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EVALUATION OF EQUINE LYMPHOCYTE 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  $400\times G$  for 30 minutes at room temperature.
- 2) The optimum condition for lymphocyte blastogenic response was obtained when the lymphocyte concentration was  $1.5\times 10^6$  cell/ml and the concentration of Phytohemagglutinin-P (PHA), Concanavalin A (Con A) and Pokeweed mitogen (PWM) were 0.025%,  $47\ \mu\text{g/ml}$  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  $^3\text{H-TdR}$  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  $1.873\pm 0.366$ , Con A  $2.511\pm 0.566$ , PWM  $2.778\pm 0.326$  in the Anglo-Norman type, and PHA  $1.866\pm 0.285$ , Con A  $2.564\pm 0.370$ , PWM  $3.016\pm 0.449$  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  $^3\text{H-TdR}$  uptake assay used previously.