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# Rapid Isolation of Mononuclear Cells from Buffy Coats Prepared by a New Blood Cell Separator [1]

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A newly developed cell separator for the preparation and fractionation of buffy coat cells from human peripheral blood is described. In this cell separator buffy coats (BC-1) as routinely obtained from blood banks were used for the preparation of a second buffy coat (BC-2) with a volume of only 5–6 ml. A special fractionation device allowed sterile isolation of almost pure platelets and mononuclear cells with recoveries of $75 \pm 10\%$ and $89 \pm 4\%$ respectively. The white blood cell contamination of the platelet suspension never exceeded $20 \times 10^6$ leukocytes (i.e., $< 1$ leukocyte per 5000 platelets). Furthermore, the mononuclear cell suspensions were shown to be contaminated with only $3 \pm 2\%$ granulocytes, whereas the white blood cell/red blood cell ratio was $2.6 \pm 1.6$, so that they could therefore be directly used for further separation by means of centrifugal elutriation.

These results indicate that this cell separator provides a rapid ($\pm 1$ h) isolation of both platelets and mononuclear cells without exposing the buffy coat cells to foreign substances like Ficoll or Percoll.

Key words: *buffy coat cell fractionation — blood cell separation — centrifugal elutriation*

## Introduction

Centrifugation over density gradients of Ficoll-Hypaque (Böyum, 1968) or more recently Percoll (Pertoft et al., 1979) is a widely used procedure to isolate mononuclear cells from human peripheral blood. However, if large numbers of mononuclear cells are required and therefore larger volumes of blood have to be processed, these techniques become rather laborious and time-consuming, even if the number of red blood cells of 1 unit blood (500 ml) is reduced by the preparation of a buffy coat.

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We previously reported that the red blood cell contamination of a buffy coat could be reduced by a factor 2 by the preparation of a second buffy coat (BC-2) (Figdor et al., 1981). The aim of the present study was to investigate whether this red blood cell contamination could be further reduced.

We report here that the preparation of a second buffy coat in a specially devised cell separator resulted in a marked reduction of red blood cell contamination. In addition we demonstrate that the buffy coat formed in this cell separator is composed of 2 layers which could be fractionated separately: the top layer containing almost pure platelets, the second layer containing almost pure mononuclear cells contaminated with low numbers of red blood cells.

