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Eosinophil Cell Separation from Human Peripheral Blood

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Summary. A density separation method is described for the isolation of viable human eosinophil leucocytes from peripheral blood. The recovery rate of the eosinophils was about 52 per cent and they made up about 93 per cent in the final preparation.

INTRODUCTION

The techniques that have been described for the separation of eosinophil leucocytes from peripheral blood are time-consuming, or need special apparatus, and are unsatisfactory in terms of yield, viability and purity of the eosinophil cell preparation.

Such separations as have been attempted have been mainly from horse peripheral blood. Separation with the aid of organic solvents gave an eosinophil suspension of nearly 100 per cent purity (Behrens and Taubert, 1952; Behrens and Marti, 1954) but the cells so obtained were not viable. A counter-streaming centrifuge has been used (Lindahl and Lindahl, 1955), but preparations of only 20–50 per cent purity were obtained. Repeated partial centrifugations gave an almost pure suspension of horse eosinophils, but only after 15 centrifugations (Behrens and Marti, 1955). Using human peripheral blood Essellier and Marti (1955) obtained eosinophil preparations of 90–95 per cent by repeated washing of leucocytes obtained from the buffy coat, but efforts to repeat this method by Archer (1963) gave much reduced purity. Archer (1963) used a specially designed centrifuge tube to obtain preparations of eosinophils from horse peripheral blood with a high purity (average 80 per cent), but low recovery (average, 30 per cent) and obtained similar results using human peripheral blood.

The eosinophil leucocyte is denser than the other blood leucocytes and therefore lends itself to density separation techniques. Archer and Hirsch (1963) obtained an eosinophil preparation of 90 per cent by centrifugation of a suspension of horse peripheral blood leucocytes applied to the top of a solution of bovine albumin, density 1·11 g/ml, while centrifugation of leucocytes from human peripheral blood suspended in a solution of bovine albumin, density 1·11 g/ml gave an eosinophil preparation of only 50 per cent purity (Archer and Blackwood, 1965).

Boyüm (1968) obtained good separation of monocytes from granulocytes of human peripheral blood by centrifugation on a mixture of sodium metrizoate and dextran with a density of 1·077 g/ml. The method presented here utilizes the advantage that sodium metrizoate, obtained as a 75 per cent solution as Triosil '75' (Glaxo Limited, Greenford, Middlesex) has a high density, 1·45 g/ml, which is easily altered by addition of water. It has a low viscosity, is nontoxic, has a pH of near neutrality, 6·8, is economic and readily available, and therefore recommends itself for use in a density separation technique. This procedure allows a separation of viable suspensions of eosinophil cells of high purity and relatively high rate of recovery from human peripheral blood.

MATERIALS AND METHODS

Solutions of sodium metrizoate of the required densities were prepared by the addition of distilled water to Triosil '75' according to the formula $X = V(a-c)/(c-b)$ where X = parts of water, V = parts of Triosil '75', a = density of Triosil '75', b = density of water and c = desired density of mixture. The solutions were prepared at 4° and all density measurements were made at 4° with a hydrometer reading to four places.

Venous blood was collected from human volunteers into heparin as the anticoagulant, giving a final concentration of 10 I.U. heparin/ml of blood. The erythrocytes were separated by sedimentation at 20° with the aid of dextran, in the proportion of 5 parts blood : one part of a 4·5 per cent dextran solution.

The eosinophil separation process was carried out by centrifugation in polypropylene centrifuge-tubes with both large and small diameters, depending on the volume of leucocyte-rich plasma available after the red-cell sedimentation. The volume of sodium metrizoate used in the centrifuge-tubes for the separation technique was that which had a height of 3 cm in the relevant sized tube. The leucocyte-rich plasma was slowly applied to the top of the sodium metrizoate solutions with the aid of a pipette and centrifuged at 400 ***g*** for 40 minutes at 20°. After centrifugation the clear plasma layer, and the interface, at which most of the leucocytes remain, are pipetted off; leaving an eosinophil-rich pellet.

The few red cells which remained as a contaminant were easily eliminated by hypotonic lysis by adding 5 volumes of 0·25 per cent saline. If this was followed within a few minutes by washing with isotonic saline, the eosinophils appeared completely unaffected.

RESULTS

A trial separation of leucocytes by centrifugation on a range of sodium metrizoate solutions of different densities, 1·11 g/ml–1·15 g/ml, was caried out and the result is shown in Fig. 1. In each case 3 ml of leucocyte-rich plasma were applied to 3 ml of sodium metrizoate solution at each density in a 13 mm diameter centrifuge-tube. After centrifugation the pellet from the tube containing sodium metrizoate at a density of 1·15 g/ml consisted of 98 per cent eosinophils and 2 per cent neutrophils, and the recovery of eosinophils in the pellet from the total in the plasma before centrifugation was 50 per cent, and 23 per cent from the total eosinophils present in the blood before red cell sedimentation. The initial eosinophil count in the plasma before centrifugation was 500 out of a total white cell count of 5,550 per mm^3 i.e. about 10 per cent.

