THE SEPARATION OF DIFFERENT CELL CLASSES FROM LYMPHOID ORGANS

III. The Purification of Erythroid Cells by pH-Induced Density Changes

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ABSTRACT

1. Mammalian erythrocytes swell as the pH of the isotonic suspending medium is lowered, as a direct consequence of the specialized permeability properties of the erythrocyte membrane. Lymphocytes and granulocytes from a variety of sources did not exhibit this property. 2. The behaviour of mouse bone marrow erythroid cells at various stages of differentiation was studied by using a change in buoyant density with pH as an index of swelling. The ability to swell with a pH drop was acquired while the cell was still nucleated. All non-nucleated cells showed swelling. Most small erythroblasts shared this property, whereas most large erythroblasts did not. 3. The density shift with pH was used to provide a purification scheme specific for erythroid cells. The bone marrow cells were first centrifuged to equilibrium in an isotonic albumin density gradient at neutral pH. Regions of the gradient containing the erythroid cells were collected, and the cells were recovered and redistributed in an albumin gradient at acid pH. The erythroid cells showed a specific density shift which removed them from contaminants. Preparations containing 90–97% erythroblasts were obtained by this technique. 4. Differentiation within the erythroid series was accompanied by a general increase in cell buoyant density at neutral pH. This density increase may have been a discontinuous process, since erythroid cells appeared to form a number of density peaks. 5. The pH shift technique, in association with established density distribution and sedimentation velocity procedures, provides a range of cell separation techniques for biological or biochemical studies of erythroid cell differentiation in the complex cell mixtures in bone marrow or spleen.

INTRODUCTION

The complexity of the cell population in lymphoid organs has limited the cellular and biochemical studies on the differentiation of lymphoid, erythroid, and myeloid cell lines. However, a technology is now developing for the separation of different types of cells, and for the separation of cells of the one type in different stages of differentiation. Separation procedures based on the physical parameters of size and of density are available. These two techniques complement each other. Separation by density may be accomplished with precision, good resolution, and

good recovery by centrifugation to equilibrium in gradients of albumin (Shortman, 1968, 1969). A simple, readily available, and effective method of exploiting the size difference between cells is sedimentation rate separation at unit gravity in the type of chamber used by Peterson and Evans (1967) and Miller and Phillips (1969). The present paper makes two contributions which widen the range of application of these established procedures. It demonstrates that the equilibrium density gradient technique, developed for lymphocytes, can effect a substantial separation between bone marrow cells of the erythroid series according to their stage of differentiation. It also demonstrates that a specialized property of certain erythroid cells, their change of volume and density with pH, can be used to separate these cells as a class from other elements in bone marrow.

The swelling of erythrocytes with decreasing pH of the suspending medium was considered in a previous publication (Legge and Shortman, 1968). The swelling is a direct consequence of the specialized properties of the erythrocyte membrane, i.e., properties related to the respiratory function of the cell. The membrane allows equilibration of external and internal pH, and allows the passage of anions but retains cations. As the pH of the external medium is lowered, protein within the cell (primarily haemoglobin) is protonated, and anions (primarily Cl^-) enter the cell in accordance with Gibbs-Donnan equilibria. The resultant increase in osmotic pressure causes the reversible swelling of the erythrocyte and the consequent volume and density changes. This paper indicates that this specialized property is acquired during differentiation at the stage when erythroid cells are still nucleated.

MATERIALS AND METHODS

Bone Marrow Cells

The marrow cells were obtained from the femurs and tibiae of 8 week old male CBA mice; eight animals were used for each experiment. The bones were scraped free of remaining tissue, the ends were cut off, and a syringe was used to expel the marrow with 20% fetal calf serum in a buffered balanced salt solution (Shortman, 1968) at 0–4°. The plugs of marrow were dispersed by sucking up and down in a wide-bore Pasteur pipette. The following procedure was used to provide a cell preparation freed of clumps and fine cell debris. The suspension was layered over fetal calf serum in a wide tube, and was left standing for 7 min at 0–4° to allow clumps to sediment out of the suspension and through the serum. The cell layer in the upper supernatant was removed, again layered over serum, and spun in a swing-out head (400 *g*, 7 min, 0–4°) to sediment the cells. Fine debris remained in the upper supernatant, clearly separated from the cell pellet.

Other Cells

"Lymphocytes" from the mesenteric lymph node and the thymus of 8 week old male CBA mice represented the cells obtained by teasing the organs with needles into 20% fetal calf serum in buffered balanced salt solution. After filtration through a stainless steel sieve, the cell suspension was treated as for bone marrow. "Erythrocytes" represented the cells from heparinized blood after rejection of the buffy coat layer, and two washings with buffered balanced salt solution. Human polymorphonuclear leucocytes were purified from defibrinated human blood, by the procedure of Hulliger and Blazkovec (1967) to sediment erythrocytes, and then the Rabinowitz (1964) column method was used to purify the polymorphs. The polymorph preparation was 96% pure. All the cell preparations described above were sufficiently pure for the volume distribution to reflect only the predominant cell type.

