MAJOR CHANGES IN SURFACE MEMBRANE PROTEINS DURING ERYTHROPOIESIS

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SUMMARY: Erythroid bone marrow cells of anemic rabbits were separated into 4 populations using velocity sedimentation at 1g. These populations contained cells having different degree of maturity. The surface membrane proteins of each population were iodinated via the lactoperoxidase catalyzed reaction and compared with those of circulating reticulocytes and erythrocytes. The electrophoretic pattern of reticulocytes was very similar to that of erythrocytes and showed 3 labelled protein bands. Medullary cells yielded at least 7 bands one of which corresponds to the main protein found on red blood cells. The expression of that protein appears to increase with the degree of maturity of the cells.

Erythrocyte membrane proteins have been extensively studied (see ref.1 for review). Using the lactoperoxidase-catalysed iodination, a restricted number of surface proteins has been detected on human and rat red blood cells (2-4). Since cellular differentiation is often associated with differences in surface membrane composition (5), it was of interest to determine the nature of the surface proteins of erythrocyte precursors. We thus studied blood and bone marrow cells of anemic rabbits, and the results obtained show that surface proteins indeed change in the process of red blood cell formation.

MATERIAL AND METHODS:

Cell suspensions: New Zealand albino rabbits (2-3 kg) were made anemic by phenylhydrazine injections as already described (6). Bone marrow was depleted of granulocytes and lymphocytes by a thyphoid-parathyphoid vac-

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Abbreviations: PBS: Phosphate buffered saline; SDS: Sodium dodecyl sulphate.

cine injection and collected from 2 femurs with a spatula (7). A single-cell suspension was prepared by 3-4 successive passages through a large opening plastic pipette and debries were removed by filtration through 2 layers of gauze. Cells were washed 3 times by centrifugation at 1500g for 3 min and resuspended in PBS. Viability, as tested by trypan blue exclusion, was over 90% in all experiments. Erythrocytes and reticulocytes were prepared from the blood of normal and anemic rabbits (6)

Iodination: Iodination was achieved by adding successively to 10^8 -10^9 cells contained in 1 ml PBS 0.1 unit of lactoperoxidase (10µl), 3 units of glucose oxidase (10µl), 0.5 mCi of [^{125}I] sodium iodide and 0.5 mg glucose (10µl). The reaction was allowed to proceed for 10 min at room temperature with gentle agitation and stopped by addition of 7 mg of cold sodium iodide. Cells were washed 5 times in PBS to eliminate free iodide.

Cell separation: Cells were separated by sedimentation velocity at unit gravity as described by Miller and Phillips (8) with minor modifications. The chamber had a surface of 100 cm^2 and the cell load was 20 ml at 10^7 cells per ml. Sedimentation time was 3 hrs and 10 ml fractions were collected. Cells were counted with a haemocytometer and pools were made of fractions under the peaks of the cell profile as shown in fig. 1, shaded areas. Aliquots from each pool were stained by standard haematological techniques or processed for protein fractionation.

Protein fractionation: The cells were lysed in distilled water, spun at 17000g for 10 min and the pellet was resuspended in 0.8 ml of distilled water (about 4mg protein per ml) SDS, sodium carbonate and β-mercaptoethanol were added, and electrophoresis was carried out according to the procedure of Neville (9). At the end of the run the gels were cut in 2mm slices and counted in a gamma well counter (Nuclear Chicago). Some gels were stained with Coomassie Blue for protein localization.

RESULTS AND DISCUSSION:

Bone marrow cells are separated by velocity sedimentation into 4 distinct populations as can be seen in fig. 1. Hematological analysis confirmed that the degree of differentiation of the cells decreases with increasing velocity, i.e. size. SDS - polyacrilamide gels stained with Coomassie Blue (fig. 2) show no striking differences between the stromal proteins of the various cell populations, even when compared with those of circulating erythrocytes. However, the results of the iodination of the surface proteins lead to 4 main observations: 1)

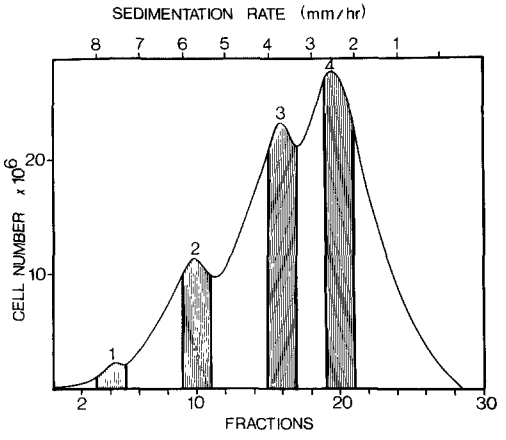

Figure 1. Sedimentation profile of anemic bone marrow cells. Radiolabelled cells in 20 ml at 10^7/ml were allowed to sediment for 3 hrs at 1g. 10 ml fractions were taken and pools were made of the fractions under the shaded areas.

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the pattern of the surface membrane proteins of erythrocytes is similar in human, rat and rabbit (fig. 3-F, ref. 2 and 4); 2) no appreciable differences in the surface membrane proteins are found between circulating reticulocytes and erythrocytes (fig. 3, E and F); 3) a minimum of 7 protein species is predominantly expressed on the surface of all medullary cells (fig. 3, A,B,C,D.) whereas only 3 are found in circulating red cells (fig. 3, E and F); 4) the main protein found on the surface of circulating cells is also present on medullary cells. Relatively to that of other proteins, its intensity also increases with the degree of maturation of the cells (fig. 3).