## Materials and Methods

*Description of the blood cell separator*

The cell separator as shown in Fig. 1 (manufactured by De Koningh, Arnhem,

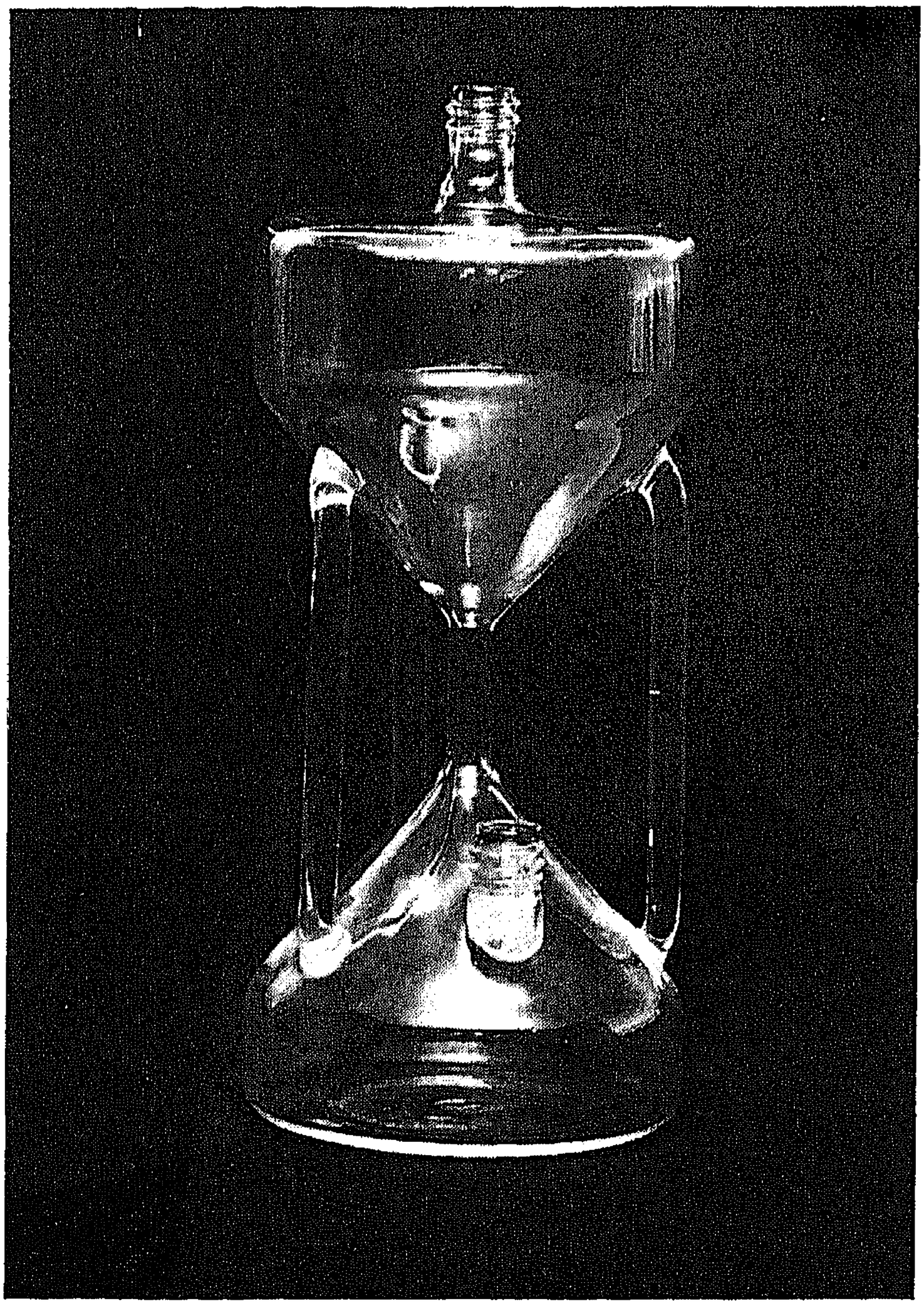

Fig. 1. The blood cell separator.

