Percoll密度勾配遠心法によるウシ赤血球の分離

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Bovine Erythrocyte Fractionation in Percoll Density Gradients

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Age-dependent fractionation of erythrocytes has been accomplished using density gradient with a variety of supporting media such as bovine serum albumin [7, 10], Stractan II [4, 9], phthalate esters [5] and dextran [6]. However, uses of serum albumin and Stractan II are technically complicated and expensive to prepare, phthalate esters are nonmiscible with water and damage the erythrocytes, and dextran causes the erythrocyte agglutination. Further, all of these methods, except those employing phthalate esters, require prolonged centrifugqation. These disadvantages have been overcome by using Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden), a commercially available polyvinyl-pyrrolidone-coated colloidal silica [11]. This method offers the advantages such as simplicity of preparation, economy, controllable osmolarity, low viscosity, and non-agglutination of erythrocytes, and this method has been applied to the fractionation of a population of human erythrocytes according to age [14]. The present report describes the method to fractionate bovine erythrocytes by centrifugation through discontinuous density gradients of Percoll. The activities of several enzymes as indications of erythrocyte age in each fraction are also determined.

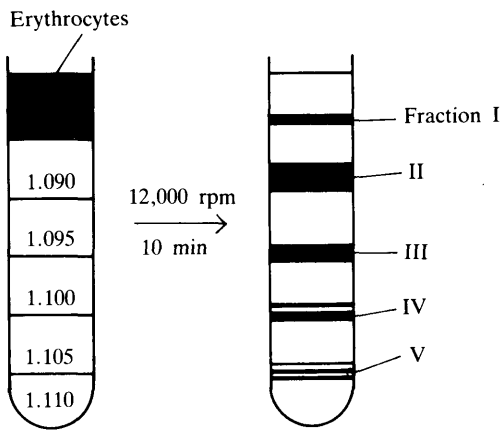

Fig. 1. Bovine erythrocytes were centrifuged at 12,000 rpm for 10 min on five Percoll layers having the specific gravities (top to bottom) of 1.090, 1.095, 1.100, 1.105 and 1.110. The cells were separated into five fractions.

Blood samples were collected from healthy Holstein Friesian cows into Alsever's solution. White cells were completely removed by passing the blood through an α-cellulose: microcrystalline cellulose (2:1 w/w) column in acid-citrate-dextrose (ACD) medium as described by Beutler *et al*. [2]. The filtered erythrocytes were washed three times with ACD. After the final washing the cells were resuspended in ACD to make a packed cell volume of 25% and stored at 4°C until use.

The fractionation of of the erythrocytes was achieved by a density layer centrifugation technique using solutions of Percoll. Five dilution steps of Percoll were made with 0.15 M NaCl solution to make specific gravities of 1.090, 1.095, 1.100, 1.105 and 1.110 at 20°C. Approximately 2 ml of each of these Percoll solutions was poured to form layers in a 15-ml glass centrifuge·tube, starting with the highest specific gravity on the bottom. Three ml of the erythrocyte suspension were carefully layered over the top of the five Percoll layers and centrifuged in the Kubota

Table 1. Enzyme activities in bovine erythrocyte fractions separated in Percoll gradients

RBC fraction	G6PD[a)]	PK[b)]	LDH[c)]
Unseparated	6.02±1.89[d)]	14.89±2.37	22.78±4.44
Fraction I	9.64±2.40	20.05±5.26	29.33±3.06
II	7.09±1.98	15.26±4.10	22.50±1.67
III	5.55±1.52	11.68±3.63	16.67±2.51
IV	3.96±1.25	8.42±2.16	14.17±0.83
V	4.36±1.86	9.74±2.10	12.12±1.33

a) Glucose-6-phosphate dehydrogenase.
b) Pyruvate kinase.
c) Lactate dehydrogenase.
d) Units per g of hemoglobin [1]; mean±SD of eight experiments.

KR-20000T centrifuge in the RA-3 rotor at 12,000 rpm for 10 min at 4°C. After centrifugation, five fractions (Fractions I- V from top to bottom) of the erythrocytes were formed on the top of five Percoll layers (Fig. 1).

The age-dependent distribution of the erythrocytes in each fraction was ascertained by the determination of the activities of three age-dependent enzymes, glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), pyruvate kinase (PK, EC 2.7.1.40) and lactate dehydrogenase (LDH, EC 1.1.1.27) [12]. Each cell fraction was removed from the tube with a capillary pipette, and cells were carefully washed three times with 0.15 M NaCl to remove Percoll. After the final washing, the cells were resuspended in ACD and the packed cell volume was adjusted to 50%. The activities of G6PD, PK and LDH were determined spectrophotometrically by the method of Beutler *et al.* [1]. Results were expressed as units per gram of hemoglobin because the hemoglobin content in the erythrocyte does not change along with cell aging [9].

The activities of G6PD, PK and LDH decreased successively from the top to the bottom fractions of erythrocytes separated in Percoll gradients (Table 1). In human erythrocytes, it has been shown that the activities of G6PD and PK decrease dramatically in the higher density fractions [3, 13]. Correlation between the age of erythrocyte and enzyme activity has been observed to occur in other animal species. In sheeps, it has been noted that the activities of G6PD and hexokinase, but not PK, decrease consistently with cell age [8]. Therefore, the results of the present study are consistent with the previous results although the changes in the enzyme activity in bovine erythrocytes with different specific activity were not so great as those reported in human erythrocytes.

In conclusion, the method described in the present report is simple enough to adapted for clinical diagnostic purposes. Further, the quantities of erythrocytes separated are sufficient for studying the physicochemical and biochemical properties of erythrocytes at different ages.

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要　約

Percoll 密度勾配遠心法によるウシ赤血球の分離：浅野隆司・保刈成男（日本大学農獣医学部獣医薬理学教室）——Percoll のdiscontinuous gradients を用いた密度勾配遠心法によって，ウシ赤血球を 5 分画に分離し，各分画についてグルコース－6－リン酸脱水素酵素，ピルビン酸キナーゼ，乳酸脱水素酵素の活性を測定した。比量の低い分画ほど，各酵素活性は高値を示した。