EVALUATION OF EQUINE LYMPPHCYTE BLASTOGENIC RESPONSE BY ETHIDIUM BROMIDE

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This study was designed to investigate equine lymphocyte blastogenic response following mitogen stimulation using ethidium bromide, a fluorescent intercalating agent.

The following results were obtained:

1) The yield of lymphocytes was highest when whole equine blood was diluted to about 15% of the hematocrit value with phosphate buffer saline, layered over the Ficoll-Conray mixture (density 1.079) and then centrifuged at 400×G for 30 minutes at room temperature.

2) The optimum condition for lymphocyte blastogenic response was obtained when the lymphocyte concentration was 1.5×10^6 cell/ml and the concentration of Phytohemagglutinin-P (PHA), Concanavalin A (Con A) and Pokeweed mitogen (PWM) were 0.025%, 47 μg/ml and 0.5%, respectively.

3) Consistent data were obtained when the concentration of sodium lauryl sulfate as solubilizer ranged between 0.125 and 0.25 mg/ml.

4) Blastogenic response following stimulation with any mitogen highly correlated with lymphocyte uptake of ^{3}H-TdR in both Anglo-Norman and hot-blooded breeds.

5) Lymphocytes from 3 Anglo-Norman types and 8 hot-blooded types of horses were stimulated with mitogens, and their stimulation indexes were determined as follows: PHA 1.873±0.366, Con A 2.511±0.566, PWM 2.778±0.326 in the Anglo-Norman type, and PHA 1.866±0.285, Con A 2.564±0.370, PWM 3.016±0.449 in the hot-blooded type.

The results suggested that evaluation of equine lymphocyte blastogenic response by using ethidium bromide is a more useful method than the ^{3}H-TdR uptake assay used previously.