STUDIES ON THE MULTIPLICATION OF *BABESIA GIBSONI IN VITRO*

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This study was carried out to clarify the factors which enhance the growth and multiplication of *Babesia gibsoni* in canine erythrocytes *in vitro*. First, *B. gibsoni* parasites were cultured together with the following three different types of canine erythrocytes, LK cells (normal canine erythrocytes), HK cells characterized by a hereditary high concentration of potassium, reduced glutathione (GSH) and glutamate, and HK/LG cells with hereditary high concentration of potassium, but without high GSH and glutamate accumulations. As a result, the highest multiplication of the parasites was observed in HK cell culture and the lowest was in LK cell culture. In addition, HK erythrocytes were fractionated on Percoll density gradients. The cells in the top layer (lowest density), compared with those in the bottom layer (highest density), had higher concentrations of GSH and glutamate. The multiplication of parasites was higher in erythrocytes from the top layer than in those from the bottom layer.

Second, when the hemolysate of reticulocytes or mature LK erythrocytes was added to the culture medium, the multiplication of the parasites was higher in the medium containing reticulocyte lysate than in that containing mature cell lysate.

Finally, parasitized erythrocytes were cultured in media containing one of the following substances, glucose, L-glutamate, L-glutamine, GSH, α-ketoglutaric acid, or sodium citrate. The multiplication of the parasites in media containing glucose and L-glutamate was higher than in the other media.

From these results, it was suggested that both glutamate and glucose are necessary nutritional elements for growth of *B. gibsoni*.