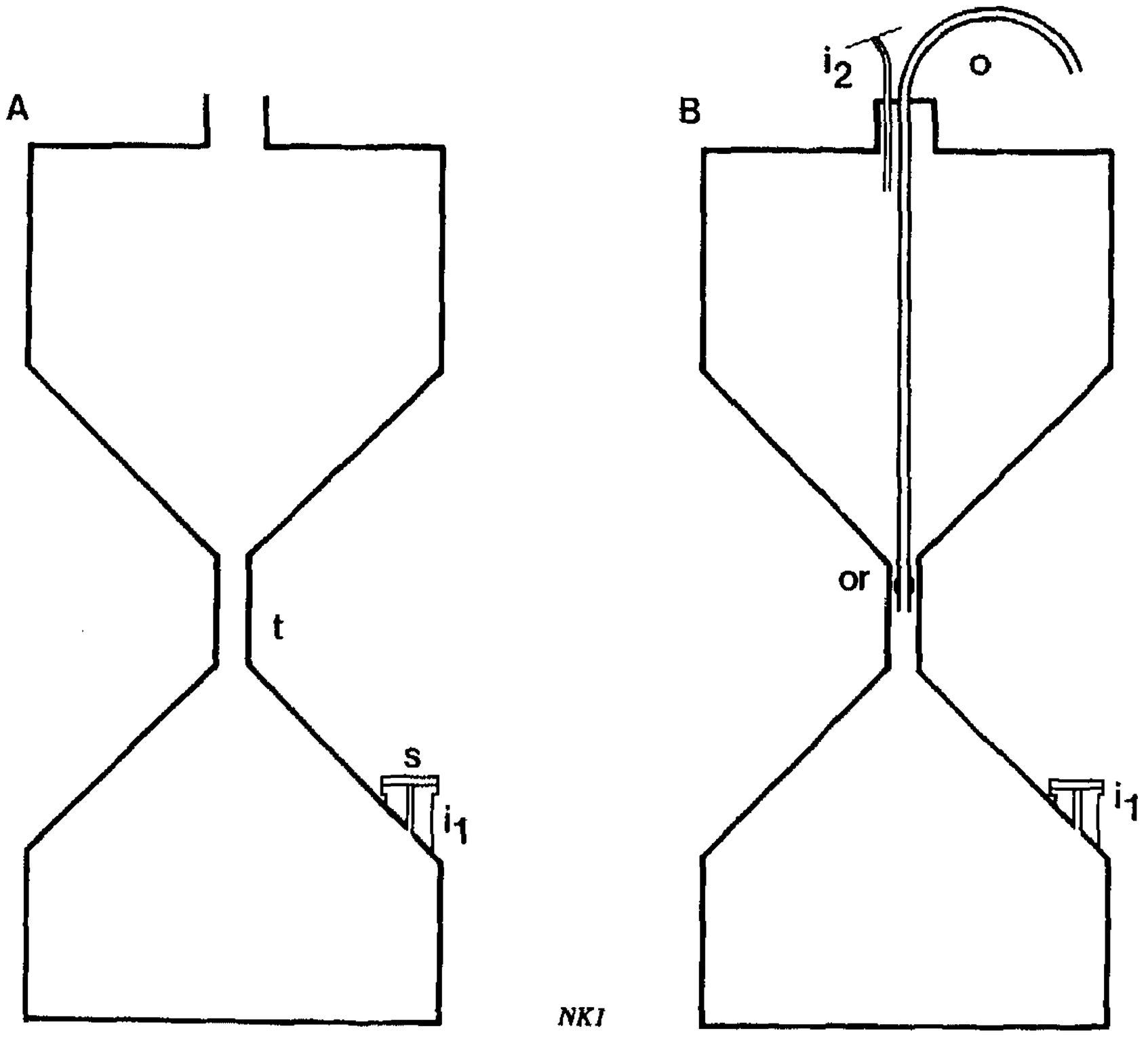


Fig. 2. The cell separator and the fractionation device in cross section. A: the cell separator consists of a lower compartment with an inlet ($i_1$) covered with a self-sealing rubber septum (s) and is connected with the upper compartment by a narrow tube (t). B: the fractionation device can be placed on the top of the apparatus and consists of an inlet tube ($i_2$) and an outlet tube (o). The lower part of the outlet tube is centered into the connection tube by an o-ring (or) in such a way that fluid still can pass.

The Netherlands) is made of glass and has a diameter of approximately 8 cm, and a height of 18 cm. It can be placed into a bucket of a refrigerated centrifuge with an inner diameter of 8.2 cm and a height of 17.5 cm (MSE-Mayor, MSE, Sussex). The separator consists of 2 compartments connected by a narrow tube (t) with an inner diameter of 5 mm (Fig. 2A). The lower compartment, which has a volume of approximately 150 ml, has an inlet ($i_1$) that permits the injection of fluid. The upper compartment has a volume of approximately 180 ml. The cell separator can be filled via an opening at the top of the upper compartment, and fractionation is achieved by means of a special device (Fig. 2B).

*Principles of the isolation of human peripheral blood cells*

After centrifugation of human peripheral blood 3 components can be distinguished: plasma at the top, red blood cells at the bottom and the buffy coat (containing platelets and leukocytes) in a very thin layer at the interface. This buffy coat is generally too thin to be harvested without considerable contamination with plasma and red blood cells. Since the thickness of the buffy coat is inversely related to its surface area, we reasoned that better fractionation would be obtained if the thickness of the buffy coat could be increased by reducing its surface area. Therefore, the isolation of white blood cells would be optimal if the buffy coat could

be formed in the connecting tube of the cell separator during centrifugation. However, pilot studies showed that, due to the small volume of the connection tube ($< 0.5$ ml), slight variations in hematocrit resulted in formation of the buffy coat either above or below the connecting tube. It was found, however, that optimal separation was obtained if the buffy coat were formed just below the connecting tube and was subsequently transported into the connection tube by addition of Maxidens, a non-toxic autoclavable fluid, immiscible with blood, which has a density of 1.9 g/ml (Nyegaard, Oslo) (see for details below).

