Ruminants have been afforded some degree of protection against nitrate toxicity by the inclusion of readily fermentable carbohydrates in the diet (Case, 1957; Crawford, 1960). In laboratory animals, various blood constituents including sulfhydryl compounds (Mortensen, 1953), glutamic acid (Genkin and Voltov, 1960, 1962) and glucose (Brooks, 1934) have been reported to limit formation or increase reduction of methemoglobin. The effect of readily available carbohydrates on nitrate toxicity in ruminants has been partially explained by an increase in the rate of disappearance of nitrate in rumen ingesta (Shapiro et al., 1949). The fact that differences in the rate of methemoglobin formation or reduction, per se, might contribute in part to the protective effect of dietary energy sources in ruminants has not been adequately explored.

In the case of the human, the fetus and the premature infant have been shown to be especially susceptible to methemoglobinemia induced by a variety of agents including nitrite (Ross, 1963; Fisch et al., 1963; Kravitz et al., 1956; Armstrong et al., 1958; Bodansky, 1951). However, the relative susceptibility of young livestock to nitrite-induced methemoglobinemia has not been experimentally defined.

The experiments reported herein were conducted to evaluate the diet of sheep and age of swine as factors influencing the rate of formation and reduction of nitrite-induced methemoglobin.

Experimental

Sheep

Trial 1. Twenty-four lambs of mixed breeding and averaging about 36 kg. were assigned to four groups of six lambs each. Rations were as follows: (1) bromegrass hay full-fed, (2) bromegrass hay plus a drench of 60 ml. of a 50% sucrose solution twice daily, (3) bromegrass hay plus 500 gm. ground corn per day, and (4) bromegrass hay plus 400 gm. ground corn and 100 gm. soybean meal per day. Trace mineral salt and dicalcium phosphate were available ad libitum. The sheep were maintained in individual pens and fed twice daily.

After about 3 weeks on experimental rations, the sheep were treated orally with a single 20-gm. dose of sodium nitrate per head. The sodium nitrate was administered as a 50% w/v aqueous solution by stomach tube approximately 30 min. after the morning feeding. Methemoglobin values were determined at 2-hr. intervals by the method of Evelyn and Malloy (1938). Two sheep from each of the four groups were treated daily until data had been obtained for all sheep (six per group). Differences between control and treatment means were tested using Dunnett's test (Steel and Torrie, 1960).

Trial 2. Twenty sheep (five per treatment) averaging 33.5 kg. were maintained on the same experimental rations as those described for trial 1. After approximately 3 weeks blood was drawn from each animal into citrate. Fresh blood samples were analyzed for hemoglobin by the method of Evelyn (1936). Within approximately 20 min. of the time of collection, 3-ml. samples of blood were placed in 15-ml. conical bottom centrifuge tubes, 0.05 ml. of a freshly prepared 0.125 M sodium nitrite solution was added, and the tubes were incubated in a 37° C. water bath. Methemoglobin was determined by the method described previously. This analysis was made at 10- to 20-min. intervals until the peak was reached and at longer intervals varying from 30 to about 120 min. thereafter.

Methemoglobin studies in vivo involved
the intravenous administration of a 2% solution of sodium nitrite into the jugular vein of each sheep at the rate of 1.5 ml. per kg. of body weight. Methemoglobin was determined at intervals varying from 30 to about 90 min. Average weights at the time of treatment were as follows: group 1, 34.6 kg.; group 2, 34.7 kg.; group 3, 37.8 kg.; and group 4, 36.6 kg. In vitro and in vivo data were obtained on successive days for the same sheep. In each instance data were obtained from only one sheep per treatment per day until data had been obtained for all sheep.

Swine

Pigs from three litters of Yorkshire or Yorkshire × Hampshire breeding were used. Male pigs were castrated at approximately 2 days of age, and all pigs remained with their dams until 6 weeks of age. At this time, they were weaned and fed ad libitum a corn, soybean meal, and tankage ration with added minerals and vitamins calculated to contain 15.3% protein. When the pigs reached about 50 kg., the protein content of the ration was reduced to 12.5%. Water containing no nitrate as indicated by chemical analysis was available at all times.

