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***Brief Communication***

# RUMINAL METABOLISM AND METHEMOGLOBIN-FORMING EFFECT OF DINOBUTON IN SHEEP

Many aromatic nitro compounds are reduced to the corresponding amines in the rumen by the activity of the rumen flora, and this may influence the effect of such compounds when given orally to ruminants. The dinitrophenol herbicide DNBP (2-(1-methyl-n-propyl)-4,6-dinitrophenol, dinoseb) is a good example of a compound with this property, as DNBP has a methemoglobin-forming effect in ruminants (*Frøslie & Karlog* 1970). These authors connected this phenomenon with the ruminal metabolism of DNBP, because it is reduced to 6-ANBP (2-(1-methyl-n-propyl)-4-nitro-6-aminophenol), and 6-ANBP successively is reduced to DABP (2-(1-methyl-n-propyl)-4,6-diaminophenol) in the rumen.

Dinobuton (2-(1-methyl-n-propyl)-4,6-dinitrophenyl isopropyl carbonate), an ester of DNBP, is also used in agriculture. This paper will present investigations into the metabolism of dinobuton in fresh rumen fluid in vitro and an experiment to demonstrate a possible methemoglobin-forming effect of dinobuton when given intraruminally to sheep.

## EXPERIMENTAL AND ANALYTICAL METHODS

The experiments were carried out as described by *Frøslie & Karlog* (1970) and *Frøslie* (in press). The analytical methods used by *Frøslie & Karlog* were modified and combined with the methods given by *Matsuo & Casida* (1970). The methanolic supernatants after centrifugation of the samples (*Frøslie & Karlog*) were evaporated in vacuum, the residue dissolved in benzene: methanol 1:1, and transferred to the aluminium oxide columns. Dinobuton, DNBP, and 6-ANBP were then eluted from the columns with benzene, methanol, and 0.1 N-NaOH, respectively. The two last mentioned compounds were determined as described by *Frøslie & Karlog,* while dinobuton was determined as DNBP after evaporation of the benzene eluates and hydrolysis

in 0.1 N methanolic NaOH. TLC were also used for identification of the metabolites.

## RESULTS

The results of one of the experiments in vitro are given in Fig. 1. Dinobuton, at concentrations of 150 µg/ml ($4.6 \times 10^{-4}$ M), is rapidly converted in fresh rumen fluid at pH 6.8. Most of the compound added is decomposed during the first half hour, and simultaneously increasing amounts of 6-ANBP were found. Ten min. after administration this metabolite amounted to 10 % of the dinobuton added, and after ½ hr. it amounted to 66 %. Then the concentration of 6-ANBP decreased and after 3 hrs. only 10 % were left. In the first 2 samples of rumen fluid small amounts of DNBP were found. Heating of the rumen fluid to 100°C for ½ hr. (in a closed bottle) destroyed its degradation capacity almost completely. Intra ruminal application of 2.65 g

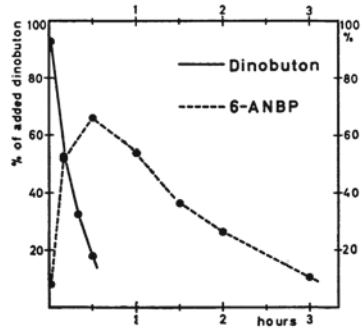


Figure 1. Metabolism of dinobuton in rumen fluid (pH 6.8) in vitro. 100 % = 150 µg/ml.

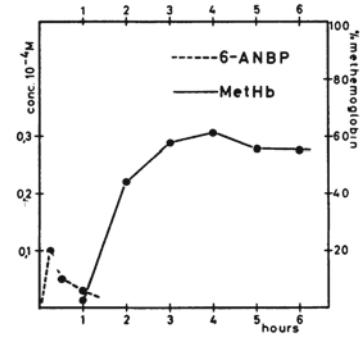


Figure 2. Methemoglobin concentrations and concentrations of 6-ANBP in plasma in a sheep after intraruminal application of dinobuton. Dose: 68 mg/kg.

of dinobuton to a sheep of 39 kg (68 mg/kg, corresponding to 50 mg DNBP/kg) lead to a serious methemoglobinemia, as seen in Fig. 2. After 4 hrs. the methemoglobin concentration was 61 % (of total 10.5 g Hb/100 ml blood). The methemoglobinemia lasted for several days, and hemolysis occurred and caused a marked drop in the hemoglobin concentration. The sheep died after 4 days. In the first hour after administration small amounts of 6-ANBP were found in plasma (max. value $0.1 \times 10^{-4}$ M), but dinobuton, DNBP, or 6-AcANBP (the N-acetylated 6-ANBP) could not be detected in plasma samples.

## DISCUSSION

Degradation of dinitrophenyl esters by hydrolysis may occur in the organism of animals (*Ernst & Bär* 1964), on plants as a photodecomposition (*Matsuo & Casida* 1970), or as a result of pure chemical reactions (alkaline hydrolysis, *Fieser & Fieser* 1956). The experiments presented here show that dinobuton is hydrolyzed to DNBP in fresh rumen fluid too, as a result of the activity of the rumen flora. DNBP, in turn, is reduced to its amino metabolites, in agreement with previous findings (*Frøslie & Karlog* 1970). It is therefore logical that dinobuton like DNBP exhibit a methemoglobin-forming effect when given intraruminally, and the results presented here confirm that this is the case.

DNBP and 6-ANBP, at doses of 50 mg/kg, both lead to a methemoglobinemia of about 50 % when given intraruminally to sheep (*Frøslie* 1971, *Frøslie* in press). It may then be concluded that dinobuton, DNBP, and 6-ANBP all show the same effect when given intraruminally to sheep, because they are metabolized in the same way. The end product of this ruminal metabolism, the diaminophenol DABP, is pointed out as the possible inducer of methemoglobinemia by DNBP poisoning in ruminants (*Frøslie* in press).

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