Cell Counts and Cell Volume Distributions

Cell counts were performed on samples diluted in buffered, balanced salt solution, by using a Model E Coulter Counter (Coulter Electronics, Chicago, Ill.), equipped with upper and lower threshhold adjustment, and a 100 μ aperture. The aperture was calibrated with human red cells as described previously (Legge and Shortman, 1968). Nonnucleated cells were counted as cells in the range 30–90 μ^3; nucleated cells were counted as cells larger than 90 μ^3. The volume distribution profiles were obtained with the Model J particle size analyser attachment. The cells were suspended in dilute (3% albumin) samples of the same media used for density gradient analysis, the final pH being checked with a glass electrode. The distributions were obtained approximately 15 min after suspension in the medium at room temperature (~25°).

Assessment of Erythroid Cells by ^{59}Fe Uptake

Because of the subjective nature of morphological criteria, haemoglobin synthesis usually was used to identify erythrocyte precursors. This was measured as the incorporation of high levels of ^{59}Fe into the cell, as detected by radioautography. Each mouse was injected intraperitoneally with 126 μc ^{59}Fe (8 μg Fe as ferric citrate, in 0.8 ml of 1% sodium citrate, Radiochemical Centre, Amersham, Eng.), and the bone marrow was obtained 17 hr later. Samples of the

original suspension and the cell fractions from density gradient centrifugation were spun through foetal calf serum, thin smears of cells in serum were made on gelatin-coated slides, and the slides were fixed with methanol. Radioautographs were prepared with Kodak NTB2 liquid emulsion, and exposed for 5 days. After developing, the slides were lightly stained with Giemsa's.

The isotope used gave a wide scatter of grains. Only cells in the center of a group of grains at least five times background were counted as labeled. No granulocytic cells were found to be labeled when slides were counted by this procedure. Around 11% of all nucleated cells in bone marrow were counted as erythroid by this technique, compared to 17% by independent morphological criteria. Lightly labeled erythroid cells may have been excluded because of the high background scatter from heavily labeled cells. Most labeled, nonnucleated cells had the grey-blue appearance of reticulocytes, and all such labeled cells are referred to as reticulocytes in this paper. The diameter of labeled, nucleated cells in the radioautographs was measured with a calibrated eye-piece micrometer, at 500-fold magnification.

Morphological Assessment of Fractions

In the few cases where erythroid cells were identified by morphological criteria, smears were prepared of cells washed and suspended in serum. The smears were fixed with methanol and stained with Giemsa's. The usual criteria were used, after checking their validity against the radioautographic method.

Media for Density Distribution at pH 5.1 and 7.0

The media consisted of bovine plasma albumin (Fraction V powder, Armour Pharmaceutical Company, Chicago, Ill.) neutralised (pH 7.0) or non-neutralised (pH 5.1), dissolved in an unbuffered, balanced salt solution. Full details of preparation are given elsewhere (Shortman, 1968; Legge and Shortman, 1968).

Density Distribution Analysis

Full details of the procedure and control experiments are given elsewhere (Shortman, 1968). Briefly, bone marrow cells from 8 mice were dispersed in a linear gradient of albumin of the appropriate pH, and spun at 4000 *g* for 45 min at 0–4°. Fractions were collected by upward displacement, and the density of each fraction was determined. After dilution, the cells were recovered from each fraction and resuspended in a known volume. The total nucleated and the total nonnucleated cells, readily distinguished on the basis of cell volume, were counted with a Coulter Counter. These categories were then further subdivided on the basis of a differential count by the radioautographic procedure, and the total number of cells of each final class was determined. The results are expressed as density distribution profiles, which plot the total number of cells per density increment against density. Recoveries of nucleated and nonnucleated cells in the gradient were in the range 89–122%.

Total Radioactivity of Fractions

A rough but independent assessment of the distribution of erythrocyte precursors in the gradient was made by counting the total radioactivity of the cell pellet from each fraction in a sodium iodide well-crystal scintillation counter. This procedure did not account for differences in the specific activity among cells, and included in the count any soluble ^{59}Fe within the cells.

RESULTS

The Effect of pH on Cell Volume

One objective of this study was to show that the swelling of cells under isotonic conditions at low pH could be used to identify and to separate erythroid cells. It was essential to show that this effect was specific for the erythroid series, and was not obtained with lymphoid or granulocytic cells, particularly. On theoretical grounds, only erythroid cells would be expected to swell by the mechanism discussed in the Introduction. However, other forms of swelling were possible (see, for example, Legge and Shortman, 1968). Accordingly, the effect of low pH on the volume distribution of several other types of cells was checked, and the results are presented in Figs. 1 and 2.

