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A RAPID SIMPLE METHOD OF BASOPHIL PURIFICATION BY DENSITY CENTRIFUGATION ON PERCOLL

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This study describes simple and relatively rapid methods of purifying basophils from human peripheral blood. Our initial studies defined the densities of basophils in several donors and helped develop strategies for selectively concentrating basophils by isopyknic centrifugation over Percoll. We layered anticoagulated blood over Percoll of appropriate density, centrifuged these for 10 min, and collected the cells held in Percoll separately from the overlying band of mononuclear cells. Most of the basophils were distributed between densities of 1.072 and 1.078 g/ml, with a peak at approximately 1.074 to 1.076 g/ml. Basophil purity of 24.4 ± 4% (range 5.6 to 50%) was achieved in a Percoll gradient of 1.076 g/ml due to both the increased numbers of basophils and the low numbers of mononuclear cells at this gradient. The cells isolated by this method are viable, appear morphologically normal, and release histamine to various stimuli in a normal manner. A second centrifugation of the cells obtained from this density over appropriate discontinuous gradients of Percoll leads to further enhancement of basophil purity. The main limitation of this technique appears to be the relatively low yield (percent of recovery) of the basophil (≤20% for the two-step procedures).

The study of allergic phenomena at the molecular level in man has been hampered by the lack of efficient ways of purifying blood basophils. For studies with radiolabeled ligands, cell preparations of sufficient homogeneity are required. Further, the cells should undergo minimum manipulations during the procedure of purification. In 1972, Day (1) reported a purification method that satisfies the above requirements. However, other groups that attempted to reproduce Day's results have failed in their attempts (2). Recently, several groups of investigators have reported various methods for obtaining basophils of high purity. All of these methods are tedious and time-consuming. The essential feature of each of these techniques is adherence of basophils (nonspecifically to glass beads (3) or specific binding to affinity columns (2, 4)), followed by selective elution of these cells. The requirement of passive sensitization of basophils in one of the latter techniques (2) is an additional source of delay.

In this study we have attempted to develop a simple method for purifying basophils by using mild conditions so as to obtain cells that are as near their natural state as possible. During the course of these studies we have defined the densities of basophils in several donors and report procedures exploiting this knowledge for obtaining basophils ranging in purity from 20 to 80%, with yields varying from 20 to 50%.

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MATERIALS AND METHODS

Reagents. Reagents used were *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES),[2] HEPES-buffered saline with 0.3 mg/ml human serum albumin (HA), HA with 2 mM Ca^{++} and 1 mM mg^{++} (HACM), EDTA disodium ethylenediaminetetraacetate (EDTA), Hanks' balanced salt solution (HBSS; GIBCO, Grand Island, NY), and anti-IgE (Calbiochem-Behring Corporation, La Jolla, CA). Histamine-releasing activity, C5a, and Alternaria mix antigen (Greer Laboratories, Lenoir, NC) were diluted with HACM immediately before histamine-release experiments.

Preparation of histamine-releasing activity. The method of production of histamine-releasing activity has been described in detail previously (5).

Preparation of C5a. C5a used for our experiments was prepared by partial purification by Sephadex G-75 chromatography of zymosan-activated fresh human serum as described previously (6).

Histamine release from isolated basophils. Active release of histamine from the leukocytes isolated by hydroxyethyl starch (HES) sedimentation and from partially purified basophil preparation from Percoll was done by challenging the cells with the above stimuli as described before (5, 6). The histamine released in the supernatant was assayed by an automated fluorometric technique (7).

Preparation of Percoll densities. Percoll stock solution was prepared by mixing 90 ml commercial Percoll solution with 8.96 ml 10× HBSS, 0.45 ml 1 N HCl, and 1 ml 10× HEPES buffer (pH 7.6). The required densities of Percoll were prepared by using the following formula (8): Percoll density (g/ml) = (% Percoll stock solution × 0.001186) + 1.0041, where 0.001186 is a constant and 1.0041 the density of physiologic media. Because the density of Percoll is altered by temperature, it is prepared before the day of experiment and kept at room temperature overnight. The final densities of Percoll were adjusted by measuring with a hydrometer at room temperature on the day of each procedure. Discontinuous gradients of Percoll were layered using a peristaltic pump (Minipuls II, Gibson) at a rate of 30 ml/hr.

Cell counts. Basophil recovery was assessed both by absolute cell counts using alcian blue, as described by Gilbert and Ornstein (9), and in some experiments by histamine assay in the basophil-containing fractions. Differential cell counts were done on cytocentrifuge (Cytospin, Shandon Elliott) smears stained by Wright stain with an automatic stainer (Hema-Tek, Ames Corporation). At least 300 to 500 cells were counted on each smear. Total white cell (WBC) counts were also done by means of a Coulter counter.

