Present Status of Spiculed Red Cells and Their Relationship to the Discocyte-Echinocyte Transformation: A Critical Review

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A VARIETY OF RED CELLS with one or more spiny projections has been described and some of these spiculed cells have been associated with specific congenital or acquired hemolytic anemias. Of these, only sickle cells are well defined morphologically, etiologically, and pathogenically. Tear drop cells are sharply defined morphologically and have been associated most prominently with myelofibrosis, although they occur in other conditions: thalassemia and abnormal erythropoiesis, both congenital and preceding leukemia. Helmet1 and triangular2 cells of microangiopathic anemia have now been well characterized by experimental studies as being due to bisection of normal red cells by fibrin strands during intravascular coagulation or injury on diseased vessel walls or on foreign surfaces presented by intracardiac prostheses.3,4 There remains a group of spiculed red cells which have been variously designated as burr cells,2,5 spur cells,6 acanthocytes,7-9 or acanthroid cells.10 Recent observations with the scanning electron microscope11-14 have provided new insights into the interrelationship of the spiculed cells occurring in a variety of diseases and artifactual distortions to which normal and abnormal red cells are equally subject. It is the purpose of the present paper to define the recognizable cell types morphologically, to discuss their pathogenesis, to define the precautions necessary to avoid artifactual distortion of normal or abnormal cells, and to attempt to reconcile a number of apparently contradictory findings on the origin of these spiculed cells.

Scanning Electron Microscope Observations and Nomenclature

The scanning electron microscope has a resolution of 200 A, or ten times that of the light microscope. In addition, it has a large depth of focus and presents a three dimensional view of red cells. These features have made it possible to delineate two prototypes of spiculed cells clearly and unequivocally: acanthocytes and echinocytes.

The prototype of the echinocyte or crenated red cell is the normal red cell, exposed to compatible plasma incubated at 37°C for 24 hr (Figs. 1–4). Addition
to 3.5 mM oleic acid or 0.3 mM lysolecithin to fresh plasma has the same effect. We shall refer to plasma capable of crenating normal red cells as echinocytogenic. A red cell exposed to such plasma transforms within minutes into an irregularly contoured disk (stage 1), a flat cell with spicules (stage 2), and finally into an ovoid or spherical cell with 10–30 spicules evenly distributed over the surfaces (stage 3). We suggest that these cells be designated as echinocytes, or crenated cells. The common synonym, burr cell, has been used for both echinocytes and acanthocytes and should, therefore, be avoided.

The identification of three stages of echinocyte formation is occasionally useful for quantification of experiments in which crenation is induced intentionally. For example, when echinocytogenic plasma is sufficiently diluted with normal plasma, the preponderance of echinocytes will shift from stage 3 to stages 1 and 2. The transformation probably occurs without change in surface area of volume, although definite proof of identity of these parameters between biconcave disks, spheres, and spiculed cells is difficult to obtain. Echino-
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cytes produced by incubated plasma are readily reversed to discocytes by fresh plasma. When red cells are exposed to plasma with 15–30 mM oleic acid, echinocytes, which have an increased volume and progressively less prominent spicules, are produced (Figs. 5–8). These may be mistaken for spheres with the light microscope. To distinguish them from other echinocytes, we have designated them sphero-echinocytes. When the concentration of oleic acid is further increased, true spheres and finally hemolysis results.

The prototype of acanthocytes are the red cells found in congenital abetalipoproteinemia. The cells have five to ten spicules of varying length, irregularly distributed over the red cell surface. The individual spicules have knobby ends (Fig. 9). Morphologically identical cells have been found in patients with alcoholic cirrhosis accompanied by hemolytic anemia. The term spur cell has been suggested for acanthocytes of liver disease, but in the absence of a morphologic distinction, the term appears both unnecessary and confusing.

The conditions which crenate normal red cells can superimpose crenation on the primary spicules of acanthocytes (Fig. 10) and other abnormally shaped red cells, such as poikilocytes or sickle cells. These cells can then be designated as acancho-echinocytes, poikilo-echinocytes and sickle-echinocytes.