*Operation*

Buffy coats (BC-1) routinely prepared by centrifugation (20 min, $1000 \times g$) of 1 unit of human peripheral blood, were collected in 60–100 ml (BC-1 were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam). After determination of the hematocrit the volume of Maxidens (md) required was calculated by the formula

$$\text{md} = 140 - \frac{\text{H}}{100} \times \text{V}$$

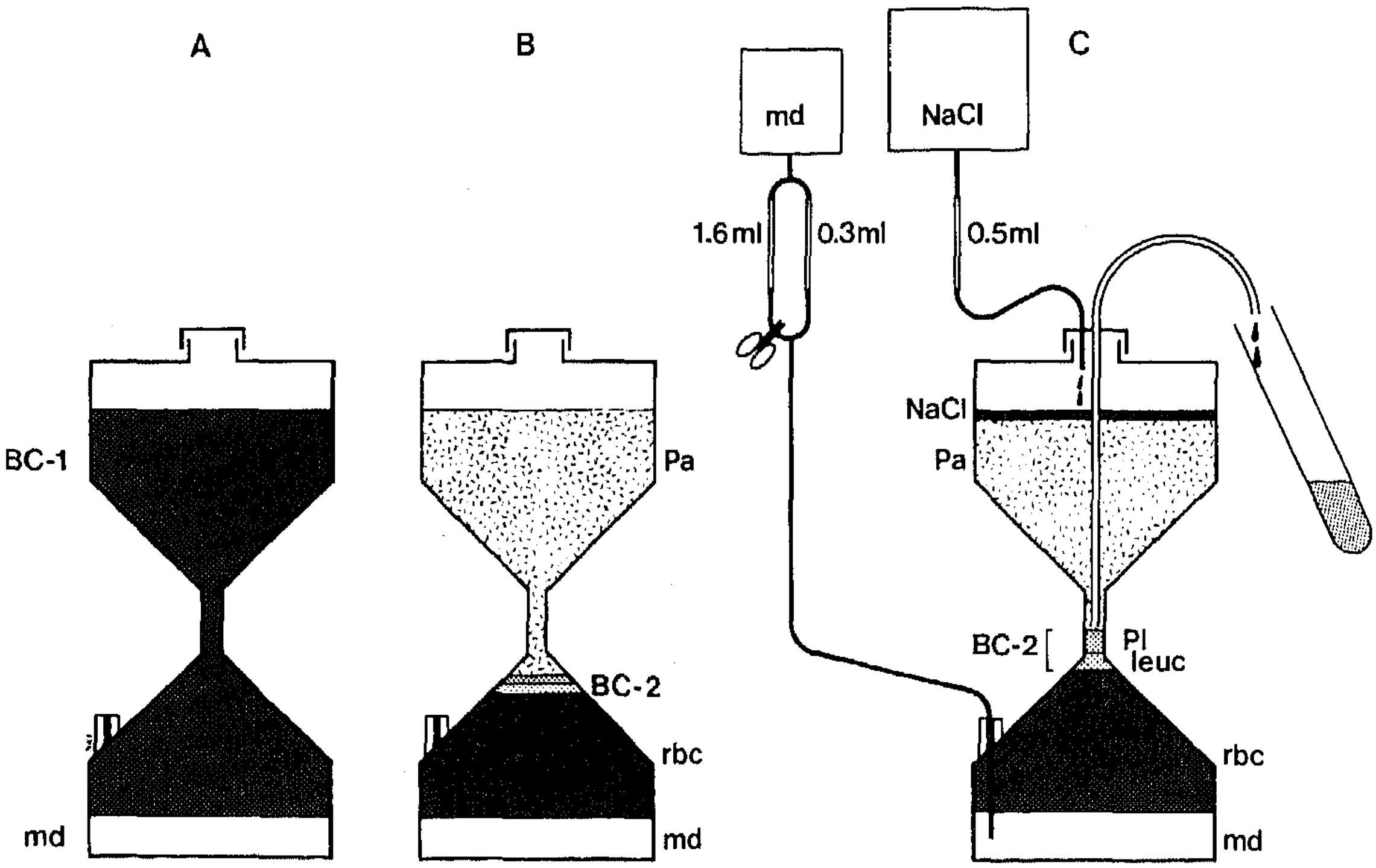


Fig. 3. Schematic representation of the separation and fractionation procedure. A: before centrifugation. The cell separator was filled with BC-1 and Maxidens (md) to adjust the volume of the lower compartment. To keep the rotor of the centrifuge in balance a second separator was filled with an equal volume of Maxidens and a sucrose solution instead of blood. B: after centrifugation. BC-2 has formed just below the connecting tube. rbc = red blood cells, Pa = plasma. C: BC-2, which consists of 2 layers, platelets (Pl) and leukocytes (leuc), fractionated by a special device as shown and simultaneous injection of Maxidens and 0.9% NaCl (see Materials and Methods). Cells were collected into tubes filled with 2.5 ml PBS (containing 1.4% bovine serum albumin and 10 U heparin/ml to facilitate accurate cell counting).

in which H = hematocrit, V = volume of BC-1 and 140 is the volume of the lower compartment that allowed BC-2 formation just below the connection tube. The cell separator was filled with Maxidens and BC-1, diluted 1 : 1 with phosphate-buffered saline (PBS) containing 15% acidic citrate dextrose (ACD) via the opening at the top (Fig. 3A). After centrifugation for 30 min, $500 \times g$, at room temperature, BC-2 formed just below the connecting tube (Fig. 3B). The BC-2 cells were collected after placing a fractionation device in the cell separator and connecting $i_2$ (Fig. 2) with a plastic bag containing 0.9% NaCl (Fig. 3C). The surface