Two barrows and two gilts from each of the three litters were selected as being representative of the respective litters. These pigs served as the experimental animals throughout the study with the exception that two pigs from one litter were killed by their dam.

Figure 1. Effect of various diets on methemoglobin formation in sheep given a single 20-gm. dose of sodium nitrate orally. Six hours after treatment values for sucrose or corn are significantly (P<.05) lower than those for soybean meal or bromegrass hay. Eight hours after treatment value for sucrose is significantly (P<.05) lower than that for bromegrass hay, and value for corn is significantly (P<.05) lower than for soybean meal or bromegrass hay. Bromegrass hay — — , bromegrass hay + sucrose ○ — ○ , bromegrass hay + corn □ — □ , bromegrass hay + corn + soybean meal Δ — Δ.  

Figure 1. Effect of various diets on methemoglobin formation in sheep given a single 20-gm. dose of sodium nitrate orally. Six hours after treatment values for sucrose or corn are significantly (P<.05) lower than those for soybean meal or bromegrass hay. Eight hours after treatment value for sucrose is significantly (P<.05) lower than that for bromegrass hay, and value for corn is significantly (P<.05) lower than for soybean meal or bromegrass hay. Bromegrass hay — — , bromegrass hay + sucrose ○ — ○ , bromegrass hay + corn □ — □ , bromegrass hay + corn + soybean meal Δ — Δ.
prior to weaning, and one pig from another litter was removed from the experiment later because of an unthrifty condition. The latter pig as well as one of the two killed by the dam had not been subjected to the nitrite treatment described under *in vivo* studies. Data pertaining to methemoglobin formation and reduction were obtained *in vitro* and *in vivo* at approximately 1 week, 3 months and 5½ months of age. At these times average weights of the pigs were 1.9, 30.8 and 94.1 kg., respectively.

**In Vitro Studies.** A sample of blood drawn into citrate was obtained from the pigs from each litter as they reached the appropriate ages. Hemoglobin was determined on the fresh samples as described previously, and glutathione was determined by the nitroprusside method (Grunert and Phillips, 1951). Three-milliliter samples of blood were incubated with 0.05 ml. of a 0.125 M sodium nitrite solution and methemoglobin was determined as described previously for sheep.

**In Vivo Studies.** One-half of the experimental animals, one barrow and one gilt from each of the three litters, were arbitrarily chosen to receive intravenous injections of sodium nitrite. The remaining pigs served as uninjected controls. A 2% aqueous solution of sodium nitrite was administered intravenously by anterior vena cava puncture at the rate of 1.5 ml. per kg. of body weight. Blood samples were obtained periodically at intervals varying from 30 to about 90 minutes and immediately analyzed for methemoglobin as described previously. These blood samples were obtained by anterior vena cava puncture for 1-week-old pigs and by ear vein puncture for pigs at later ages.

**Results and Discussion**

**Sheep.** Methemoglobin values for sheep following oral administration of 20 gm. of sodium nitrate per head in trial 1 are plotted in figure 1. Peak methemoglobin values were reached in 4 to 8 hr. in most instances. The highest average value of 5.3 gm. methemo-
globin per 100 ml. blood was reached in 8 hr. by the control group fed only bromegrass hay. Drenching sheep twice daily with 30 gm. of sucrose significantly (P<.05) suppressed the average peak methemoglobin value to about 43% of that in the control group. When compared with the control group, feeding 500 gm. of ground corn daily resulted in the greatest (P<.05) suppression of methemoglobin with the peak value being only 25% of the control. However, the feeding of a concentrate in which 20% of the corn was replaced by soybean meal resulted in methemoglobin values which were not significantly different from the bromegrass hay-fed controls.