Fig. 5-8. Sphero-echinocyte and spherocytes, characterized by shortened spicules and progressing to complete spheres.
Fig. 9. Acanthocytes characterized by a small number of irregularly distributed spicules, generally with knobby ends.

Fig. 10. Acantho-echinocyte defined as an acanthocyte with secondary spicules superimposed by echinocytic factor.

Light Microscopy

Once the details of echinocyte and acanthocyte morphology as seen by the SEM are appreciated, the different types of spiculed cells can usually be distinguished in ordinary smears (Fig. 11). The typical echinocytes have a serrated outline, with small projections more or less evenly spaced over the circum-

Fig. 11. Acanthocytes and echinocytes in smear from a patient with cirrhosis and hemolytic anemia.

Fig. 12. Echinocytes and two acantho-echinocytes. Supravital preparation from a splenectomized man. Lysolecithin was added to fresh blood to transform discocytes into echinocytes and acanthocytes into acantho-echinocytes. Note contrast between regularly serrated edges of echinocytes and few irregular processes of acanthocytes some of which have developed bifurcation due to superimposed secondary spicules.
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ference of the red cells, while acanthocytes have a few spicules of varying length and thickness projecting irregularly from the red cell surface.

Wet preparations have the obvious advantage that red cells can be seen in side views, and hence the biconcave shape readily distinguished from any of the three stages of echinocytes transformation. Spherical or cup-shaped cells are also most readily distinguished from biconcave disks in wet preparations.

Particular difficulties arise when both echinocytes and acanthocytes are present in a preparation. It may then be helpful to add plasma containing lysolecithin deliberately and to examine wet preparations with the phase microscope. The acanthocytes are transformed into acantho-echinocytes and stand out prominently because all other cells have been transformed into echinocytes (Fig. 12). Some acantho-echinocytes may be seen to have bifurcation of their spicules, as would be predicted from the SEM images.

The greatest difficulties are encountered during experiments in which red cells are exposed to plasma from patients with acanthocytes. Any stored plasmas may acquire the capacity to crenate fresh red cells, and this effect may then be mistaken for an inherent capacity of the patient’s plasma to induce echinocytes or acanthocytes. To avoid such fallacies, a detailed knowledge of the variety of conditions which can induce echinocyte transformation becomes crucial.

Discocyte-Echinocyte Transformation in Physiologic Saline and the “Glass Effect”

Ponder provided extensive studies and detailed descriptions of the phenomenon of crenation as preceding sphering and lysis, yet few investigators have appreciated that crenation can take place without any change in tonicity.* Washing in saline probably acts by removing a protective coating of plasma from the red cells. This is suggested by the fact that three washes are needed to crenate 100% of cells. After two washes, when certainly less than 5% of plasma remains, only 1%–10% of red cells are crenated. In contrast, for subsequent reversal of echinocytes to discocytes, addition of 10%–20% fresh plasma to saline is necessary. This would be expected if the maintenance of biconcave shape depended on a thin plasma layer on the red cell surface which could only be reconstituted by a relatively high percentage of plasma in saline.

As pointed out by Furchgott, the findings just described depend entirely on the observation of red cells between glass and cover slip. If thrice washed red cells in saline or Hank’s solution are observed between two plastic cover slips, the red cells are seen to have maintained their biconcave shape. Apparently close contact with glass or a high ratio of glass to red cell surfaces is needed to produce crenation of thrice washed cells. Exposure to the glass in a test tube is insufficient, as can be verified by observation of the red cells through the wall using an objective with long working distance. Only when

*Discocytes exposed to hypertonic solutions become flattened discocytes (leptocytes), echinocytes become shrunken echinocytes. In both cases the volume is decreased. In hypotonic solution, discocytes become cup-shaped, before they become swollen spherocytes. Echinocytes in hypotonic solutions become spher-o-echinocytes and spheres.
cells are washed 12 or more times will crenation result even in a test tube or between plastic surfaces and then only in part of the red cell population.