Previous work showed that under conditions for which rat erythrocytes increased 55% in volume with a pH drop from 7.5 to 5.1, rat thymic lymphocytes showed at most a 2% swelling (Legge and Shortman, 1968). Fig. 1 demonstrates that a pH change from 6.8 to 5.1 in albumin-buffered isotonic media caused 40% swelling of mouse erythrocytes, but only 2% swelling of mouse thymic lymphocytes and only 2% swelling of mouse lymph node lymphocytes, Thus, two quite different types of lymphocytes failed to swell significantly with pH. Fig. 2 demonstrates that the same pH drop caused 29% swelling of human erythrocytes but only 3% swelling of granulocytes. The reduced swelling of human erythrocytes, compared to rat and mouse erythrocytes, has been discussed elsewhere (Legge and

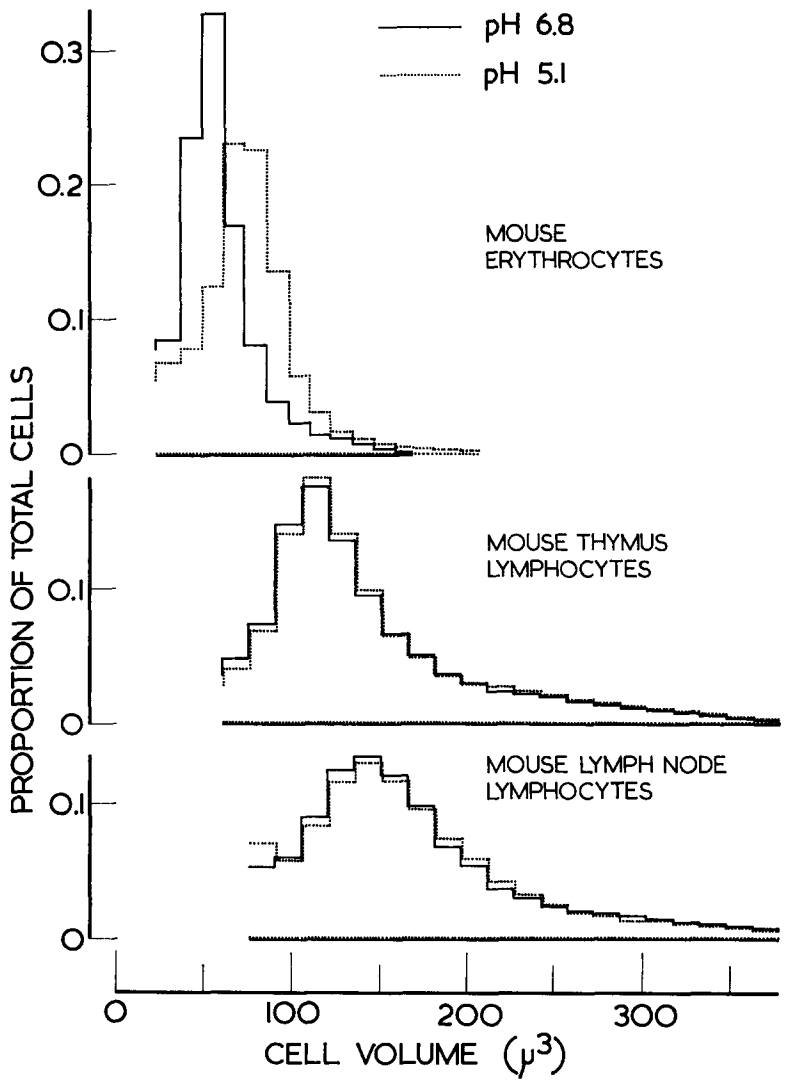

FIGURE 1 The volume distribution of mouse erythrocytes and lymphocytes at neutral and acid pH. The volume distributions were obtained with the Coulter Cell Counter. The suspension medium was an isotonic solution containing 3% albumin of the appropriate pH, dissolved in unbuffered balanced salt solution. Volume distributions were determined at room temperature, 15 min after suspension in the media. pH values were determined directly on the cell suspension after the volume distributions were performed. Further details are given in the text.

Shortman, 1968). In summary, erythrocytes from three different mammalian species increased markedly in volume as the pH was lowered. Although small increases in volume were always observed at low pH with other cell types, these increases were negligible in comparison with the effect on erythrocytes.