Cell viability. Viability of cells was assessed by trypan blue exclusion and expressed as a percentage.

Statistics. A linear regression analysis of basophil counts and histamine concentrations in 40 samples of Percoll-separated basophils was determined using the "best fit" least squares method. A log-log transformation of the data was performed because of the wide range of values. The significance of the correlation coefficient was estimated by calculating the value of t by means of standard techniques.

RESULTS

Determination of basophil density. Our initial experiments with Percoll gradients of various densities suggested that basophils were less dense than neutrophils, but denser than monocytes and most of the lymphocytes. With this information, experiments were done to screen a series of volunteers to identify the precise density of basophils. In these experiments, 10 ml of blood, anticoagulated with EDTA (1 ml 0.1 M EDTA/

[2] Abbreviations used in this paper: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; HA, HEPES-buffered saline with 0.3 mg/ml human serum albumin; HACM, HA with 2 mM Ca^{++} and 1 mM Mg^{++}; EDTA, disodium ethylenediaminetetraacetate; HES, hydroxyethyl starch.

10 ml blood) were layered very carefully over 10 ml of Percoll of required density in a 50-ml conical-bottomed plastic tube. A steady rate of flow of blood was assured by applying the tip of a 10-ml plastic pipette to the side of the tube about 5 to 10 mm above the meniscus of Percoll and controlling the flow very carefully. When run in properly, the column of blood layers above Percoll, making a sharp interface. If during layering even a small amount of blood enters the Percoll, or if the pipette penetrates the Percoll layer inadvertently, the density of Percoll is affected (decreasing the density). In such cases the procedure is repeated. After layering blood over Percoll, the tubes are centrifuged using a swing-out rotor (IEC HN-S II Centrifuge, Damon/IEC Division) and manually operating the controls. This permits a gradual acceleration and deceleration of the centrifuge. Centrifugation at 600 × G for 10 min leads to a clear band of mononuclear cells at the interface between plasma and Percoll (10), and a "buffy coat" of neutrophils and eosinophils immediately above the packed RBC at the bottom. After centrifugation, the plasma layer and the mononuclear cell layer at interface are removed together (designated M), and most of the Percoll layer is removed separately (designated P). Great care is taken to remove all of the mononuclear cell layer and to avoid disturbing the neutrophils situated just beneath the Percoll layer, because admixture of even small numbers of these cells in the Percoll leads to a decrease in basophil purity due to a "dilution effect."

In our pilot experiment, we used 10 ml each of Percoll densities 1.070, 1.072, 1.074, 1.076, 1.078, and 1.080 g/ml in one donor (Fig. 1). Examination of the result indicated that most of the basophils had densities between 1.072 and 1.078 g/ml. Therefore, further screening of donors employed only densities from 1.072 to 1.078 g/ml. As shown in Figure 1, basophil purity (as assessed by differential cell counts on cytosmears) was highest at 1.076 g/ml for most donors and averaged 24.4 ± 4% (range 5.6 to 50%). Table I gives the complete differential WBC count from 12 donors of cells recovered from the Percoll layer (1.076 g/ml) and compares

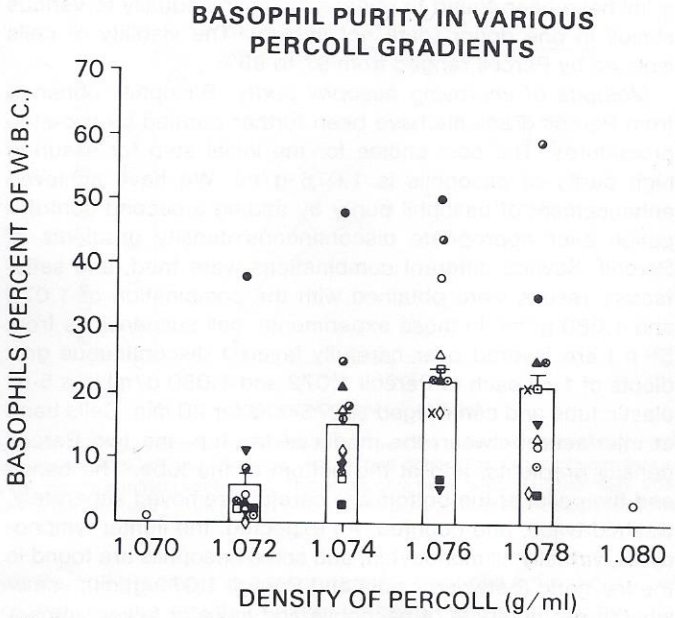

Figure 1. Ten milliliters of anticoagulated blood were carefully layered above a 10-ml gradient of Percoll 1.072 to 1.078 g/ml (except in one donor, 1.070 to 1.080 g/ml) in separate 50-ml tubes and centrifuged at 600 × G for 10 min. The cells held in the Percoll layer at the end of the centrifugation were collected and counted. This figure shows the basophil percentage (of the WBC) as assessed by cytosmears stained by Wright stain in 12 donors.