Data from trial 2 concerning nitrite-induced methemoglobin formation *in vitro* and *in vivo* following the intravenous administration of sodium nitrite are presented in figures 2 and 3, respectively. The data appear similar for all groups. Thus, the dietary treatments used in these studies appear to have had no effect on the rate of methemoglobin formation or reduction *per se*. This suggests that the protection afforded sheep by a carbohydrate source against intoxication from orally-administered sodium nitrate in trial 1 was related mostly, if not entirely, to a lowering of nitrite absorption from the digestive tract, presumably as a result of lowered nitrite accumulation in the rumen.

These results agree with previous observations that feeding readily available carbohydrates provides ruminants some degree of protection against nitrate toxicity symptoms (Shapiro et al., 1949; Case, 1957; Crawford, 1960). While the apparent action of soybean
meal in counteracting the protective effect of corn under these conditions has not been reported previously, feeding potassium nitrate at levels that were increased with time up to 5% of the ration was reported by Hatfield and Smith (1963) to lower gains in sheep when the ration contained soybean meal but not when it contained urea. Weichenthal et al. (1963) reported that 1% of sodium nitrate reduced gains of cattle on a high-concentrate ration with no difference being observed between two levels of soybean meal, and Crawford (1960) reported that soybean meal as a supplement to a forage ration offered more protection against nitrate toxicity than low-protein concentrates. Further work is needed to define the conditions under which nitrogenous feeds may be antagonistic to orally administered nitrate.

Swine. Average methemoglobin curves obtained in vitro for pigs at various ages are presented in figure 4. Peak methemoglobin values were obtained 30 to 60 min. after the addition of sodium nitrite and blood from the oldest pigs yielded the highest values. However, peak methemoglobin values appear to have been influenced by initial hemoglobin levels, which were highest in the oldest pigs as shown in table 1.

Data following the methemoglobin peaks (figure 4) indicate differences in the rate of methemoglobin reduction in vitro for the pigs at various ages. Average rates of methemoglobin reduction in blood from the pigs at approximately 1 week, 3 months and 5½ months of age were 0.85, 1.18 and 0.68 gm. per 100 ml. per hour, respectively. When in vitro methemoglobin values were calculated as percent of the initial hemoglobin (figure 5), differences in peak values between the
TABLE 1. HEMOGLOBIN VALUES (GM./100 ML. BLOOD) OF PIGS AT VARIOUS AGES*

<table>
<thead>
<tr>
<th>Litter</th>
<th>1 week</th>
<th>3 months</th>
<th>5½ months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In vitro</td>
<td>In vivo</td>
<td>In vitro</td>
</tr>
<tr>
<td>1</td>
<td>10.11 (3)</td>
<td>9.66 (2)</td>
<td>12.01 (3)</td>
</tr>
<tr>
<td>2</td>
<td>10.17 (4)</td>
<td>10.09 (2)</td>
<td>12.14 (2)</td>
</tr>
<tr>
<td>3</td>
<td>8.52 (4)</td>
<td>7.68 (2)</td>
<td>12.16 (4)</td>
</tr>
</tbody>
</table>

Values are averages for the pigs used in the *in vitro* or *in vivo* studies. The numbers of pigs represented are shown in parentheses.

various ages were almost absent. However, differences in the rate of methemoglobin reduction between ages were still apparent.

Methemoglobin data obtained *in vivo* following the intravenous administration of sodium nitrite to pigs are shown in figure 6. Average peak methemoglobin values for the pigs at 1 week, 3 months and 5½ months of age were 3.1, 8.4 and 11.7 gm. per 100 ml. of blood, respectively. These values are equivalent to 32, 69 and 81% of the initial hemoglobin.

In contrast to results concerning methemoglobin reduction *in vitro*, data obtained during the *in vivo* recovery phase showed a more rapid rate of methemoglobin reduction in the oldest pigs. However, the rate of methemoglobin reduction at 5½ months appeared to be decreasing in the range equivalent to about 30 to 50% methemoglobin (figure 6). This is the range in which the low rate of methemoglobin reduction was observed *in vitro* for the pigs at the same age.

