BIOCHEMICAL ANALYSES OF CANINE ERYTHROCYTES ASSOCIATED WITH MULTIPLICATION OF *BABESIA GIBSONI IN VITRO*

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In order to clarify the pathogensis of anemia caused by *B. gibsoni* infection, biochemical changes in erythrocytes infected with *B. gibsoni* during *in vitro* cultivation of the parasites were investigated.

Infected erythrocytes from dogs with acute babesiosis were suspended in α-medium supplemented with L-glutamine (0.3 mg/ml), sodium bicarbonate (2 mg/ml), sodium pyruvate (0.11 mg/ml), penicillin G (100 unit/ml), streptomycin (0.1 mg/ml) and 40% normal canine serum to yield a PCV of 10% and incubated at 37°C under an atmosphere of 5% CO_2 for 6 days. Every 24 hours, a portion of the culture's extracellular medium was removed and replaced with an equal volume of fresh medium.

Under these conditions, the cultures showed a 6～20-fold increase in parasitemia at day 6. At this time, the concentrations of methemoglobin and malondialdehyde, an end product of lipid peroxidation in erythrocytes, significantly increased. In addition, glucose consumption and lactate production by infected erythrocytes, and 2, 3-diphosphoglycerate (2, 3–DPG) concentrations also increased. The erythrocytes deformability decreased, whereas osmotic fragility of infected erythrocytes did not change. The concentrations of reduced glutathione (GSH), ATP and methemoglobin reductase activity in infected erythrocytes were almost unchanged.

Significant increases of both methemoglobin and malondialdehyde concentrations were also observed in *B. gibsoni* infected dogs with high parasitemia.

These results strongly indicate that erythrocytes infected with *B. gibsoni* suffered from oxidative damage by the parasites. This may play a major role in the anemia of *B. gibsoni* infection.