TABLE I

Comparison of differential WBC count in Percoll 1.076 g/ml with peripheral blood basophils[a]

Donor	Percoll Density 1.076 g/ml[b]					Peripheral Blood Basophils[c]	
	Neutro.	Lymph.	Mono.	Eos.	Baso.	Count (10^3/ml)	Percent
1. S.A.	14	35	6	0	50	81.4	1.5
2. D.T.	28	42	5	1	24	N.D.[d]	N.D.
3. D.P.	13	46	1	2	38	23.6	1.0
4. M.L.B.	5	70	3	0	22	N.D.	N.D.
5. C.H.	20	67	6	0	7	22.0	0.3
6. D.Z.	26	52	3	2	17	34.4	0.5
7. P.M.	53	26	4	0	17	46.9	1.2
8. P.N.	19	54	4	1	22	25.0	0.9
9. L.S.	16	55	7	0	22	59.4	1.5
10. V.R.	3	52	1	0	44	118.9	1.9
11. R.P.	1	69	8	0	22	75.1	1.1
12. C.T.	21	73	0	0.3	5.6	31.3	0.6

[a] Ten milliliters of anticoagulated blood were layered above 10 ml Percoll 1.076 g/ml and centrifuged at 600 × G for 10 min. The cells held in the Percoll layer were collected separately from the overlying mononuclear and the underlying RBC layers. The table gives the differential counts from 12 donors in the Percoll layer, and the absolute basophil count and basophil percentage from peripheral blood.

[b] Differential count was done using Wright stain on cytosmears.

[c] Counts from 1 ml of peripheral blood.

[d] N.D. = not done.

them with initial basophil percentages and absolute basophil counts. Lymphocytes were the most frequent "contaminating" cells affecting basophil purity, although in some donors neutrophils were also prominent. Lymphocytes averaged 51 ± 4.5% (range 26 to 70%), neutrophils averaged 18 ± 4% (range 1 to 53%), monocytes averaged 4.5 ± 1% (range 0 to 12%), and eosinophils averaged 0.6 ± 0.2% (range 0 to 2%). The percentage of recovery of each cell type from eight different donors in Percoll densities ranging from 1.072 to 1.078 g/ml is shown in Figure 2. Basophils are distributed between 1.072 and 1.078, with a peak at 1.076. Close scrutiny of Figure 2 permits one to conclude why, despite almost equal basophil recovery in 1.074 and 1.076 g/ml, the purity peaks at 1.076 g/ml. It is clear that at 1.074, although basophil recovery is high, lymphocytes and monocytes are present in substantial numbers, and this reduces the purity of the basophils considerably. For the same reason, one could predict that day-to-day variations in numbers (particularly of lymphocytes) could influence basophil purity in any gradient without any change in basophil number or density. This prediction is borne out by further experiments (see below). Further corroborative information on basophil density and recovery is obtained by examining both mononuclear (M) and Percoll (P) layers from the different gradients (Fig. 3*a* and 3*b*). As shown in Figure 3, basophil recovery in the Percoll layer is maximum between 1.074 and 1.076 g/ml, whereas at densities of 1.076 g/ml and above, the majority of basophils are retained at the interface between plasma and the Percoll layer (i.e., in M). Total basophil recovery combining interface and Percoll layers is maximal at 1.078 g/ml (approximately 80%).

Absolute basophil counts and routine assessment of basophil recovery were done with alcian blue (9), but in several experiments histamine content of the cell fraction was used in addition, in order to verify the validity of the former mode of assessment. For measuring histamine from peripheral blood, 0.5 ml 6% perchloric acid was added to duplicates of 0.5 ml anticoagulated blood, mixed thoroughly, and spun down, and the supernatant was assayed for histamine. From each cell fraction obtained during the experiments, 0.3-ml aliquots were treated in duplicate with 0.3 ml 6% perchloric acid and processed as above for histamine assay. The results of histamine