Although fresh plasma protects fresh red cells from becoming echinocytes between glass and cover slips, echinocytes will frequently form at the edge or in thin portions of supravital preparation after a few minutes. In some plasmas the cells will remain discocytes in the test tube, but become echinocytes between glass and cover slips. The importance of these observations lies in the realization that we may alter the appearance of red cells by even minimum preparation for observation or, so to speak, in the very process of observing them.

**Discocyte-Echinocyte Transformation in Plasma**

The simplest way of producing echinocytogenic plasma is to incubate normal plasmas at 37°C for 24 hr. The echinocytogenic factor can be quantitated by dilution with normal plasma.25 The mechanism by which the plasma acquires the echinocytogenic factor is enzymatic. Heating of fresh plasma to 56°C for 30 min or addition of an enzyme inhibitor, para-hydroxymercuribenzoate, destroys the ability of plasma to become echinocytogenic on subsequent incubation at 37°C for 24 hr. Heating to 56°C of plasma after it has become echinocytogenic does not destroy the echinocytogenic factor.2627

Addition of oleic, other fatty acids or lysolecithin makes normal fresh plasma echinocytogenic. We have suggested but not proven that the echinocytogenic factor in incubated plasma might be lysolecithin26 and the enzyme involved in its production lecithin-cholesterol-acyl transferase which is known to esterify cholesterol at a slow rate and to produce lysolecithin.28 The fatty acid content of plasma is not changed significantly by incubation at 37°C for 24 hr.

Plasma containing large amounts of lipoproteinlipase (LPL) can become echinocytogenic on brief incubation in vitro. De Vries29 studied the conditions under which this occurs. He produced alimentary hyperlipemia in volunteers who then received an injection of heparin known to release LPL. Blood was drawn within 15 min and incubated at 37°C. The continued activity of LPL, i.e., the production of fatty acids from chylomicrons was evident from continued clearing of the plasma. Free fatty acids reached levels of 1.6 to 2.0 mEq in approximately 30 min and crenation of red cells supervened. Crenation of red cells is not, however, routinely observed in vivo because fatty acids clear rapidly and only accumulate on subsequent incubation of blood in vitro. Possible exceptions to this rule have recently been observed by Ulliot30 in surgical patients on cardiac bypass, possibly because low levels of albumen and simultaneous cooling slowed clearance of free fatty acids.

**Discocyte-Echinocyte Transformation in Aged Red Cells**

It has long been known that red cells lose their ability to sediment normally in banked blood. It is less generally appreciated that this is due to failure to form rouleaux secondary to transformation of discocytes to echinocytes. Echinocytes form within 3 to 4 days in whole blood kept at 4°C,31 and within 24 hr in blood incubated at 37°C. However, while the supernatant plasma
SPICULED RED CELLS becomes echinocytogenic after 24 hr at 37°C, plasma separated from blood kept at 4°C for days or even weeks only rarely becomes echinocytogenic. Consequently, it appears that aging of red cells promotes echinocyte transformation quite apart from any plasma changes. It may be useful to think of the shape changes produced by incubated or altered plasma as due to extrinsic factors, and of the aging effect as due to intrinsic factors, possibly reduced adenosine triphosphate.32-35

Reversibility of Echinocytes

Echinocytes produced by saline washes or by echinocytogenic plasmas are readily reversed to discocytes by resuspension in fresh normal plasma. Albumen in buffered saline can be substituted for normal plasma. Other compounds and conditions which revert echinocytes are phenergan,36,37 polyvinyl pyrrolidone, gelatine, a host of other compounds, and low pH. According to Deuticke,38 crenated cells produced by a number of anions and cup shapes produced by certain cations are interconvertible, with appropriate mixtures restoring the disc shape.

Echinocytes produced by aging of red cells are not usually reversible by fresh plasma, but may be reversible on incubation with adenosine which allows regeneration of ATP.32-35 Echinocytes present in banked blood may become irreversible in fresh plasma, yet appear to revert to discocytes in the circulation. This may be inferred because echinocytes are not seen in the circulation of recipients even when their percentage is high in the transfused blood and the bulk of transfused red cells is known to circulate in the recipient.