The Density Distribution of Bone Marrow Erythroid Cells

The previous section showed that mature erythrocytes swell in isotonic solutions when transferred from high to low pH. The possibility that more primitive erythroid cells share this

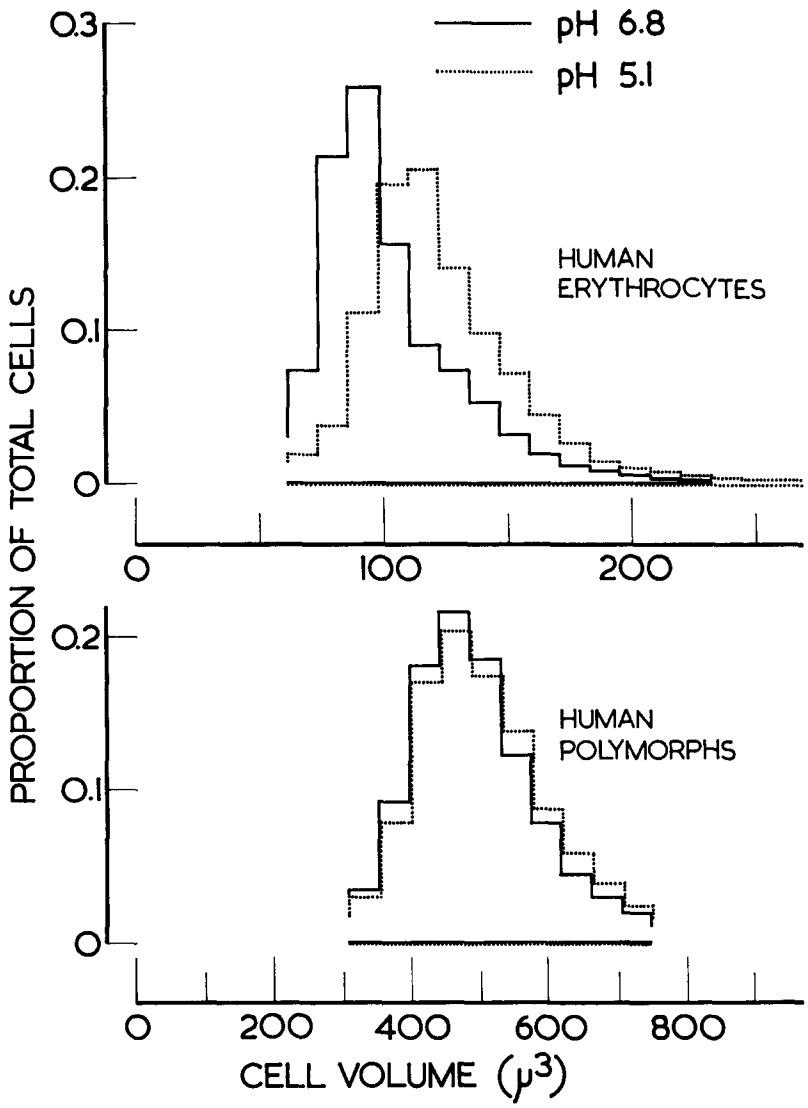

FIGURE 2 The volume distribution of human blood erythrocytes and polymorphonuclear leucocytes at neutral and acid pH. Details as for Fig. 1.

property could not be tested directly with the Coulter Counter, since pure preparations of erythroblasts were not initially available. However, swelling is accompanied by a decrease in density (Legge and Shortman, 1968), and the density distribution of cells may be obtained by separation in isotonic albumin gradients at various pH values (Shortman, 1968; Legge and Shortman, 1968). Since the cells in the individual density fractions could be counted and separated into various size classes with the Coulter Counter, and smeared preparations of the fractions allowed counting of various morphological categories as well as radioautographic assessment of cells showing haemoglobin synthesis, the density distribution of various erythroid elements could be obtained despite the presence of other cells. The properties of the erythroid cells studied are summarized in Table I. The density distribution, at two different pH values, of all nucleated and non-nucleated cells are given in Fig. 3, and of the various members of the differentiating erythroid

TABLE I

Properties of Erythroid Cells

Erythroid cell	Definition			Other terms	Cell division	Proportion of all (nucleated and nonnucleated) bone marrow cells	Average density	Average density
	Haemoglobin synthesis	Size	Nucleus					
							pH 7.0	*pH 5.1*
Large erythroblast	+	>90 μ^3 >9.8 μ diam.	+	Proerythroblasts and basophilic erythroblasts	+	0.003	1.069	1.064
Small erythroblast	++	>90 μ^3 <9.8 μ diam.	+	Polychromatophilic and orthochromatophilic erythroblasts	±	0.072	1.081	1.071
Reticulocyte	++	30–90 μ^3	−		−	0.22	1.084	1.065
Erythrocyte	−	30–90 μ^3	−		−	0.28	>1.092	1.073

series in Fig. 4. It must be emphasized that these were relatively low resolution gradients, since they covered a wide density range with relatively few fractions, and the runs at pH 7.0 were complicated by the enhanced cell-to-cell association which occurs at neutral pH (Shortman, 1968). Nevertheless a number of general points were established, both from the results at pH 7.0 alone, and by the pH shift.