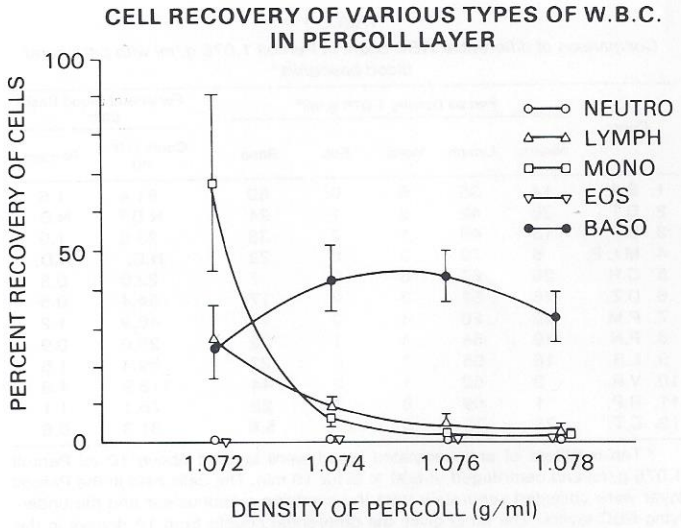

Figure 2. This figure shows the percent of recovery of all types of WBC in a Percoll gradient of 1.072 to 1.078 g/ml. The mononuclears steadily decline in densities above 1.072 g/ml; basophils are distributed widely between 1.072 and 1.078 g/ml, with a peak at approximately 1.074 to 1.076 g/ml; and neutrophils and eosinophils appear in very low numbers in this range of densities.

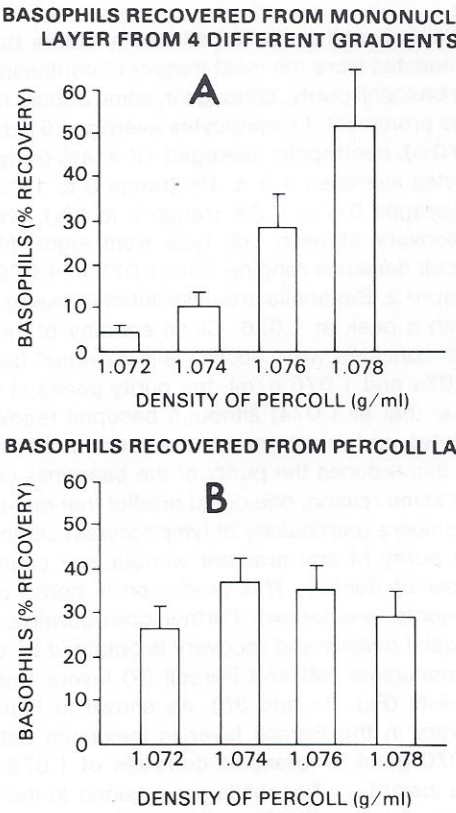

Figure 3. This figure shows the basophils recovered from the mononuclear layer (*Fig. 3a*) and the Percoll layer (*Fig. 3b*) separately at Percoll densities of 1.072 to 1.078 g/ml. A steady increase in the number of basophils appears in the mononuclear layer as the Percoll density increases whereas the number of basophils recovered from the Percoll layer peaks at approximately 1.074 to 1.076 g/ml and declines beyond 1.076 g/ml.

assay from 40 samples from four different donors are plotted in Figure 4, and this shows a statistically significant correlation (r 0.90, $p < 0.001$) with basophil counts made by the alcian blue stain method.

Morphologic and functional studies of basophils obtained by Percoll density centrifugation. Basophils obtained from the different densities of Percoll appear identical under light microscopy. We were unable to detect differences in either cell

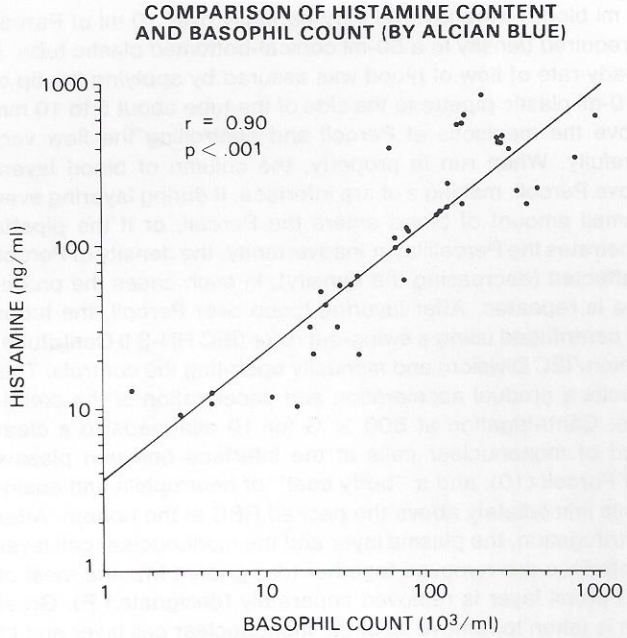

Figure 4. Basophil count as determined by the alcian blue method is plotted against histamine content assayed by an automated fluorometric method on 40 fractions from four donors. A statistically highly significant correlation is found ($p < 0.001$).

sizes or number of granules in the basophils obtained from different gradients that could explain the differences in their densities. Further, the basophils obtained by Percoll density centrifugation appeared identical to those obtained by HES sedimentation. Figure 5 shows an example of basophils isolated by Percoll density centrifugation. Studies of histamine release from basophils in response to various stimuli in several donors are given in Table II. Histamine release has been found to parallel that from basophils obtained from HES sedimentation (Fig. 6). Spontaneous release of histamine from cells isolated by Percoll density centrifugation was less than 5%. Cells isolated from Percoll densities 1.074, 1.076, and 1.078 g/ml have been found to release histamine equally to various stimuli in one donor (data not shown). The viability of cells isolated by Percoll ranged from 97 to 99%.

