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Formation of free radicals by oxidative damage induced  
by alk(en)ylthiosulfates derived from Allium plants

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Sodium *n*-propylthiosulfate (NPTS), sodium 2-propenylthiosulfate (2PTS) and sodium methylthiosulfate (MTS) are thought to be causative compounds of onion and garlic poisoning. Since these compounds are known to induce oxidative damage in canine erythrocytes, free radicals produced by reactions of these alk(en)ylthiosulfates with a canine hemolysate were investigated by using an electron spin resonance (ESR) combined with a spin-trapping technique. 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was used as a spin-trapping reagent to stabilize short-lived free radicals.

When each alk(en)ylthiosulfate was incubated with the hemolysate in the presence of DMPO, ESR signal consisting of hyperfine coupling constants,  $AN=1.46$  mT and  $AH^{\beta}=1.62$  mT, was detected, indicating the existence of sulfite radicals ( $\cdot\text{SO}_3^-$ ). The order of intensity of ESR signals due to DMPO-sulfite spin-adducts was 2PTS>NPTS>MTS. Furthermore, the incubation of each alk(en)ylthiosulfate with the hemolysate resulted in increases of methemoglobin (MetHb) and the turbidity index in an order similar to that of the sulfite-radical formation (2PTS>NPTS>MTS). The DMPO-RS spin adducts derived from thiyl radicals (RS $\cdot$ ) were also produced

when alk(en)yl thiols were dissolved in ice-cold hemolysate containing DMPO. However, the addition of DMPO as a scavenger of thiyl radicals and sulfite radicals did not affect the formation induced by alk(en)ylthiosulfate of MetHb or the increase of the turbidity index. These results suggested that the increases of MetHb and the turbidity index induced by alk(en)ylthiosulfate were mainly due to the reaction of Hb with alk(en)ylthiosulfate itself, not thiyl radicals and sulfite radicals. Furthermore, it was shown that the addition of catalase reduced the MetHb formation by 2PTS, suggesting that hydrogen peroxides and/or hydroxyl radicals were partly involved in this oxidative damage.

From these results, it was concluded that sulfite radicals and thiyl radicals were produced in the reactions of alk(en)ylthiosulfate with the hemolysate and that thiyl and sulfite radicals were not involved in the oxidation of Hb. Since superoxides ( $\text{O}_2^-$ ) and lipid peroxides were reported to be produced in the reactions of alk(en)ylthiosulfate with erythrocytes, the sulfate-related radicals identified in this study might play an important role in the further oxidative damage of canine erythrocytes.