These results confirm and extend previous observations that cell size decreases gradually with maturation in erythroid cells (7), and that the electrophoretic pattern of the surface proteins of circulating cells is very similar in various mammalian species (2,4). The transition from the marrow to the blood seems to be a very important step in red cell membrane differentiation, and furthermore, it may be a final one

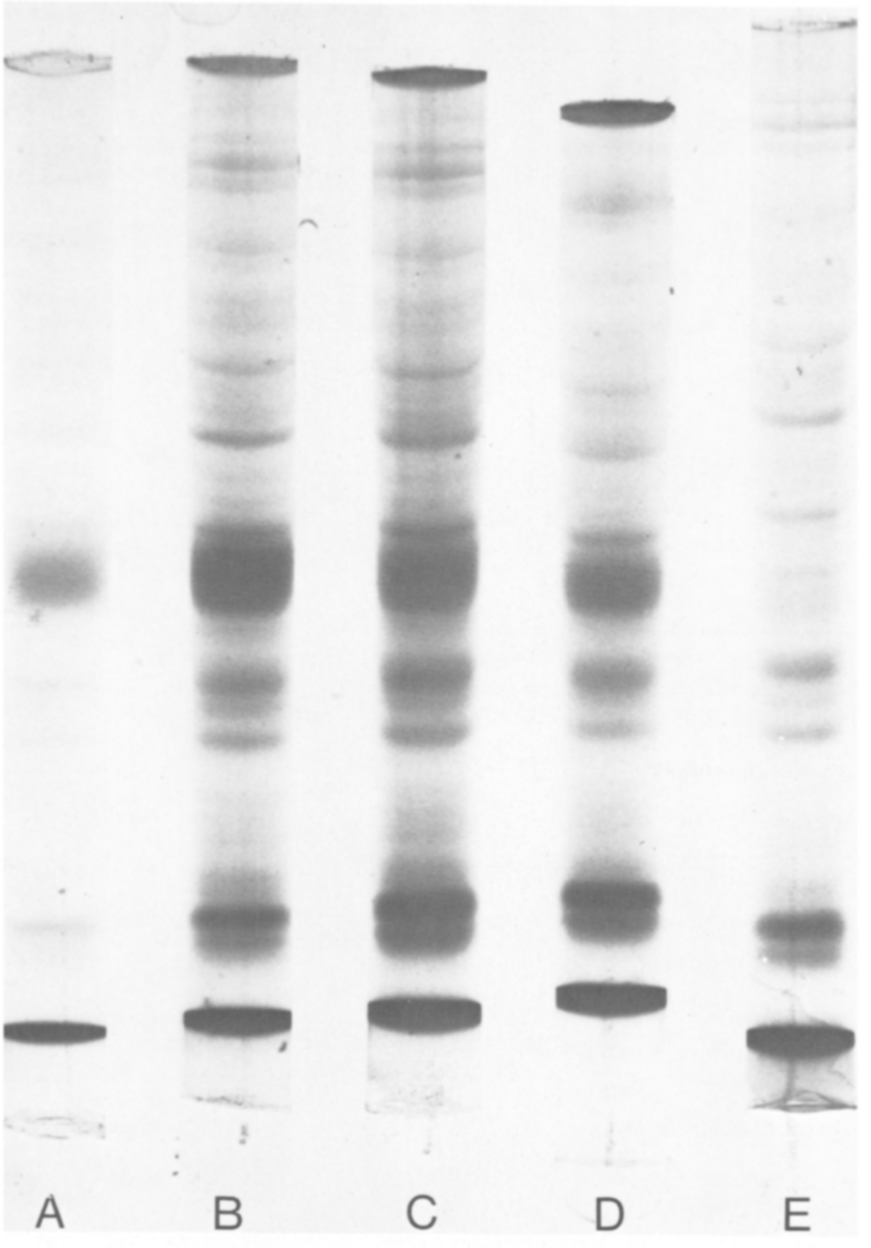

Figure 2. Coomassie Blue staining of SDS - polyacrylamide gels. Cell stroma of the pools shown in fig. 1 (A-D) and erythrocyte ghosts (E) were solubilized and subjected to electrophoresis in 7.5% SDS polyacrylamide gels.

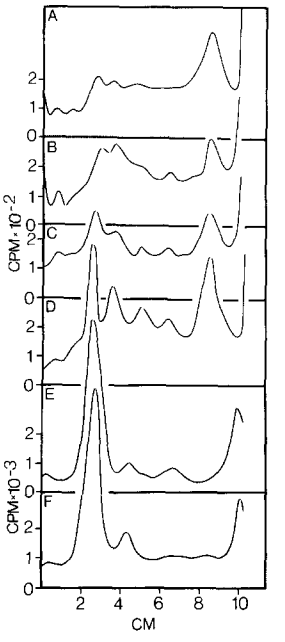

Figure 3. Radioactive profile of SDS-polyacrylamide gels of stromal proteins. Cell stroma of the pools shown in fig. 1 (A-D), reticulocytes (E) and erythrocytes (F) ghosts were solubilized and subjected to electrophoresis in 7.5% SDS-polyacrylamide gels. The gels were cut into 2mm slices and counted for radioactive iodide.

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since the degradation rate of surface proteins parallels the life span of whole red blood cells (4). Erythropoiesis is thus a useful and simple model system to study the importance of the surface membrane in the control of cell differentiation and circulation.

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