Methods of improving basophil purity. Basophils obtained from Percoll gradients have been further purified by two-step procedures. The best choice for the initial step for assuring high purity of basophils is 1.076 g/ml. We have achieved enhancement of basophil purity by adding a second centrifugation over appropriate discontinuous density gradients of Percoll. Several different combinations were tried, and satisfactory results were obtained with the combination of 1.072 and 1.080 g/ml. In these experiments, cell suspensions from Step I are layered over carefully layered discontinuous gradients of 1 ml each of Percoll 1.072 and 1.080 g/ml in a 5-ml plastic tube and centrifuged at 275 × G for 20 min. Cells band at interfaces between the media at the top, the two Percoll density gradients, and at the bottom of the tube. The bands and the pellet at the bottom are carefully removed separately, washed twice, and counted. As expected, the lighter lymphocytes, virtually all monocytes, and some basophils are found in the top band (between media and Percoll 1.072 g/ml); somewhat larger numbers of basophils and more or fewer lymphocytes constitute the middle band (between Percoll 1.072 and 1.080 g/ml); and large numbers of RBC, most of the neutrophils, and varying number of lymphocytes and basophils remain at the bottom of the tube (Table III). Thus, the band between

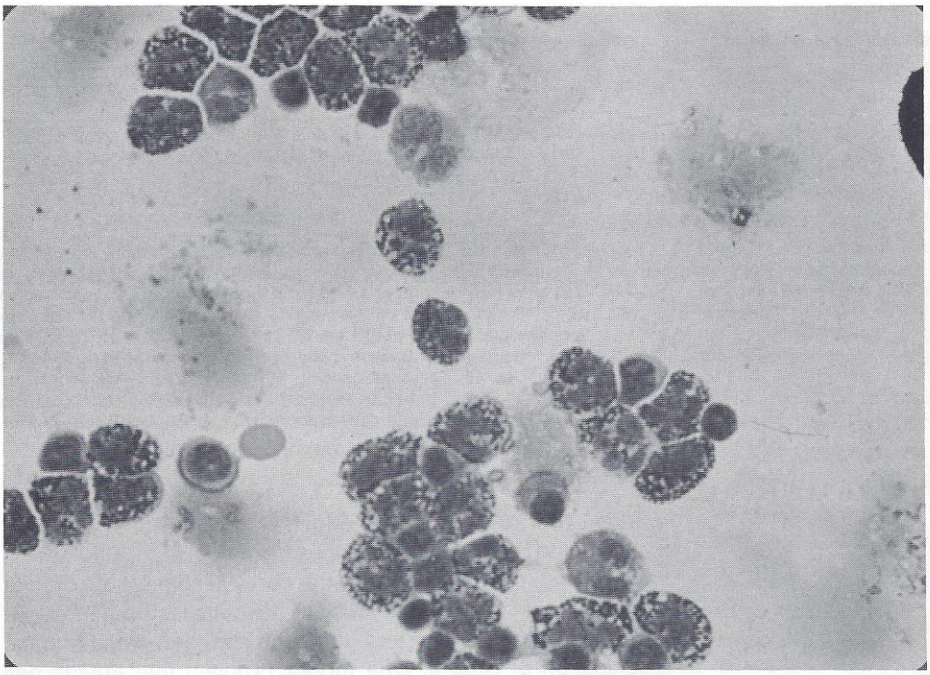

Figure 5. This photomicrograph shows basophils isolated by a two-step density centrifugation over Percoll as described in *Results* (magnification × 1000). The cells appear morphologically normal and in this donor were 64% in purity.

TABLE II

Comparison of histamine release to several stimuli in four donors[a]

Donor	Percoll Density (g/ml)	Stimulus												
		Alternaria			C5a				Anti-IgE			H.R.A.		
		10^{-5}	10^{-4}	10^{-3}	1:60	1:40	1:20	1:10	10^{-5}	10^{-4}	10^{-3}	1:8	1:4	1:2
M.L.B.	1.076	40	60	78	—	5	38	48		—			—	
R.P.	1.076		—		—	31	43	45	6	53	69		—	
C.T.	1.076		—		—	53	72	90	15	54	86	90	103	—
V.R.	2-Step[b]		—		19	30	32	—	1	13	65	—	39	113

[a] This table lists the percent of histamine release from basophils purified over Percoll in four donors (three one-step and one two-step procedures). Two stimuli were used in two donors, and three stimuli were used in the other two donors. The doses of alternaria were dilutions made from commercial extract (w/v), and the doses of the other stimuli were all dilutions made from stock solutions. The cells isolated and purified over Percoll were still able to actively release histamine in response to the various stimuli.