The Density Distribution of Erythroid Cells at pH 7.0

The points to be noted about the density distribution at pH 7.0 were:

1. Most nonnucleated cells were much more dense than the bulk of nucleated cells (Fig. 3).

2. The bulk of haemoglobin synthesis (as assessed by the total ^{59}Fe incorporation into cells in the various fractions) was carried out by cells which, at least at the end of the 17 hr incorporation period, were denser than most nucleated cells but lighter than most nonnucleated cells. These cells were therefore substantially enriched in the intermediate region 1.078–1.089 (Fig. 3).

3. In very general terms, differentiation within the erythroid series was reflected in the pH 7.0 gradient as an increase in density (Fig. 4; Table I). Large erythroblasts, which should have included most of the dividing elements, were found in the upper regions of the gradient. The more mature, smaller erythroblasts were more dense, and after loss of the nucleus the density increase continued until the fully mature erythrocyte that had ceased haemoglobin synthesis had a density greater than the normal gradient range.

4. The density increase, although generally paralleling other parameters of differentiation, did not always follow these parameters step by step. Thus a few large erythroblasts were found with high density, and many small erythroblasts had the same density as many reticulocytes (Fig. 4).

5. The density increase with differentiation appeared to be a discontinuous process, since even the individual subcategories of erythroid cells formed density peaks, rather than a smooth profile (Fig. 4).

The Effect of Low pH on Erythroid Cell Density

The effect of a shift in pH from 7.0 to 5.1 on cell density is included in Figs. 3 and 4, as well as in Table I, from which the following main points can be drawn:

(1) The decrease in pH caused a small but definite shift in the total nucleated cell population (Fig. 3). This may have corresponded to the very small (2–3%) swelling of nucleated cells at this pH, together with more extensive swelling of the minor population of erythroblasts.

(2) A major shift in density of the cells responsible for the bulk of haemoglobin synthesis (Fig. 3) indicated that these precursors of the mature erythrocyte could swell with pH.

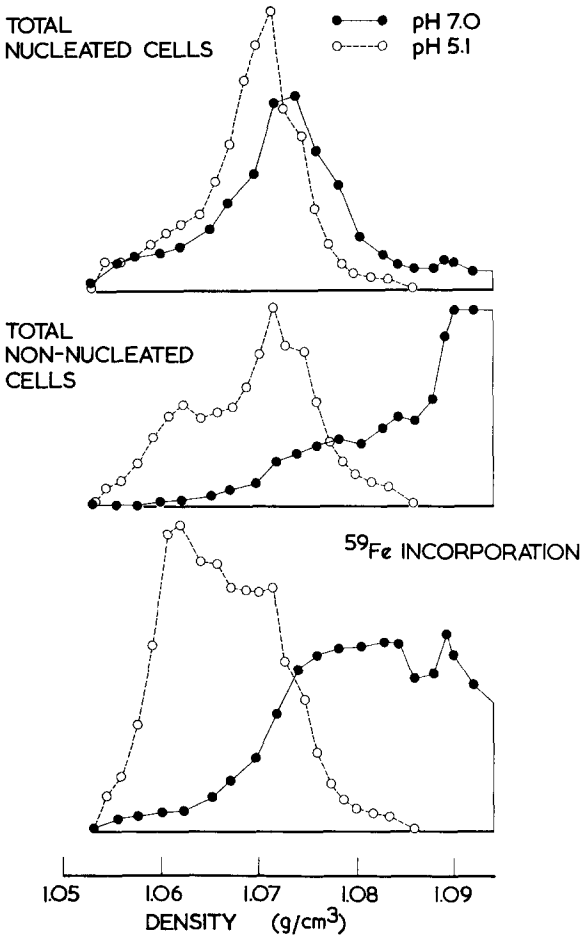

FIGURE 3 The density distribution of mouse bone marrow cells at neutral and acid pH. The curves are based on two separate density gradients, one at each pH. The number of cells (or radioactivity) per density increment is plotted against density. The peak is normalised to the same height, for all curves at neutral pH. The curves for acid pH are normalised to have the same area under the curve as the corresponding value at neutral pH. Nucleated and nonnucleated cells were counted separately, with the Coulter Counter. The total ^{59}Fe incorporation into the various fractions 17 hr after injection into the animal was used as one marker for those cells engaged in extensive haemoglobin synthesis over this period. Further details are given in the text.

(3) All nonnucleated cells, reticulocytes, and erythrocytes, showed extensive, low pH-induced swelling (Fig. 3 and 4).

