbic acid in the deficient dams were lower than the plasma ascorbic acid levels of their fetuses. Goldsmith (1961) showed a preferential reserve of fetal ascorbic acid at the expense of the maternal reserve.

Maternal hemoglobin and PCV values were also lowered during ascorbic acid deficiency, whereas the fetal levels of hemoglobin and PCV were similar to those of the control group. Apparently the fetal hematologic values were not greatly decreased during ascorbic acid deficiency.

The deficient pregnant guinea pigs injected with 40 mg./kg. $NaNO_2$ had higher levels of methemoglobin formation at 60 minutes than the controls. Fetal samples of blood collected at this same time (60 minutes) showed levels of methemoglobin that were nearly identical in both the deficient and control groups. This indicated that the increased fetotoxic effects of $NaNO_2$ in the ascorbic acid deficient guinea pigs were due to higher levels of methemoglobin in the dam. Apparently the partial placental barrier to $NaNO_2$ (Sinha, 1968) was not altered during ascorbic acid deficiency.

## SUMMARY AND CONCLUSIONS

In this investigation the toxicity of $NaNO_2$ was studied in the ascorbic acid deficient guinea pig. Levels of methemoglobin formed as a result of oxidation of hemoglobin by nitrite were determined. Effects on the reproductive performance of pregnant guinea pigs with subnormal plasma ascorbic acid levels were studied. Maternal and fetal levels of methemoglobin induced by maternal nitrite toxicosis were also compared. From these experiments the following conclusions were made:

1. Injection of 50 mg./kg. $NaNO_2$ caused higher levels of methemoglobin formation in guinea pigs deficient in ascorbic acid.

2. A mortality rate of 83.3% occurred in the ascorbic acid deficient group after treatment with 50 mg./kg. $NaNO_2$. This dose caused no mortalities in the control group.

3. Pretreatment with 10 mg./kg. methylene blue prevented the formation of high levels of methemoglobin in ascorbic acid deficient guinea pigs treated with 50 mg./kg. $NaNO_2$. The methemoglobin levels were slightly higher than those of a similarly treated control group.

4. No mortalities occurred in the deficient group that was pretreated with 10 mg./kg. methylene blue prior to administration of 50 mg./kg. $NaNO_2$.

5. Pregnant guinea pigs with plasma ascorbic acid levels below 0.40 mg./100 ml. aborted following treatment with 45 mg./kg. $NaNO_2$. This dose did not cause abortion in guinea pigs with higher levels of plasma ascorbic acid.

6. Methemoglobin levels were higher in ascorbic acid deficient pregnant guinea pigs than in controls following the injection of 40 mg./kg. $NaNO_2$. Fetal blood samples obtained by laparohysterotomy showed levels of methemoglobin comparable to those of the similarly treated control group. This indicated the partial placental barrier was not altered during ascorbic acid deficiency in the pregnant guinea pigs.

7. The increased fetotoxic properties of $NaNO_2$ in the ascorbic acid deficient guinea pigs were due to increased levels of methemoglobin formation in the maternal blood.

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