[b] Step I was Percoll 1.076 g/ml, and step II was discontinuous gradients of 1.072 and 1.080 g/ml.

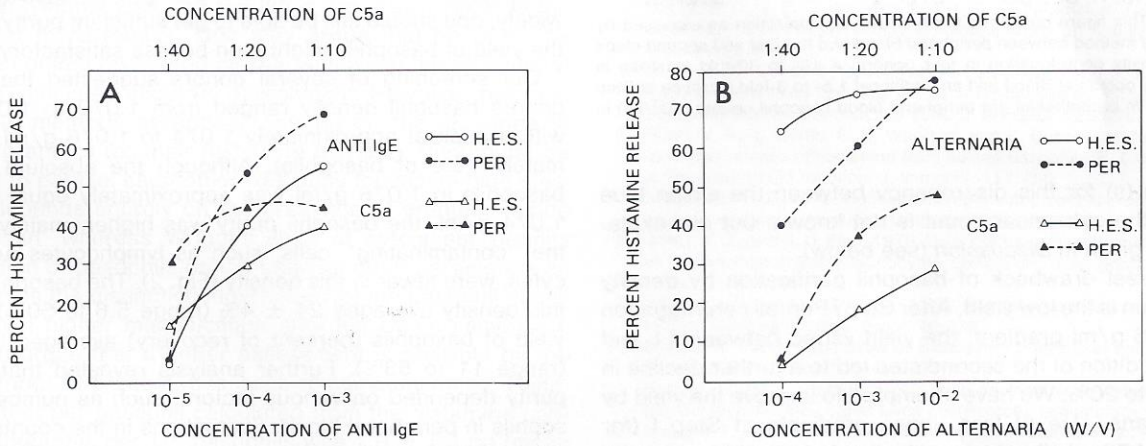

Figure 6. This figure shows histamine release (percent of total) from basophils isolated by H.E.S. compared with those purified by Percoll density centrifugation from two donors. Anti-IgE and C5a were used in one donor (*Fig. 6a*), and alternaria and C5a in the other donor (*Fig. 6b*). HES = hydroxyethyl starch-sedimented cells. PER = cells purified over Percoll. The doses of anti-IgE were dilutions made from stock commercial solution, and those of C5a were dilutions of stock solution prepared in our laboratory from human serum. Cells isolated over Percoll actively release histamine in response to the three stimuli.

the two Percoll gradients is appropriate for basophil collection. Figure 7 shows the results of experiments on four subjects by using the two-step procedure described above and basophil percentages as assessed by alcian blue staining. This shows that compared with peripheral blood, the basophil purity after Step I is 10- to 30-fold higher, and an additional 1.5- to 3-fold enhancement is obtained by the addition of the second step. It is obvious from this figure that the initial (peripheral blood) basophil percentage affects the final basophil purity greatly. On cytosmear preparation, the basophil percentages have been much higher (for example, the Step II basophil purities have been mainly around 40%, with a range from 15 to 83%).

TABLE III

Differential WBC counts from fractions of Step II discontinuous gradients[a]

WBC	Step I	Step II Fraction No.		
		1	2	3
Neutro.	18.2 ± 5	2 ± 2	31.5 ± 7	69 ± 14
Lymph.	46.3 ± 15	70 ± 7	20 ± 10	16.5 ± 11
Mono.	3.3 ± 2	11 ± 8	0.4 ± 0.4	0.1 ± 0.1
Eos.	0.3 ± 0.3	0	0.2 ± 0.2	5 ± 2
Baso.	31.8 ± 15	16 ± 7	47.5 ± 12	9.6 ± 4.5

[a] This table shows the results of two-step procedures from five donors with a Percoll density of 1.076 g/ml used for Step I and discontinuous gradients of Percoll 1.072 and 1.080 g/ml for Step II. The cells from Step I were layered above the discontinuous gradients, then centrifuged at 275 × G for 20 min, and the cells banding between all the interfaces and the pellet at the bottom of the tubes were collected separately and counted. The figures are mean ± SEM for Step I and the three fractions from Step II.