(4) Of the nucleated erythroid cells, most of the smaller elements showed low pH-induced swelling, while most of the larger elements did not. For the largest erythroblasts, only the more dense peaks below density 1.075 g/cm^3 at pH 7.0 and about 10% of the total, were shifted by the pH change. Because of the overlap in the pH 7.0 and pH 5.1 profiles it was not clear whether all small elements showed a density shift with pH drop, or whether it was confined to the region below 1.075 at pH 7.0.

The Purification of Erythroid Cells

The observation that erythroid cells, including many of the nucleated elements, underwent marked density shifts when the pH changed, suggested a relatively simple and specific procedure for their purification. If a fraction containing erythroid cells was isolated from a gradient at one pH, then redistributed at another pH, the erythroid elements should shift to a region now cleared of other cells, and leave the nonerythroid cells of the original fraction near the original density position. It remained to establish that erythroid cells would withstand repeated changes of pH and the associated changes in volume, and to test if other factors such as aggregation would reduce the effectiveness of the technique. The remainder of the experimental work represents a brief assessment of this approach.

Rebanding of a Single Peak at a Different pH

In one such test experiment, bone marrow cells from non ^{59}Fe injected mice were distributed in a gradient at pH 7.0. The overall cell profile

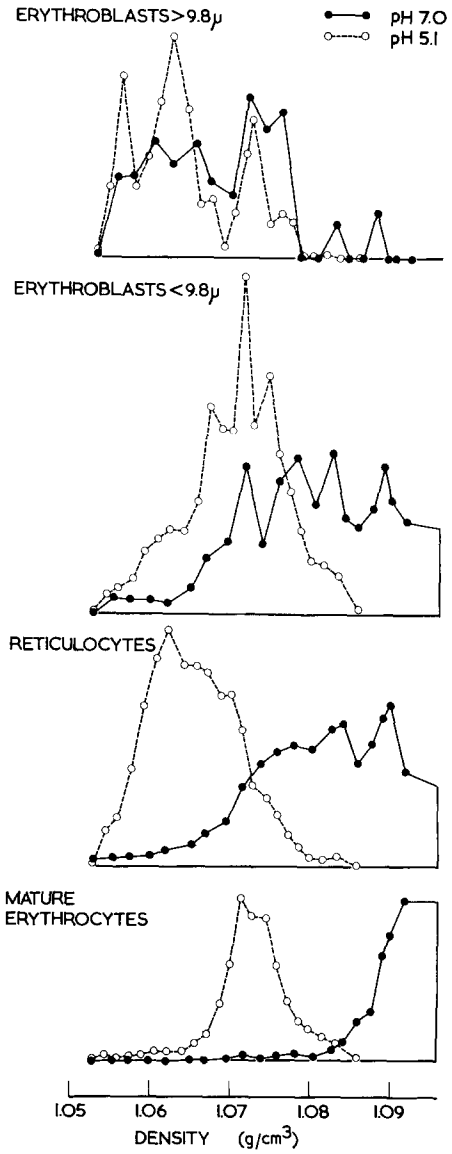

FIGURE 4 The density distribution of erythroid cells of mouse bone marrow at neutral and acid pH. The curves represent the distribution of all cells of a given type, quite independently of each other. This was obtained from density gradient runs on mouse bone marrow by total cell counts on fractions, combined with differential counts with radioautography after ^{59}Fe uptake. Definition of the cells studied, together with the absolute numbers of each in bone marrow, is given in Table I. Other details are as in Fig. 3.

was similar to the results in Figs. 3 and 4. Fractions covering the region from density 1.0806 g/cm³ to density 1.0848 g/cm³ were selected, corresponding to a major peak in the erythroblast and reticulocyte profile (Fig. 4). Cells in this region were then isolated and redistributed in a gradient at pH 5.1. Virtually all of the nonnucleated cells showed a marked density shift, rebanding in the upper region of the gradient with a calculated recovery of 103%. Nucleated cells formed two bands in the second gradient, with a recovery of 94%. The upper band (peak at 1.066 g/cm³) contained 74% erythroblasts, as opposed to 20% in the original marrow and 38% in the fraction from the first gradient. Contaminants were primarily polymorphs, and phase microscopy showed most of these to be associated with erythroblasts in clumps of 3 or more cells. The lower band (peak at 1.077 g/cm³) contained only 7% erythroblasts. The small density shift of the nonerythroid cells in this second band may have been due to any of the following: slight osmotic pressure differences between the two albumin preparations; the detrimental effects of repeated centrifugation; cell aggregation effects in the first pH 7.0 gradient; or the small swelling observed for nonnucleated cells in the Coulter Counter experiments.