Acanthocytes and Echinocytes In Vivo

The conditions in which acanthocytes have been studied most extensively are congenital abetalipoproteinemia and liver cirrhosis with hemolytic anemia.7-10,14-20 In addition, they have been found in a familial disorder with neuronal degeneration, but without abetalipoproteinemia,39 in severe neonatal liver disease with hemolytic anemia,40,41 and in patients with no recognizable hematologic disorder, in whom the spleen had been removed for traumatic rupture or incidental to kidney transplantation.42 The percentage of acanthocytes is high in abetalipoproteinemia, very low in splenectomy, and variable in cirrhosis.

Although it has been reported that acanthocytes have a reduced filtrability in vitro19 which may suggest that they are removed by the spleen in vivo, hemolytic anemia is not associated with acanthocytosis in abetalipoproteinemia. Moreover, when normal cells are transfused into patients with cirrhosis and hemolytic anemia, they have a shortened survival, although they do not always transform into acanthocytes.20 Thus it appears that the bizarre shape of acanthocytes does not necessarily interfere with their normal survival and function.

The condition under which echinocytes occur in vivo are less well defined. "Burr" cells have been found in uremia, bleeding peptic ulcer, carcinoma of the stomach, and heart disease.5 The original report depicts a variety of spic-
uled cells some of which resemble those later identified in microangiopathic anemia. Since then, burr cells have been equated with acanthocytes by some and with echinocytes by others. The occurrence of echinocytes in smears of patients with uremia has been reasserted recently. A critical examination of the work is indicated, particularly since it had not been fully appreciated how commonly crenation can be induced after withdrawal of the blood. Dacie warned about artificial crenation in blood smears, but made no recommendation how to avoid them. Present knowledge of echinocyte formation suggests that fresh blood should be examined immediately between plastic cover slips to give adequate assurance that the crenated cells were already present in the circulation. Examination of stained smears is less reliable, even when they are prepared immediately from free flowing blood. We have, on occasion, observed crenated cells in half a dozen cover slip preparations while none were present in others prepared at the same time and ostensibly handled in the same fashion.

Schwartz and Motto found them occasionally in only part of a smear. This is not to suggest that echinocytes do not occur in vivo. In addition to uremia, echinocytes have been shown to occur in pyruvate kinase deficiency and phosphoglycerate kinase deficiency. In general they are irreversible, except for the heparinized patients on cardiac bypass recently studied by Ullyot in whom in vivo crenation was almost certainly due to high fatty acids and reversible by fresh plasma.

Pathogenesis of Spiculed Cells

Because abetalipoproteinemia and liver disease are clearly associated with plasma abnormalities, it was tempting to speculate that acanthocytes may be the result of altered plasma constituents. Normal red cells transfused into patients with cirrhosis become acanthocytes after several days in the recipients circulation. Evidence has been presented that acanthocytes in both abetalipoproteinemia and cirrhosis contain an excess of cholesterol, presumably reflecting an increased surface area of acanthocytes. Moreover, as recently reviewed by Keller et al., it has been claimed by some, though denied by others, that the plasma of patients with cirrhosis and acanthocytes can induce transformation of normal red cells to acanthocytes on incubation for 24 hr. However, according to the published sequential pictures echinocytes rather than acanthocytes are present at 1 hr, which develop progressively more pronounced spicules after 24 hr of observation. This is in keeping with prior observations of Grahn et al. that plasma of about 50% of cirrhotic patients can transform normal red cells into echinocytes on incubation for 15 min, even though the patients have neither hemolytic anemia nor acanthocytes or echinocytes in their own circulation. Again, critical repetition of these experiments in the light of newer knowledge of echinocyte and acanthocyte morphology appears indicated.