TABLE IV

Differential WBC counts from fractions of Step II discontinuous gradients[a]

WBC	Step I	Step II Fraction No.			
		1	2	3	4
Neutro.	3.0 ± 2.4	0	1.1 ± 1.2	12.3 ± 12.7	38.9 ± 26.6
Lymph.	74.6 ± 5.6	45.5 ± 7.3	92.9 ± 3.3	43.0 ± 7.3	46.5 ± 21.2
Mono.	16.1 ± 1.5	52.9 ± 8.7	0.8 ± 0.5	0.3 ± 0.1	0.3 ± 0.2
Eos.	0.1 ± 0.1	0	0	0.1 ± 0.1	0.8 ± 0.5
Baso.	2.8 ± 0.9	0.2 ± 0.1	3.8 ± 1.4	44.2 ± 5.9	12.6 ± 5.5

[a] The results of a modified two-step procedure in three donors are given in this table. Step I involved layering 10 ml anticoagulated blood above 10 ml Percoll 1.080 g/ml (two donors) or 1.076 g/ml (one donor). After centrifugation at 600 × G for 10 min, the cells in the mononuclear layer and the Percoll were collected together and layered above the discontinuous gradients of Step II. In Step II, discontinuous gradients were made using 1 ml each of Percoll 1.065, 1.072, and 1.080 g/ml. The cells banding at interfaces and pelleting at the bottom after centrifugation at 275 × G for 20 min were carefully collected and counted. The results are mean ± SEM from Step I and the four fractions from Step II.

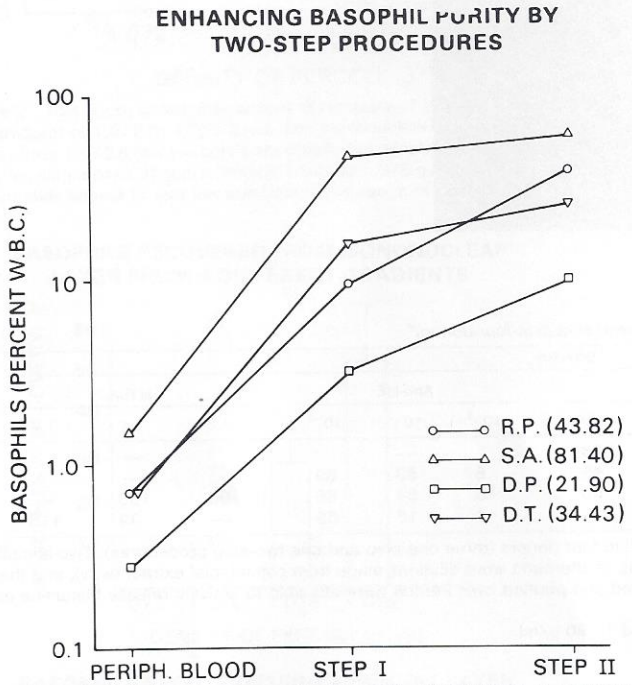

Figure 7. This figure compares the basophil concentration as assessed by the alcian blue method between peripheral blood and the first and second steps of Percoll density centrifugation in four donors. A 10- to 30-fold increase in basophil purity occurs at Step I and an additional 1.5- to 3-fold increase at Step II. The figures in parentheses are peripheral blood basophil counts (10^3/ml) in each donor.

The reason (s) for this discrepancy between the alcian blue count and the cytosmear count is not known, but our explanations are given in *Discussion* (see below).

The greatest drawback of basophil purification by density centrifugation is the low yield. After Step I Percoll centrifugation in the 1.076 g/ml gradient, the yield varied between 11 and 53%, but addition of the second step led to a further decline in yield, to 10 to 20%. We have attempted to improve the yield by several means. Use of less dense gradients at Step I (for example, 1.075 g/ml) led to a reduction in basophil purity with only a slight improvement in yield (data not given). When mononuclear and Percoll layers from either 1.076 g/ml or 1.080 g/ml were combined with the hope of taking through the second step 60 to 70% of the basophils, most basophils ended up above the 1.072 g/ml density in the second step. Thus, the yield had in fact deteriorated, and the purity was also adversely affected. It appeared that this was the result of the dense band formed between media and 1.072 g/ml Percoll by the enormous numbers of mononuclear cells, physically impairing the movement of cells downward. To remedy this, a gradient of 1.065 g/ml was added at the top (thus, with the second step now comprising three gradients, 1.065, 1.072, and 1.080 g/ml). This restored the purity (Table IV) but did not improve the yield, because some of the cells distributed above the 1.065 g/ml gradient and offset the intended benefit of this modification of Step II. One further problem that affects cell recovery during two-step Percoll gradient procedures is loss of cells during washes (approximately 30% loss); some of these losses can be reduced by keeping the volume of washes to less than 2 ml.

DISCUSSION

These studies were designed to develop a rapid method of purifying peripheral blood basophils. In doing so, we have purposely avoided using techniques that rely on selectively concentrating basophils by adhesion to surfaces nonspecifically or in affinity columns (2–4). We worked on the premise that like all other WBC (8, 10, 11), most basophils would share a relatively narrow range of density. If they did, then separating them from the other cells should be possible by isopyknic centrifugation. We also argued that if their densities varied widely, one should still be able to get sufficient purity, although the yield of basophils might then be less satisfactory.