No deaths occurred when the pigs were treated with intravenously administered sodium nitrite at 1 week of age, but later treatments resulted in the death of one pig each at the 3- and 5½-month treatment...
periods. While the possibility of a carry-over effect of previous nitrite treatments cannot be completely dispelled on the basis of data presented here, the length of time allowed between treatments makes such an effect appear unlikely. Further, there was no difference in average body weights of 94.7 and 93.5 kg. for the treated and untreated, respectively, at 5½ months, and there appeared to be no difference between in vitro methemoglobin data for these pigs. This comparison is shown in figures 7 and 8 for pigs at 3 and 5½ months of age, respectively.

Average values for reduced glutathione in milligrams per 100 ml. of blood for the pigs at various ages were as follows: 1 week, 11.2; 3 months, 14.2; and 5½ months, 15.5. Thus, variations in reduced glutathione cannot be interpreted as having contributed to the apparently greater susceptibility of the older pigs.

Hansard et al. (1953) have reported the blood volume per unit of body weight in pigs 2 weeks of age to be about 1.25 times that of pigs 4 to 5 months of age. While differences in blood volume would be expected to influence the response to parenterally-administered sodium nitrite, the slower rate of methemoglobin reduction demonstrated in vitro may have contributed in part to the greater susceptibility of the pigs at later ages. The data indicate that healthy pigs 1 week of age were no more, and possibly less, susceptible to nitrite-induced methemoglobinemia than older pigs. However, this should not be interpreted as being indicative of the relative susceptibility of the fetus or premature young of this species.

Summary

Drenching sheep twice daily with a sucrose solution providing 60 gm. of sugar per day,
or the addition of 500 gm. of corn per day to a forage ration, offered a large degree of protection against the methemoglobinemia induced by 20 gm. of orally-administered sodium nitrate. Substitution of 100 gm. of soybean meal for an equal amount of corn appeared to reduce the protection provided by the corn. When 0.03 gm. of sodium nitrite per kg. of body weight was given by intravenous injection, no differences in methemoglobin values were observed for sheep on the various rations. No differences in the rate of methemoglobin formation or reduction were apparent when blood from these sheep was incubated in vitro with sodium nitrite. These data indicate that the effect of dietary variations on the severity of nitrate toxicity in sheep was probably due almost entirely to variations in nitrite accumulation in the digestive tract and not to differences in the rate of methemoglobin formation or reduction per se.

Pigs given intravenous injections of 0.03 gm. sodium nitrite per kg. of body weight developed a lower degree of methemoglobinemia when treated at 1 week of age than the same pigs when similarly treated at approximately 3 months and 5½ months of age. While peak methemoglobin values (percent methemoglobin) obtained under in vitro conditions for the pigs of various ages were similar, a slower rate of methemoglobin reduction was observed for pigs at 5½ months of age. This difference in rate of methemoglobin reduction could not be explained on the basis of differences in reduced glutathione content of the blood, which tended to increase with increase in age, nor was it confirmed under in vivo conditions. These data indicate that healthy pigs 1 week of age were

![Figure 7. In vitro nitrite-induced methemoglobin formation in blood from pigs 3 months of age not previously treated with nitrite and those previously subjected to intravenous nitrite treatments at 1 week of age. Previously treated ———, not previously treated ———. ○, □ and △, values for pigs previously treated of litters 1, 2 and 3, respectively. ●, ■ and ▲, values for pigs not previously treated of litters 1, 2 and 3, respectively.](image-url)
Figure 8. In vitro nitrite-induced methemoglobin formation in blood from pigs 5½ months of age not previously treated with nitrite and those previously subjected to intravenous nitrite treat-
ments at 1 week and 3 months of age. Previously treated ----, not previously treated ---. O, □ and △, values for pigs previously treated of litters 1, 2 and 3, respectively.
, ■ and △, values for pigs not previously treated of litters 1, 2 and 3, respectively.

no more susceptible to nitrite-induced methemoglobinemia than the same pigs when older.

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