area of BC-2 was reduced by moving the BC-2 cells into the connecting tube by injection of Maxidens via $i_1$ (Fig. 2) with a flow rate of 1.6 ml/min generated by hydrostatic pressure. When the BC-2 cells arrived in the connecting tube, the flow rate of Maxidens was reduced to 0.3 ml/min and at the same time the 0.9% NaCl solution was introduced at a flow rate of 0.5 ml/min. In this way BC-2 cells and plasma were simultaneously transported through the outlet tube, and the different cell fractions were collected without disturbance of the various cell layers in BC-2. This procedure has the advantage that the plasma acts as a sheath flow stream for the BC-2 cells and thus prevents sticking of these cells to the inner side of the outlet tube.

In all, 7 fractions were collected visually. The white top layer of the BC-2 was collected in fraction 1. The pink middle layer was collected arbitrarily in 5 different fractions, and the remaining red blood cell layer was collected in fraction 7.

## Results

The results of isolation and fractionation of BC-2 cells after dilution of BC-1 cells are shown in Tables I and II. On average, $103 \pm 6\%$ of the leukocytes present in BC-1 were recovered in the 7 fractions. $75 \pm 10\%$ of the thrombocytes were recovered in fraction 1 in a volume of $2.6 \pm 0.7$ ml. This thrombocyte fraction was almost pure, the white blood cell contamination being always less than $20 \times 10^6$ cells (i.e., < 1 white blood cell per 5000 thrombocytes). $89 \pm 4\%$ of the mononuclear cells were recovered in fractions 2–5 in a total volume of $2.7 \pm 0.7$ ml. The average contamination with granulocytes of these mononuclear cell fractions was only $3 \pm 2\%$. Fraction 6 contained relatively few white blood cells, including both mononuclear cells and granulocytes. Fraction 7 contained the packed red blood cells and most of the granulocytes present in BC-1.

