SEPARATION OF CANINE LYMPHOCYTES BY GRADIENT CENTRIFUGATION AND SHEEP ERYTHROCYTES ROSETTE FORMATION ASSAY

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This study was designed to investigate the one-step separation of canine lymphocytes by gradient centrifugation on a Ficoll-Conray gradient and sheep erythrocytes rosette formation by canine lymphocytes.

The following results were obtained.

1) According to the increase of density of the Ficoll-Conary mixture (1.100 g/ml − 1.135 g/ml), lymphocyte yield was increased but lymphocyte purity was decreased.

2) The most preferable result was obtained when 8 ml of 1 : 8 diluted blood was layered onto the Ficoll-Conray with a density of 1.120 g/ml and centrifuged at room temperature for 15 minutes at 400 G.

3) Using separated lymphocytes, sheep erythrocytes rosette formation was performed in the presence of dextran. Dextran of different molecular weights ranging between 59 and 500×10^3 enhanced sheep erythrocytes rosette formation and that of molecular weight 500×10^3 gave the best enhancement. As for the dextran concentration, 6% dextran gave the best enhancement. At concentrations higher than this, enhancement effect was weakened.

The percentage of E rosettes was increased from 3.8% and 3.7% in PBS to 16.8% and 16.4% in 6% dextran of molecular weight 500×10^3 (mongrels and beagles, respectively).

4) Whether dextran was present or not, rosette forming cells were only lymphocytes, and no rosette formation by dead lymphocytes was seen. Nylon fiber effluent lymphocytes significantly raised the rosette forming percentage as compared to the peripheral blood lymphocytes ($p < 0.05$).