We are not aware of any attempts to elucidate the pathogenesis of echinocytes in vivo. Nevertheless, some speculative inferences on the biochemical and structural membrane changes connected with red cell crenation may be permissible. Crenation of red cells in incubated plasma was found associated
with a significant increase in lysolecithin and labeled membrane fatty acids, although the plasma fatty acid concentration was normal.\textsuperscript{53} It is known that plasma fatty acids and plasma lysolecithin equilibrate with the red cell membrane within minutes.\textsuperscript{48} Albumen is known to have receptor sites for both fatty acids and lysolecithin and reversibility of echinocytes with albumen is well documented. One may thus speculate that reversible crenation of the red cell can be brought about by accumulation of fatty acid or lysolecithin or both due to a number of different plasma changes or alterations in red cell metabolism.\textsuperscript{53,54} In contrast, aging of red cells is known to be associated with loss of ATP and the crenation of aged red cells is not reversible by fresh plasma. Preliminary experiments in our laboratory suggest that aged crenated red cells have a slightly increased volume. Incubation with adenosine sometimes restores the ATP and discoid shape.\textsuperscript{33-35} The crenated cells of pyruvate kinase and phosphoglycerate kinase deficiency may also be crenated because of reduced levels of cellular ATP.\textsuperscript{49,50} There is evidence that the loss of ATP and increased calcium\textsuperscript{34} are associated with a stiffening of the membrane.

The crenation of red cells due to saline washings and the glass effect may reflect yet another aspect of the constitution of the red cell membrane. Bessis and Prenant\textsuperscript{51} have recently confirmed earlier observations made by Furchgott.\textsuperscript{24} They have found that a red cell in a microdrop of Hank's solution, which in turn is surrounded by silicone oil, will crenate on approach of a glass pipette and revert to a disk on withdrawal of the pipette. The individual spicules reappeared again and again on identical sites of the red cell surface during as many as 40 consecutive cycles of crenation and reversal. The experiments do not preclude the possibility that the spicules appear at random sites on the first occasion and that a spicule once formed leaves on reversal a locus of increased vulnerability at which spicules subsequently reform. It seems more likely, however, that there is some structural differentiation of the red cell membrane which accounts for the occurrence of spicules as a result of the glass effect, once the protective protein coating has been removed.

Although the concepts presented here are speculative, they suggest that studies of the discocyte-echinocyte equilibrium may be of value in building models of the structure of the red cell membrane.

**SUMMARY**

The literature on spiculed red cells contains a redundant nomenclature and contradictory claims on the pathogenesis of the abnormal red cells. A resolution of these difficulties requires knowledge of the many conditions that induce crenation in normal and abnormal red cells because these artifacts have frequently been confused with spiculed cells in the patient's circulation.

The biconcave red cells (discocytes) can be transformed into crenated red cells (echinocytes) (1) by extrinsic factors (plasma incubated at 37°C for 24 hr, lysolecithin, high levels of fatty acid or physiologic levels of fatty acid in the presence of lysolecithin and many others); (2) by intrinsic factors, such as aging of red cells, which are probably related to depressed ATP; and (3)
by washing in saline and the “glass effect” of observing cells between slide and cover slip. The extrinsically induced discocyte-echinocyte transformation is generally reversible by washing in fresh plasma, the intrinsically induced transformation is not. The discocyte-echinocyte transformation due to glass contact is prevented by observation between plastic cover slips. Echinocytes probably occur in various diseases, but such claims must be reevaluated because examination of fresh cells between plastic cover slips is necessary to exclude artifactual crenation during preparation of smears.

Sphero-echinocytes and spherocytes may develop with higher concentration of echinocytogenic agents. The relationship of these cells to echinocytes, on one hand, and prelytic spheres, on the other, needs further clarification.

The spiculed cells in the circulation of certain patients with liver disease are indistinguishable from the acanthocytes of abetalipoproteinemia. Acanthocytes can develop crenation superimposed on their own spicules and become acantho-echinocytes. It is suggested that the term burr cell for echinocyte and spur cells for the acanthocytes of liver disease be abandoned because they are redundant and do not allow for designation of the mixed forms of acantho-echinocytes which are of diagnostic importance.

Speculations are presented on the pathogenesis of echinocyte formation and their importance for an understanding of the structure of the red cell membrane.

REFERENCES

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