Our screening of several donors suggested that in most donors basophil density ranged from 1.072 to 1.078 g/ml, with a peak at approximately 1.074 to 1.076 g/ml (approximately 75% of basophils). Although the absolute count of basophils in 1.076 g/ml was approximately equal to that in 1.074 g/ml, the basophil purity was higher, mainly because the "contaminating" cells, such as lymphocytes and monocytes, were fewer in this density (Fig. 2). The basophil purity in this density averaged 24 ± 4% (range 5.6 to 50%), and the yield of basophils (percent of recovery) averaged 35 ± 5% (range 11 to 53%). Further analysis revealed that basophil purity depended on various factors, such as numbers of basophils in peripheral blood, fluctuations in the counts of other types of WBC, particularly of lymphocytes and neutrophils, and certain technical factors. Atopic status did not seem to influence basophil counts; although the two subjects in our study with high basophil counts were allergic subjects, others who were also atopic had low basophil counts. The effect of leukocytosis was evident from repeat screening of two donors who incidentally were recovering from mild infections (one donor recovering from an upper respiratory tract infection and another from a urinary tract infection), whose basophil purity declined dramatically without significant reduction in basophil count or basophil recovery in the appropriate gradient. With

regard to the technical problems, if abrupt disturbance of Percoll is made while layering blood either by the pipette tip accidentally being advanced into Percoll or by a column of blood being dropped into the gradient, the basophil purity is adversely affected. Also, if while withdrawing the mononuclear layer even a small quantity is left behind, the basophil purity declines dramatically. The latter can be avoided if the Percoll layer is selectively aspirated through the side of the tube with a wide-bore needle. In spite of the technical difficulties, with a little practice the technique can be mastered and reproducible results obtained. Thus, basophil purity adequate for morphologic studies such as those using electron microscopy can be achieved with one-step Percoll density centrifugation by using a density of 1.076 g/ml in most subjects.

In selected donors, basophil purity adequate for studies at the molecular level with the use of radioligands may be obtained by a two-step procedure as outlined in *Results*. Here, selection of donors on the basis of peripheral blood basophil counts is the most fruitful initial approach. Basophil percentages of 1.5 or above seem adequate, and if such individuals are subjected to further screening by using a Percoll density of 1.076 g/ml, one could identify suitable subjects. As shown in Figure 7, such individuals can be expected to yield basophil purities above 60% by using additional density centrifugations over discontinuous gradients of 1.072 and 1.080. When such high purity is not required, the two-step procedure may still be used to avoid the initial screening of donors. For this purpose, an initial centrifugation over a Percoll density of 1.080 g/ml, removing both mononuclear and Percoll layers together, and then a Step II Percoll centrifugation over discontinuous gradients comprising 1.065, 1.072, and 1.080 g/ml may be adequate for any donor.

Finally, mention should be made of the discrepancy between assessment of basophil purity by the alcian blue method of Gilbert and Ornstein (9) and by cytosmear. Several possible explanations exist for this. It is possible that cytosmear somehow yields an abnormal picture by concentrating basophils selectively, perhaps because basophils are more adherent to glass than lymphocytes and the latter are drawn towards the filter paper more readily. One may argue that because the histamine content correlated closely with alcian blue counts of basophils, this is an accurate assessment. However, because both these methods essentially depend on the presence of basophil granules (or their histamine content), they might be expected to parallel each other. Because the alcian blue stain method depends entirely on the presence of granules taking up the stain, whereas Wright's stain of cytosmear permits identification of basophils on the basis of other parameters as well, such as the distinctive morphology of the basophil nuclei under much higher magnification afforded by oil immersion, one could argue that a better assessment is possible by the latter method. At present, this issue remains unsettled. Histamine content per basophil calculated from the histamine content in nanograms per 10^3 basophils was 1.9 ± 1.4 pg (mean ± 1 SD). Attempting to derive the histamine content per basophil from the *y* intercept in Figure 4 yields an abnormally high value of 3.6 pg. We believe this is an artifact introduced by determining a least squares line from log/log data. On final analysis, if one accepts that the measurement of histamine in the nanogram range and the per basophil content of histamine widely accepted in the literature (12) are accurate, one could conclude that the alcian blue method is more accurate than the basophil counts made from cytosmears stained by Wright's stain.

In conclusion, we have described a methodology for obtaining preparations of high basophil purity from human peripheral blood. The one great drawback is the low yield of basophils, particularly in the two-step procedures. However, if candidates are selected on the basis of high peripheral blood basophil counts, satisfactory numbers of purified basophils can be obtained for electronmicroscopic studies or for studies at the molecular level. The method is relatively rapid (less than 1 hr for one-step and 1½ to 2 hr for the two-step procedures, depending on the volume of blood handled and the number of washes), and the cells are subjected to the least manipulations.

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