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HAEMORHEOLOGICAL CHANGES DURING HUMAN ERYTHROCYTE LIFE-SPAN

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ABSTRACT

The investigation of erythrocyte deformability changes during cell ageing might give one insight on circulatory behaviour of young and old red blood cells.
Erythrocytes of healthy subjects of both sexes had been separated into 3 fractions of increasing age on a Percoll discontinous density gradient and their different age has been tested by pyruvate kinase (PK) and glucose-6-phosphate dehydrogenase (G6PD) activities.
Each fraction, free of leucocytes, suspended to 10% haematocrit, was filtered through Nuclepore filters 5 micron diameter; erythrocytes lying on filters were observed by Scanning Electron microscopy.
Erythrocyte deformability has shown small decrease from middle aged cells to old and youngest ones.

INTRODUCTION

Red blood cell physical and chemical modifications throughout its life-span, such as osmotic fragility increase and metabolic impairment, have been described since long (5). Density increase with age is well documented, and several techniques based on this assumption permit the separation of different age erythrocyte populations to be used for various investigations.
Erythrocyte deformability might undergo important changes during its life-span, mainly due to ATP depletion, which could significantly affect the circulatory behaviour of these cells.
Significant increase in viscosity has been described during erythrocyte agening (6).
Human and rabbit red cells were reported not to alter their membrane rigidity while aging when subjected to micropipette analysis (9).
Filtration through 3 um pore membrane failed to prove an useful tool to concentrate young erythrocytes (7).

KEYWORDS: Erythrocyte life-span, Percoll, G6PD, PK, erythocyte filterability, washed red blood cell filterability

The aim of this study is to investigate the filterability behaviour of red blood cells at different stages of their life-span, in order to identify possible modifications of deformability linked to cell aging.

MATERIALS AND METHODS

Blood was drawn from 5 healthy adult subjects of both sexes, aged 26 to 36, using EDTA as anticoagulant; none of the donors was affected by haematological disorders, as verified determining the usual haematologic parameters (haematocrit, red blood cell and white blood cell count, haemoglobin content).

Density separation of red blood cells was obtained by centrifugation of whole blood on a discontinous density gradient of Percoll, as described elsewhere (8).

Gradient densityranged from 1068 to 1108 g/ml; the lowest density indicated was used merely to isolate white blood cells from red ones.

Three to six fractions of different mean age could be obtained by this method; usually three fractions of increasing density, indicated as "young", "middle-aged" and "old" cells, were isolated for filterability determinations.

Separated cell fractions were washed (10 min at 1000XG) three times with Ringer's buffer; the washed red cells were then resuspended at 10% haematocrit.

Leucocytes in the washed blood samples were $100 \pm 30/mm^3$.

Aliquots of whole blood were centrifuged to isolate erythrocytes, which were washed and resuspended as described above. Erythrocytes suspensions were then filtered through Nuclepore filters 5 micron diameter at 5 cm H_2O negative pressure at room temperature (2-3-4).

Erythrocytes lying on filters were fixed by commonly used procedures and examined by Scanning Electron microscopy.

Glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase (PK) activities were tested on density separated cells in order to verify the effectiveness of the separation according to age.

In fact, the progressive loss of both enzymes activities during red cell aging has been documented.

The activities of G6PD and PK were assayed by spectrophotometric methods on erythrocyte lysates obtained by water dilution (1).

RESULTS

Blood centrifugation on Percoll gradients yields cell fractions of increasing age, as shown by the activity decrease of two age-sensitive

enzymes: G6PD and PK (fig. 1).