A much narrower range of densities was then tried to find out if the eosinophil recovery could be increased without too much reduction in the purity of the eosinophil-rich pellet. Fig. 2 shows a typical profile obtained in this experiment. Three ml of leucocyte-rich plasma were loaded onto 3 ml of sodium metrizoate at each density in a 13 mm diameter centrifuge-tube. By increasing the density of the sodium metrizoate solutions from 1·14 g/ml to 1·15 g/ml a high purity of eosinophils was obtained in the pellet, from 45 per cent at density 1·14 g/ml to 99 per cent at density 1·15 g/ml. However, as the purity increased so the recovery of the eosinophils decreased, from 100 per cent of the total eosinophils present in the leucocyte-rich plasma before centrifugation at density 1·14 to 50 per cent at density 1·15 g/ml.

The optimum density of sodium metrizoate for eosinophil separation was 1·148 g/ml giving a high purity, in this case consisting of 95 per cent eosinophils and 5 per cent

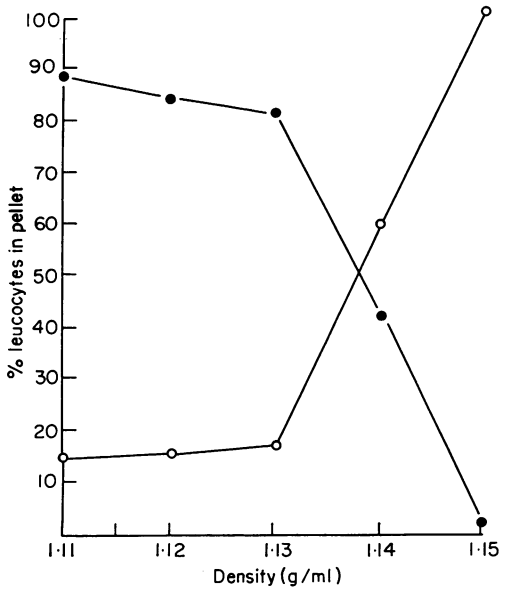

FIG. 1. Per cent of leucocytes in the pellet after centrifugation on sodium metrizoate solutions of increasing density. ●, Neutrophils; ○, eosinophils.

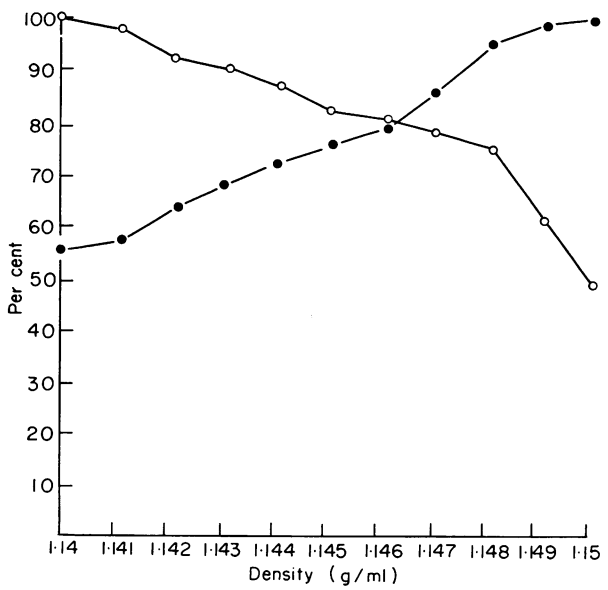

FIG. 2. Centrifugation of leucocyte-rich plasma on a narrow range of densities of sodium metrizoate; ●, Per cent eosinophils in pellet '(purity); ○, per cent recovery of eosinophils from leucocyte-rich plasma.

TABLE 1

ISOLATION OF EOSINOPHIL LEUCOCYTES FROM HUMAN PERIPHERAL BLOOD

Absolute count of eosinophils in blood of each donor (per mm^3)	Total number of eosinophils processed ($\times 10^6$)	Volume of leucocyte-rich plasma applied to Triosil (ml)	Internal diameter of centrifuge-tube (mm)	No. of eosinophils recovered ($\times 10^6$)	Per cent recovery of eosinophils from total eosinophils	Per cent purity
75	3	31	25	1·9	63	99
210	8·4	33	25	5·04	60	91
550	22	32	25	8·58	39	95
1000	40	33	25	19·2	48	89
1300	52	33	25	26·52	51	91
80	1·6	16	13	0·656	41	96
350	7	16	13	3·29	47	97
850	17	10	13	10·54	62	85
1100	22	14	13	12·76	58	93
1630	32·6	12	13	15·6	48	90
Mean					51·7	92·6

neutrophils, with a high rate of recovery, 75 per cent of the total eosinophils present in the plasma were in the pellet after centrifugation (48 per cent of the total eosinophils in the blood before red cell sedimentation).

Using a solution of sodium metrizoate of density 1·148 g/ml a number of eosinophil isolations have been carried out. Results of some typical runs are shown in Table 1. A high purity of eosinophils, averaging about 93 per cent, was regularly obtained and a reasonably high recovery was also achieved, averaging about 52 per cent of the total eosinophils present in the blood before red cell sedimentation. Neither the size of centrifuge tube used nor the initial eosinophil count made any difference to purity or yield of eosinophils. Viability, as tested with trypan blue, was satisfactory, about 1 per cent of the eosinophils were stained, and never more than 3 per cent.

The eosinophils separated by this technique appeared normal exhibiting active motility when examined by phase contrast microscopy. On fixation and staining they were not distinguishable from the eosinophils of circulating blood.

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