The Separation of Peripheral Blood Cells of the Horse

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ABSTRACT

The peripheral blood cells from Standard bred horses were subjected to procedures which will separate equine peripheral blood cells with good precision and efficiency into red cell, leukocyte, and platelet fractions. The separated cells have normal morphology and the differential count of the separated granulocytes and lymphocytes is unchanged from that of the original sample.

RÉSUMÉ

On a soumis les cellules du sang circulant de chevaux "Standardbred" à des procédés susceptibles de les séparer avec précision et efficacité, de façon à ce que qu'on obtienne: hématies, leucocytes et plaquettes. La morphologie des cellules ainsi séparées est normale et le comptage différentiel des granulocytes et des lymphocytes s'avère identique à celui de l'échantillon original.

INTRODUCTION

A kinetic study of the peripheral blood cells necessitated an efficient means of separating these cells prior to labelling them with ^{75}Se-labelled selenomethionine or ^{3}H-labelled diisopropylfluorphosphate. Since autoradio-radiographic studies were to be done, it was necessary that the separation procedures did not alter cellular morphology sufficiently to prevent their individual recognition.

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MATERIALS AND METHODS

ERYTHROCYTES

A pure population of erythrocytes was obtained using the following steps:

1. Ten ml of whole blood were collected into EDTA[1] (0.2 ml of a 5% solution in normal saline) in a siliconised gamma counting vial[2] (14 x 100 mm) and allowed to sediment at room temperature for 30 minutes. The supernatant plasma, leukocytes and platelets were then aspirated from the red cell sediment using a Pasteur pipette.
2. Cold homologous plasma (EDTA anticoagulant, 4°C) was added to the red cell sediment to fill the gamma counting vial (to a total volume of 15 ml), and the contents of the tube were mixed by inversion 30 times.
3. The red cells were again allowed to settle 30 minutes and the supernatant plasma, leukocytes and platelets were again removed by aspiration.
4. Steps 2 and 3 were repeated.
5. The sediment was then centrifuged[3] at 3000 rpm (2510 rcf) for ten minutes and the residual plasma removed.

Leukocyte and platelet counts were performed on erythrocyte sediments which were suspended in an equal volume of homologous plasma (packed cell volume approx 50%). With this technique no leukocytes and no platelets could be found on hemocytometer[4] count. When smears of this suspension were made, only one to three leukocytes (usually eosinophils) and no platelets were present on an entire slide.

[1]Tripotassium salt of ethylenediamine tetra-acetic acid, BDH Chemicals, Poole, England.

[2]Nuclear Chicago, Des Plaines, Illinois.

[3]Sorvall R-C3 centrifuge with HG-4 swing head, Ivan Sorvall Inc., Newark, Conn.

[4]Spencer Brightline Hemocytometer with a Neubauer Ruling.

LEUKOCYTES

Ten ml of whole blood were collected into a 50 ml polycarbonate tube containing EDTA (0.2 ml of a 5% solution in normal saline). Thirty-five ml of a saponin-dextran-dextrose solution[5] at 4°C were added to the blood to hemolyse the red cells. The contents of the tube were mixed gently by inversion ten times and allowed to stand for ten minutes, followed by centrifugation at 300 rmp (2510 rcf) for ten minutes at 10°C. The supernatant containing hemoglobin, red cell stroma, and most of the platelets was poured off by rapidly inverting the tube, leaving the leukocyte button adherent to the bottom of the tube (3). With the tube inverted, the walls of the tube were washed with cold (4°C) physiological saline from a plastic wash bottle. The leukocyte button was then resuspended in 10 ml of cold physiological saline by gentle refluxing with a Pasteur pipette and the suspension transferred to a 15 ml conical polycarbonate centrifuge tube. The suspension was then centrifuged as above and the supernatant again poured off. At this stage, a thin film of red cell stroma and platelets remained on top of the leukocyte button. This film was floated from the button by gently adding a few ml of a physiological saline down the side of the inclined tube with a wash bottle while slowly rotating the tube. The final saline wash together with the suspended red cell stroma was then poured off. The leukocyte button was then resuspended in plasma, and smears were prepared. The efficiency of the recovery of leukocytes was judged to be about 80%. There were very few unidentifiable leukocytes, and the proportion of lymphocytes to granulocytes was unchanged. Leukocyte counts could not be performed on the resuspended cells because of the clumping caused by the dextran in the saponin solution. There was a low level of platelets present in the leukocyte button that could be further reduced by additional washing in cold physiological saline. The amount of saponin required for optimum leukocyte morphology appeared to vary with the age and lot number of the product. A fresh vial of saponin was used at 0.0067%, but an older product (five years on shelf) required a concentration of 0.0178%.

[5]0.0067% Saponin (BDH Chemicals, Poole, England) in 6% Dextran — 5% Dextrose (Dextran 55, Abbott Laboratories, Chicago, Illinois).

PLATELETS

Ten ml of whole blood were collected into EDTA in a gamma counting vial. The tube was placed in the centrifuge and spun at 10°C (2) until the rotor speed reached 4200 rpm (5650 rcf). At this stage (approximately two minutes from starting the centrifuge), the centrifuge rotor was turned off with the electric brake turned on. The supernatant contained a mean of 66.7% of the platelets present in the original blood on 240 samples. Leukocyte counts on the platelet rich supernatant ranged from 0-300/m^3. Red cells could not be detected with a red cell counter[6], and very few or none could be seen on smears of the platelet rich supernatant. For optimum morphology the platelets were resuspended in homologous plasma for preparation of smears.

DISCUSSION

The rapid settling of the red cells in normal equine blood allows efficient separation of the red cells themselves. It is interesting that in some horses made anemic by hemorrhage the red cells settled readily in their own plasma but did not settle normally in homologous plasma from normal horses. (J. H. Lumsden, University of Guelph, personal communication). The saponin-dextran-dextrose hemolysing solution represents a level of hemolytic activity which does not alter the leukocyte morphology. Too little saponin results in an excessive layer of red cell stroma and hemoglobin on top of the leukocyte button, and too much saponin causes leukocyte destruction. The dextran increases the cohesion between the leukocytes, causing them to form a firm button which can be washed without dislodgement from the tip of the centrifuge tube. This cohesion can be increased by the use of heparin rather than EDTA anticoagulant due to the effects of free Ca^{++} and Mg^{++} ions on the leukocyte (primarily granulocyte and monocyte) membranes (1). With the present technique even repeated refluxing of the sedimented leukocytes with a Pasteur pipette was in-

[6]Coulter Counter, Model B, Coulter Electronics/nc, Hialeah, Florida.

sufficient to break up the cellular clumps so that an accurate total count could be made. When smears were made of leukocytes and platelets that were washed and suspended in normal saline, there was normal morphology on wet examination and many injured and degenerate cells on examination after drying. Consequently, it was necessary to resuspend the cells in plasma prior to smearing to protect the cells from osmotic shock on drying. The platelet separation technique is also applicable to cattle and represents a departure from the traditional method of low rcf for a longer time to produce platelet rich plasma.

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