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PHOTHEMOLYSIS OF GOAT ERYTHROCYTES
BY PHEOPHORBIDE A

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Vegetable diets provided for commercial livestock sometimes cause so-called "ingestive photosensitization." Pheophorbide a (PPa), a decomposition product of chlorophyll a, is considered to be a causal substance. To clarify the cause of photosensitization by PPa, this substance was first prepared by acid-hydrolysis of chlorophyll a that was extracted from spinach, and the effect of PPa on *in vitro* photohemolysis of goat erythrocytes was then studied as a model experiment. PPa, excited by being exposed to light wavelengths of over 600nm, increased the hemolysis of the erythrocytes in proportion to its incubation time with the cells. The addition of N_3^- , an effective scavenger of singlet oxygen (1O_2), to the medium markedly inhibited the hemolysis in a concentration-dependent manner, whereas the addition of superoxide dismutase and catalase, inhibitors of superoxide (O_2^-) and hydrogen peroxide (H_2O_2), respectively, to the medium had little effect on it. These results suggest that 1O_2 participates in the photohemolysis sensitized by PPa, and that both O_2^- and H_2O_2 are not produced by the photo-excited PPa or are not involved in the photosensitizing processes.

Electron spin resonance spectroscopy combined with a method converting 1O_2 to nitroxide radical by 2,2,6,6-tetramethyl-4-piperidone and the spin-trapping method of O_2^- and H_2O_2 by 5,5-dimethyl-1-pyrroline-1-oxide were employed to get direct evidence for the production of these activated oxygens (1O_2 , O_2^- and H_2O_2). The results showed that only 1O_2 was produced by PPa which was excited by light wavelengths of over 600nm.

From these results, it was concluded that 1O_2 produced by the photo-excited PPa was exclusively responsible for the hemolysis of erythrocytes.