Separation was shown to be less efficient if BC-1 was not diluted, when the concentration of granulocytes in the fractions 2–5 doubled and the recovery of mononuclear cells was slightly decreased (Table III).

## Discussion

Red blood cells were efficiently removed from BC-1, as routinely prepared by blood banks, by the preparation of a second buffy coat (BC-2) in a specially designed cell separator. Careful fractionation of the BC-2 preparation resulted in

TABLE I

COMPOSITION OF THE FRACTIONS OBTAINED FROM BC-2

BC-1 cells were diluted 1 : 1 with PBS/ACD, centrifuged at 1500 rev/min for 30 min, and fractionated into 7 fractions. The fractions were differentiated after staining for MGG and non-specific esterase. The

| | Experiment 1 | | | | Experiment 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | WBC [a] $\times 10^6$ | Percentage | | | WBC $\times 10^6$ | Percentage | | |
| | | Ly | Mo | Gr | | Ly | Mo | Gr |
| BC-1 | 1720 | 30 | 12 | 58 | 1980 | 35 | 16 | 49 |
| Fraction 1 | <1 | 100 | 0 | 0 | 17 | 100 | – | – |
| 2 | 86 | 77 | 20 | 3 | 31 | 96 | 3 | 1 |
| 3 | 225 | 69 | 30 | 1 | 76 | 87 | 12 | 1 |
| 4 | 384 | 72 | 25 | 3 | 397 | 60 | 39 | 1 |
| 5 | 109 | 68 | 27 | 5 | 312 | 79 | 20 | 1 |
| 6 | 42 | 49 | 39 | 12 | 80 | 43 | 20 | 37 |
| 7 | 1060 | 8 | 2 | 90 | 1105 | 1 | 2 | 97 |
| % Recovery | 111 | | | | 102 | | | |

[a] WBC = white blood cells.

TABLE II

YIELD AND CONTAMINATION OF THE MONONUCLEAR CELL SUSPENSIONS FROM BC-2 OBTAINED FROM BC-1 DILUTED 1 : 1

BC-2 was prepared from diluted BC-1 (1 : 1). After centrifugation the fractions 2, 3, 4 and 5 containing predominantly mononuclear cells were recombined. Granulocyte contamination and white blood cell/red blood cell ratio was determined by cytospin centrifuge preparations after MGG staining.

| Experiment no. | White blood cells $\times 10^6$ | % Granulocytes | % Recovered mononuclear cells | White blood cell/ red blood cell ratio |
|---|---|---|---|---|
| 1 | 805 | 3 | 82 | 3.0 |
| 2 | 816 | 1 | 89 | 1.3 |
| 3 | 635 | 5 | 93 | 2.8 |
| 4 | 443 | 2 | 88 | 1.8 |
| 5 | 670 | 6 | 95 | 0.3 |
| 6 | 593 | 2 | 84 | 4.1 |
| 7 | 473 | 6 | 90 | 0.7 |
| 8 | 608 | 2 | 91 | 4.9 |
| Mean ± S.D. | | 3 ± 2 | 89 ± 4 | 2.4 ± 1.6 |

almost pure thrombocytes and mononuclear cell suspensions, with recoveries of 70–90% and 89% respectively. The rather large variation in number of white blood cells present in the buffy coats did not affect the separation. Optimal cell separation

rather large variation in the number of white blood cells that contaminated the platelets in fraction 1 was due to the fact that cuts between the various fractions were made visually.

