A Simple Rapid Method for the Removal of Leukocytes From Human Blood

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Filtration of human blood cells through lamb's wool columns removed more than 96% of all leukocytes in a series of experiments, while the retention of erythrocytes by the column averaged 6.4%. This method should prove extremely useful for obtaining pure erythrocyte preparations for use in biochemical and physiological studies, and for removing leukocytes from blood prior to transfusion.

INTRODUCTION

Various types of leukocytes have been separated from one another or from erythrocytes by differential leukoadhesion on glass beads, glass wool, nylon fibers, and cotton–wool mixtures, or by methods employing sedimentation through aqueous solutions, density gradients, and solutions of high density (1–5). Many of these methods were not developed primarily to remove leukocytes from whole blood, and none is entirely satisfactory for this purpose. The methods currently in use tend either to be time consuming, to yield unpredictable results, to be relatively ineffective, or to cause considerable cell destruction.

This report describes a rapid and efficient method for the removal of leukocytes from human blood. It should be quite useful for biochemical and physiological studies which require extremely pure erythrocytes, as well as for purification of blood prior to transfusion.

MATERIALS AND METHODS

Normal human blood from different individuals was obtained by venous puncture, and clotting was inhibited by the addition of 1 mg of EDTA per ml of whole blood. An 0.9% saline solution was made by adding NaCl to distilled water; while no repeated pH measurements were made during the separation process, occasional data indicated the saline solution had a pH of approx 5. Approximately 1 ml of whole blood was washed twice with 0.9% saline solution, and then divided into

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2 equal parts. After washing, one of the resulting pellets was suspended to its original volume in its own plasma as a control. Cells were pelleted on a slide by use of the cytocentrifuge (Shandon Scientific Co., London, England), and total white and red cell counts performed after further dilution. The pellet from the other half of the sample was suspended in 10 vol of saline solution prior to addition to the column.

Unbleached lamb's wool (Scholl Mfg. Co., Chicago, Ill.), washed thoroughly with distilled water and dried, was used in all experiments. Several other commercially available bleached lamb's wool preparations proved ineffective. The lamb's wool was purchased commercially in small quantities; because of this and because of the essentially continuous manufacturing process, batch or lot numbers have no significance and are not specified. Bleaching of the fibers thus apparently alters their ability to adsorb white cells. 0.5 cm lengths of lamb's wool fibers were packed tightly into the bottom 5 cm of a column (0.8 × 15 cm) and quite loosely for an additional centimeter. The weight of lamb's wool used was 0.40–0.45 g. The column was washed with 20–30 ml of saline solution; care was taken to avoid trapping bubbles in the column during the initial wetting of the fibers or letting the level of the solution fall below the top of the fibers. The column was washed just prior to use in order to prevent the formation of small bubbles on the wool fibers.

The saline solution was allowed to run out of the column until it was only slightly above the level of the lamb's wool. The suspension of blood was then added to the column, and the flow rate adjusted to approximately 1.5 ml/min. After the cell suspension had entered the column, the red cells were eluted with 12–15 ml of additional saline. The cells in the eluent were pelleted and resuspended in plasma to their original volume prior to making cytocentrifuge preparations and determining white and red cell counts, as described above.

Although the entire process was usually performed at room temperature, it was found also to work well when carried out in the cold. In addition, sterilization of the lamb's wool (by autoclaving) was found to have no effect upon the subsequent results of the procedure.

Total leukocyte counts were made by diluting the sample in 2% acetic acid containing 0.1 mg/ml gentian violet and counting white cells in a counting chamber; red cells were diluted appropriately in saline and counted in the chamber. Cytocentrifuge preparations were fixed in absolute methanol for 5 min, dried and stained with Wright–Giemsa solution (6) prior to making differential white cell counts.

RESULTS

Table 1 shows the results of 12 separate experiments in which washed human blood cells were filtered through lamb's wool columns. In all cases greater than 92% of the leukocytes were removed from the sample by the filtration procedure, while the average leukocyte removal was 96.3%. The erythrocyte losses did not exceed 8% in any experiment; an average erythrocyte loss of 6.4% was obtained. In addition, the morphology of the red cells did not appear to be altered by the filtration procedure.

Differential white cell counts (Table 2) indicate that all types of leukocytes are removed from the blood cell preparations, and no leukocyte type appears to be preferentially removed.


**TABLE 1**

**Effect of Filtration through Lamb’s Wool on Blood Cell Counts**

| Control | Leukocytes (mm^3) | Leukocytes removed (%) | Erythrocyte loss (%) |
|---------|-------------------|------------------------|----------------------|
| 7200    | 300               | 95.8                   | 7.5                  |
| 6600    | 450               | 92.5                   | 7.9                  |
| 6250    | 125               | 98.0                   | 6.9                  |
| 6000    | 400               | 93.4                   | 8.0                  |
| 6800    | 500               | 92.7                   | 7.5                  |
| 6600    | 190               | 97.1                   | —                    |
| 5400    | 125               | 97.7                   | —                    |
| 6250    | 88                | 98.6                   | 4.1                  |
| 6350    | 160               | 97.5                   | —                    |
| 6000    | 100               | 98.4                   | 3.9                  |
| 6250    | 112               | 98.3                   | 5.3                  |
| 6700    | 225               | 96.6                   | —                    |
| Average | 6317              | 96.3                   | 6.4                  |

**TABLE 2**

**Mean Differential Leukocyte Count following Filtration through Lamb's Wool**

| Leukocyte types (%) | Monocytes | Lymphocytes | Granulocytes |
|---------------------|-----------|-------------|--------------|
| Control             | 5.7       | 30.1        | 64.2         |
| After filtration    | 7.6       | 29.9        | 62.5         |

**DISCUSSION**

Attempts to separate leukocytes from red cells using other isotonic solutions, including isotonic sodium phosphate buffer, pH 7.4 or 7.7 and mammalian Ringer’s solution, pH 7.7, were unsuccessful. The composition of the suspending medium thus appears to be extremely important for optimal separation. Once the white cells are adsorbed to the lamb’s wool, we have been unable to elute them using any of the above buffers.

This procedure has also been shown to work extremely well for removing leukocytes from mature tadpole, frog, and chicken blood, as well as from preparations of tadpole erythroblasts (7). The latter cell preparations have recently been shown to incorporate tritiated-uridine at high levels (Waldman and DeWitt, unpublished results). It thus appears to be generally applicable for the removal of leukocytes from immature and mature red blood cells in a wide variety of systems.

This technique, developed for the removal of leukocytes from human blood, may also prove useful in preparing whole blood prior to transfusions, as it is often important to remove leukocytes, especially from whole blood given to patients who have been sensitized to leukocytes by transfusions or pregnancy. Although relatively small volumes of blood were filtered in the experiments reported here, it is possible to purify larger numbers of erythrocytes by using lamb’s wool columns of greater volume.
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