A Simple Procedure for Purification of Erythroblasts

The previous rebanding experiment showed that erythroid cells could be enriched by a density shift technique, but suggested that repetitive shifts would be needed to remove contaminants carried over by association effects. The following procedure was designed to purify erythroid cells of density greater than 1.080 g/cm³ at pH 7.0, without the need for gradient generation, fraction collection, or density measurements. It could readily be adopted for any other density zone. All procedures were carried out at 4°. The steps involved were:

1. SELECTION OF CELLS > 1.080 AT PH 7.0: Bone marrow cells from 10 mice were thoroughly dispersed in 4 ml of pH 7.0 albumin medium, density 1.080 g/cm³, layered above 0.5 ml of the same medium in a centrifuge tube, and spun in a swing-out head of a refrigerated centrifuge at 4000 *g* for 5 min. The supernatant was rejected, with care to remove all nonsedimented cells.

2. SELECTION OF CELLS < 1.075 AT PH 5.1: The cell pellet from the previous step was resuspended in 4 ml of pH 5.1 albumin, density 1.075 g/cm³. 3 ml of unbuffered balanced salt

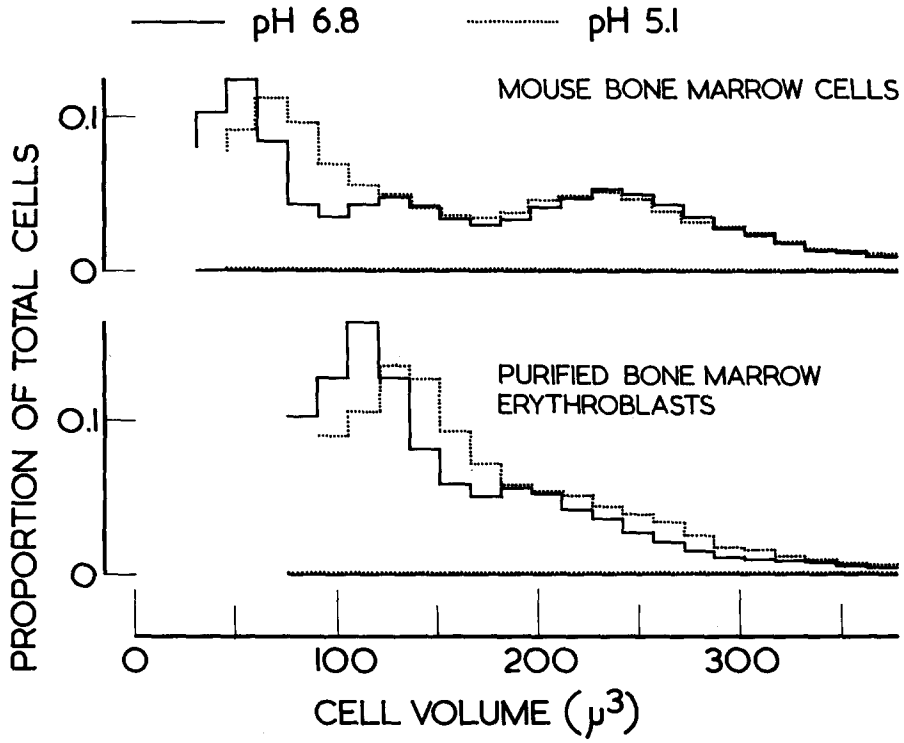

FIGURE 5 The volume distribution of purified bone marrow erythroblasts at neutral and acid pH, compared to the original bone marrow suspension. The erythroblasts were prepared by the simplified separation procedure described in the text. Other details are as in Fig. 1.

solution was layered above this suspension and the interface was stirred to produce a more diffuse zone. The tube was spun at 4000 *g* for 5 min. The supernatant was removed and retained, except for 1.0 ml above the pellet, with care to recover all cells at the upper interface.

3. RESELECTION OF CELLS > 1.080 AT PH 7.0: The supernatant from the previous step (cells, albumin, and salt solution, average density ~1.037 g/cm^3) was mixed and layered above 2 ml of pH 7.0 albumin, density 1.080 g/cm^3, and the interface was stirred to produce a more diffuse zone. The tube was spun at 4000 *g* for 5 min, and the supernatant was removed. The pellet represented the erythroblast preparation, now returned to neutral pH conditions.

The final preparations contained 20–30% nonnucleated erythroid cells. Of the nucleated cells, 90–97% were erythroblasts. Viability of the nucleated cells by the eosin exclusion technique was 90%, compared to around 80% in the original suspensions. Recovery was low, amounting to only 20% of erythroblasts of the selected density range.

pH Dependent Volume Changes of Purified Erythroblasts

The purified erythroblasts were selected on the basis of density and of density shift following a pH change. It was of interest to know the volume distribution of the final product, since there had been no direct selection on this basis. In addition, it was necessary to confirm that a pH drop would cause swelling of erythroblasts as assessed directly by a volume change.