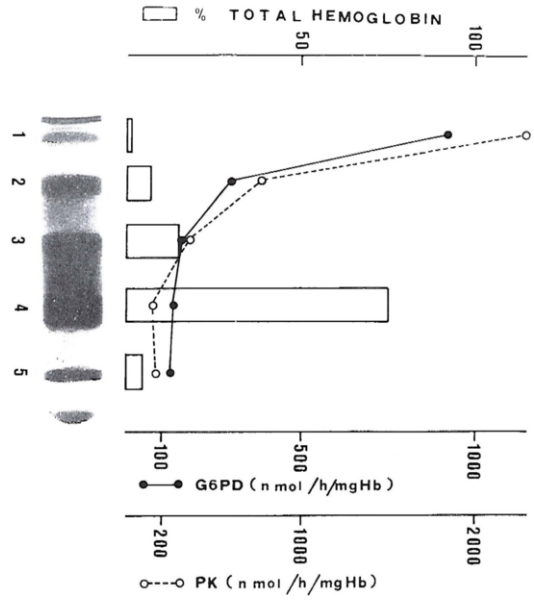

Fig 1: G6PD and PK activity in red blood cells separated on Percoll density gradient (increasing density from fraction 1 to 6).

Density separated red blood cells look in good conditions on SEM examination, showing no appreciable morphological modifications between light and heavy cells as illustrated in fig. 2.
All whole red blood cells population was treated with Percoll (52%) to check possible effects on their filterability; treated red blood cells did not show significant differences compared with untreated cells.
Red blood cell filterability (V_{WRBC}) fails to show any significant change increasing erythrocyte age, as shown in fig. 3; in fact, age-separated filterability ranges within the total RBC population values.

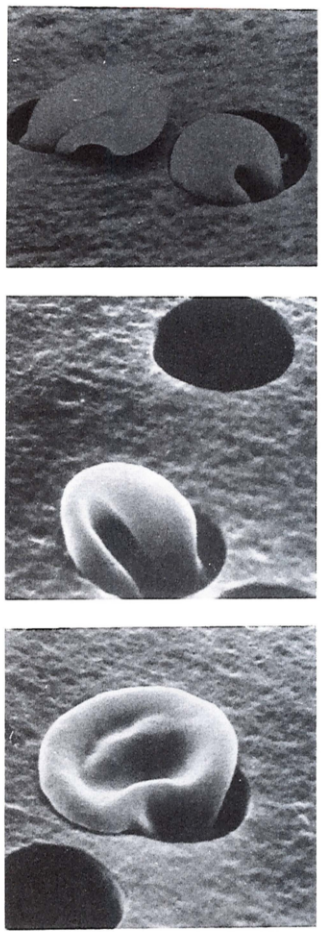

Fig. 2: Scanning Electron microscope photogrphs of density separated human red blood cells on micropore membranes (Nuclepore) for filtration.
A: "Young" cells; B: "Middle-age" cells; C: "Old" cells.

DISCUSSION

Some rheological properties of red blood cells had been described to undergo age-related changes in human and rabbit samples (6-9). Present data suggest that the modifications linked to cell-ageing do not involve deformability as tested by filtration technique on Reid and Dormandy apparatus (10).
We can hypothesize that filterability modifications, if any, might involve only a very small amount of oldest cells.
This small "cohort" could not be able to maintaine normal flexibility, without significant affecting the deformability of the density separated subpopulations.

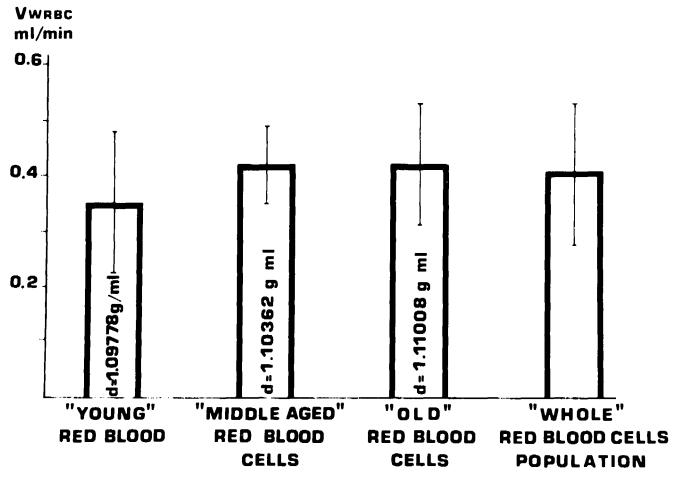

Fig. 3: Filterability of red blood cells of different age separated on Percoll density gradients.
Values (V_{WRBC}) are expressed as mean±S.E.

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