EDITORIAL

The Discocyte-Echinocyte Equilibrium of the Normal and Pathologic Red Cell

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ERIC PONDER'S careful observations of erythrocyte behavior under a variety of in vitro environmental conditions led to his descriptions of the "disc-sphere transformation," a reversible phenomenon characteristic of the normal red cell.1,2 The term "sphere" was unfortunate, because this form is actually a "crenated sphere," as Ponder himself stated in his later papers.3,4 Despite Ponder's clarification of this transformation, stated in his monographs of 1948 and 1955,5,6 many investigators and clinicians have failed to recognize this simple concept and have thus introduced a number of misconceptions regarding the specific relationships of altered red cell morphology and disease states. It is evident that the present views of the crenated erythrocyte are in a state of marked confusion. Although, generally speaking, we are opposed to the addition of new terminology, we strongly believe that the introduction of new terms to denote the two forms of the normal erythrocyte will serve to bring this concept into focus: "discocyte"—for the biconcave disc, the usual circulating form of the red cell—and "echinocyte" (Greek: ἐχῖνος, sea urchin) for the crenated, spiculed form produced by alterations in the intra- or extracellular environment (Fig. 1).

The biconcave disc form of the discocyte is dependent on a delicate equilibrium of multiple forces, both internal and external, as yet poorly defined. They are such that minor environmental alterations can shift the balance in the direction of the echinocyte. The end point of this transformation is not reached simultaneously by all the cells in the population and many intermediate forms of echinocytes appear. Under certain circumstances, the echinocyte state becomes "irreversible" so that suspension in fresh plasma fails to restore the discocyte form. The echinocyte state occurs in red cells stored for more than 14 days (at 4°C) and after addition of traces of substances as lecithin or viper venom.7,8 The molecular events accompanying these irreversible shifts of the equilibrium are unknown.

There appear to be certain disease states in which the circulating red cells assume spiculed configurations. To characterize these cells, a number of terms, including burr cells, spur cells, acanthocytoid, acanthroid, pyknocytes and helmet cells have been introduced. (See editorial by R. Silber.)9 When such cases are suspected, the first step is to insure that the spiculed forms observed are not the product of in vitro discocyte-echinocyte transformation.

The preparation of dried, stained films of erythrocytes introduces numerous
morphologic alterations. At the beginning of the smear, as drying begins, many normal cells are transformed into echinocytes. At the end of the smear, nearly all the erythrocytes, including echinocytes, are spread and flattened by surface tension.1

In vitro discocyte-echinocyte transformation in "wet" preparations can be avoided by insuring clean glass surfaces, adequate volume between slide and cover slip and immediate inspection of the specimen. Glassware must be impeccably clean, since alkaline ions from the glass and traces of grease or detergent can produce discocyte-echinocyte transformation. A.C.D. or heparin should be employed as anticoagulant in minimal concentrations Red cells suspended in native plasma must be observed immediately after venipuncture. It is imperative that a spacer be placed between slide and coverslip, so as to maintain an adequate surface:volume ratio. If the spacer is omitted, plasma substances which determine the shape of the red cells may be absorbed by the glass surfaces. Alterations in cell shape may be induced by substances used for cleaning slide and cover slip and by the nature of the glass.3

The blood should be inspected under these conditions in a "four tube" preparation consisting of: (1) patient's cells/patient's plasma, (2) patient's cells/normal plasma, (3) normal cells/patient's plasma, and (4) normal cells/normal plasma. In all of these preparations, fresh plasma (less than 4 hours old) must be employed. This simple study provides important information on the reversibility of observed echinocytes and the presence of an "echinogenic" or "anti-echinogenic factor" in the pathologic plasma and prevents errors of interpretation. Cells which were discocytes at the time the blood sample was taken may become echinocytes as the plasma ages.
Echinocyte transformation of normal red cells is invariably induced by repetitive saline washings. Restoration of the discocyte state can be achieved in vitro by suspension in normal plasma, albumin, glucose, gelatin, polyvinylpyrrolidine and fixatives such as glutaraldehyde, osmic acid, etc. This provides a useful test for evaluation of the percentage of irreversible echinocytes in a blood sample.

The discocyte-echinocyte transformation is exhibited in all pathologic forms. Three examples will be briefly described below: the sickle cell, the thalassemic poikilocyte and the acanthocyte.

When hemoglobin-S is polymerized in the discocyte, it produces the familiar sickle or holly-leaf form. When polymerization occurs in the echinocyte, it produces a characteristic “S-echinocyte.” As with the normal echinocyte, this can be best appreciated with the scanning electron microscope. Its long depth of focus and 200 Å resolution has permitted exceptional three-dimensional characterization of the surface configuration of the red cell (after aldehyde fixation and thin palladium coating) (Fig. 2). Examination of echinocytes with this instrument permits delineation of the spicules and evaluation of their length, form and surface appearance. By using this technique, striking differences between the normal echinocyte and the S-echinocyte can be seen. The latter exhibits a stellate form possessing spicules longer than those of the normal echinocyte, with truncated points and fibrillar surfaces. These observations correlate with the polymerized state of hemoglobin-S molecules in the cell interior as seen in standard electron microscopy, freeze-etch preparations and previous description of sickle cell deformation seen with the scanning electron microscope.

Aldehyde and other fixation techniques unfortunately tend to favor reversion of the echinocyte to the discocyte stage. In order to observe the reversible echinocyte, it is necessary to employ the freeze-dry technique for cell preparation.
Fig. 3.—Poikilocytes from a patient with thalassemia major in discocyte form (top) and echinocyte form (bottom).

The thalassemic poikilocyte assumes multiple forms including the ovalocyte, the “tailed,” “tear-drop,” “tennis racquet” cell, and the “cup-like” or “doughnut” form. All of these forms correspond to the discocyte state, and each produces a specific echinocyte.12 (See Fig. 3.)

We must now consider the “acanthocyte” and its relation to this equilibrium of form. It is our opinion that, at present, this term must be restricted to the red cell abnormality peculiar to abetalipoproteinemia.18 The acanthocyte is morphologically quite different from the echinocyte. In the scanning electron microscope, it displays four to eight large, smooth surface projections from an irregular central mass.19 After several saline washings, the acanthocyte may develop into a crenated form with unique spicule pattern, and can be termed “echino-acanthocyte.” This form reverts to the acanthocyte after washing with fresh plasma.

Thus, it is only when in vitro discocyte-echinocyte transformation has been carefully excluded that specific associations of altered erythrocyte morphology and clinical disease states can be made. Only then can the application of specific terms, other than “crenated red cell,” be considered. The investigator searching for biochemical alterations in the pathologic red cell and, particularly, in its membrane, as regards lipid composition, enzyme activity, cation content, etc., must always have this physiological discocyte-echinocyte equilibrium in mind.

REFERENCES

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