The volume distribution of the purified erythroblasts compared to the original bone marrow suspension is given in Fig. 5. The size distribution of the original mouse bone marrow cells at neutral pH showed three peaks or zones: the low volume peak corresponding to nonnucleated cells; the next peak corresponding mainly to small erythroblasts and small lymphocytes, and the largest peak corresponding primarily to granulocytic elements. The shift in nonnucleated cell and small erythroblast volume with pH caused a reduced resolution at pH 5.1, but it was clear that no significant swelling occurred among the bulk of

the large cells. The purified erythroblast preparation showed three size zones. The smallest zone corresponded to the 30% nonnucleated cells, and is not included in Fig. 5. Two volume peaks of erythroblasts were isolated: small (110 μ^3) and medium (200 μ^3) sized. Both sizes of purified erythroblast increased in volume with the drop in pH, directly confirming the conclusions from the density shift of Fig. 4.

DISCUSSION

Density Separation

The differentiation of erythroid cells can be divided into a number of stages: (i) large, dividing erythroblasts; (ii) small, nondividing erythroblasts; (iii) the nonnucleated reticulocytes; (iv) mature erythrocytes. Hemoglobin synthesis and accumulation continues throughout this sequence, stopping at the final stage. Distribution in a density gradient at neutral pH effects partial to complete separation of these various stages from each other, as well as partial to complete separation from other types of cells in bone marrow. The increase in cell density with cell maturation had already been established for the terminal stages of this sequence in circulating blood, by Kabat and Attardi (1967), Glowacki and Millette (1965) and Leif and Vinograd (1964), but not before the present study had it been established for the more primitive elements in bone marrow. Cell density depends on the average chemical composition of the cell (including water content) and the change in density with differentiation could result from a vast number of different events. To a large extent, however, the density increase must reflect the accumulation of hemoglobin, which reaches a high concentration in the mature erythrocyte. Other factors that make major contributions to the density changes and that complicate a simple, step-by-step density increase would be the rise and fall in RNA associated with hemoglobin synthesis, and especially the loss of the relatively dense nucleus. The peaks in the density distribution profiles suggest that certain aspects of the differentiation process occur in distinct steps, rather than continuously. It would be of interest to study more closely the metabolic state of cells in the various density peaks.

Size Separation

The volume distribution curves for bone marrow cells at neutral pH illustrate the potential value of a second separation procedure based on size, to complement the density fractionation. A sedimentation rate separation, such as the technique of Peterson and Evans (1967) and Miller and Phillips (1969), should, when applied to individual density fractions, give a separation based on size alone. The Coulter Counter profiles of the erythroblast preparation, purified on the basis of density and the ability to swell with pH, showed two size classes of nucleated cells, as well as the smaller nonnucleated elements. These would all be separable by a final sedimentation step.

Separation Based on Density Changes with pH

The ability to swell and shrink with pH appears to be acquired while the erythroid cell is still nucleated. However, since many of the larger and supposedly dividing elements have not yet acquired this property, the purification technique based on a pH change is only useful for relatively mature erythroblasts. The advantage of the technique is its specificity for erythroid cells, and this is especially valuable when other cells in bone marrow (or spleen) have a size and density similar to those of erythroblasts. For example, small lymphocytes and small erythroblasts are similar in appearance and have roughly similar size and density, but the two cell lines can readily be distinguished and separated from each other by a pH shift. Used as an analytical tool, density distribution at two pH levels could help decide whether a particular biological or biochemical activity of bone marrow is associated with the erythroid component. Used as a purification technique, it could provide a markedly enriched preparation of any of the more dense (at pH 7.0) peaks of erythroid cells. In practical terms, this would require a prior study to decide which density regions at pH 7.0 to reband, since the various peaks may well be quite different in their biological and biochemical properties. It would also involve a three step procedure similar to the "simplified erythroblast purification," in order to minimise contamination by cells trapped in the "wrong" density zone by aggregation effects.

Applications

The aim of this paper is not to provide a detailed protocol for optimum separation of any given cell, but rather to give the necessary background for an approach involving three techniques. Sedimentation rate separation and equilibrium density sep-

aration are based on different parameters of a cell. Rather than choose between them, they should be used together to provide a complete analysis of the cell population. When erythroid cells are studied, the pH shift technique provides a separation procedure reflecting a specific property of the erythroid series. One additional procedure useful as a preliminary step would be the specific elimination of granulocytic and monocytic elements on the basis of their adhesion to glass surfaces, by using a modification of the Rabinowitz (1964) technique.[1] This approach would be of potential value in any biochemical or biological study of erythroid cell differentiation, such as investigation of the effects of erythropoietin, or the study of Friend and Rauscher viruses which selectively infect and stimulate erythroid cells.

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