| Experiment 3 | | | | Experiment 4 | | | | Experiment 5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WBC $\times 10^6$ | Percentage | | | WBC $\times 10^6$ | Percentage | | | WBC $\times 10^6$ | Percentage | | |
| | Ly | Mo | Gr | | Ly | Mo | Gr | | Ly | Mo | Gr |
| 1773 | 27 | 11 | 62 | 972 | 49 | 16 | 35 | 1081 | 41 | 17 | 42 |
| 19 | 69 | 25 | 6 | 10 | 89 | 11 | – | 14 | 56 | 41 | 2 |
| 16 | 76 | 19 | 5 | 74 | 73 | 27 | – | 84 | 69 | 28 | 3 |
| 206 | 56 | 38 | 6 | 110 | 79 | 20 | 1 | 184 | 69 | 25 | 6 |
| 210 | 62 | 30 | 8 | 144 | 70 | 28 | 2 | 183 | 72 | 24 | 4 |
| 203 | 68 | 27 | 5 | 115 | 75 | 22 | 3 | 257 | 76 | 15 | 9 |
| 70 | 9 | 2 | 89 | 31 | 66 | 21 | 13 | 23 | 11 | 6 | 83 |
| 1133 | – | 2 | 98 | 420 | 4 | 4 | 92 | 372 | 5 | 1 | 94 |
| 105 | | | | 93 | | | | 103 | | | |

TABLE III

YIELD AND GRANULOCYTE CONTAMINATION OF THE MONONUCLEAR CELL SUSPENSIONS FROM BC-2 OBTAINED FROM UNDILUTED BC-1

BC-2 were prepared from BC-1 that were not diluted. After centrifugation the fractions 2, 3, 4 and 5 containing predominantly mononuclear cells were recombined. The % granulocytes was determined by cytospin centrifuge preparations after MGG staining.

| Experiment no. | White blood cells $\times 10^6$ | % Granulocytes | % Recovered mononuclear cells |
|---|---|---|---|
| 1 | 730 | 5 | 97 |
| 2 | 400 | 2 | 83 |
| 3 | 569 | 11 | 81 |
| 4 | 495 | 6 | 73 |
| 5 | 650 | 6 | 89 |
| 6 | 525 | 7 | 90 |
| Mean ± S.D. | | 6 ± 3 | 86 ± 8 |

was obtained if the BC-1 were diluted before centrifugation, which may be explained by the reduction of both the density and the viscosity of the plasma. In addition, centrifugation at room temperature resulted in better separation than centrifugation at a lower temperature (10°C), which accords with observations of Freireich et al. (1965).

The cell separator was also used for preparation of buffy coats from 300 ml of whole blood. In a single centrifugation step pure thrombocyte and mononuclear cell preparations were obtained as described above (data not shown). A smaller version of the cell separator, constructed to process small volumes (50 ml) of whole blood, also allowed efficient separation of thrombocytes and mononuclear cells, although red blood cell contamination was slightly higher. These findings show that the technique allows isolation of mononuclear cells with low red blood cell contaminations from buffy coats as well as whole blood.

Furthermore, we have shown that mononuclear cells (derived from one unit of blood) isolated by this technique could be used directly for efficient separation of monocytes and lymphocytes by centrifugal elutriation (Figdor et al., 1982) whereas whole blood and buffy coats as routinely prepared by blood banks, result in overloading of the separation chamber of the elutriator rotor (Van Es and Bont, 1980). Isolation of lymphocytes and monocytes in this way has the advantage that contact between the buffy coat and body foreign substances like Percoll and Ficoll-Hypaque is avoided.

Although other techniques such as intermittent flow centrifugation (Aisner et al., 1976) and continuous flow centrifugation (Freireich et al., 1965; Judson et al., 1968; Hester et al., 1979) allow processing of larger volumes of blood, our cell separator has the advantage that the buffy coats are isolated in much smaller volumes (5–6 ml) and that the time required to prepare and carry out a separation is relatively short. Furthermore, compared to the techniques mentioned above, better cell separation was obtained, and recovery of mononuclear cells was much higher while contamination with granulocytes and red blood cells was similar or less. It may also be mentioned that buffy coats formed in the cell separator may be kept sterile since all parts of the device, and the Maxidens, are autoclavable.

This device extends the range of techniques available to separate large as well as small volumes of blood, since it allows efficient separation of platelets and mononuclear cells under sterile conditions. Further research is necessary to optimize the technique in such a way that it can be used routinely for isolation of different blood components.

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