MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

13 September 1973

RED CELL MEMBRANE ALTERATIONS AS DIAGNOSTIC AND PATHOPHYSIOLOGIC CLUES

Case I. "Hyposplenism"

During a routine evaluation by her family internist this 58 yr old WF was noted to have abnormal red cells on her peripheral smear characterized by anisocytosis with marked variation in shape (poikilocytosis), targeting, Howell-Jolly bodies and spiculated red cells.

On evaluation her clinical history was unremarkable. Of interest was a long history of dutiful attendance at the Duke University Clinic under guidance of Dr. Klempner with the usual rice-fruit diet for "possible impending diabetes with a 2 hr p.p. of 105 mgm%"! Of interest is the absence of previous data from that clinic regarding her blood counts or the character of her peripheral red cells!! She had been in a minor auto accident 2 yrs earlier but denied significant abdominal injury.

Physical examination was completely within normal limits. Pertinent laboratory data revealed a hemoglobin of 13.6 gms%, hematocrit of 42 vol% and a RBC of 4.1 million. Her WBC was 8,050 with a normal differential and platelets were 332,000/mm^3. The peripheral smear revealed poikilocytosis characterized by target cells, spiculated red cells and Howell-Jolly bodies in the red cells.

On the basis of her peripheral smear a liver-spleen scan with multiple imaging positions and multiple scanning agents was done. The asplenic status was corroborated. (All other laboratory data was within normal limits.)

Characteristics of the Hyposplenic State were first reviewed by:

1.) Eppinger, H.: Zur pathologie der melzfunktion. Klin. Wchnschr. 50: 1572, 1913.

The classically documented effects of the functional spleen on the circulating erythrocytes:

1. Site of maturation of the reticulocyte (loss of reticulum):

a.) Total surface area decreases from about 250 μ^2 to 135 μ^2.
b.) The amount of lipid diminishes in direct proportion to the loss of surface area.
c.) There is a major loss of intracellular water.
d.) The relative concentration of fatty acids and cholesterol increases.

e.) Surface becomes less "sticky".

f.) There is an increase in zeta potential with a change in isoelectric point of cell from 3.5 to less than 2.

2. "Culling" function:

Removes cells with altered surface characteristics (spherocytes, antibody coated cells, etc.).

3. "Pitting" function:

Ability to remove a solid particle from the cytoplasm of the red cell without destroying the cell itself. (Nucleoprotein fragments [so-called Howell-Jolly bodies], hemosiderin granules, Heinz bodies, organisms [plasmodia, Bartonella], etc.).

4. Reservoir function:

Physiologic significance is trivial since normal human spleen only holds 30-50 ml of red cells.

5. Site of demise of senescent erythrocytes.

2.) Crosby, William H.: Normal function of the spleen relative to red blood cells. Blood 14:399, 1959.

The erythrocyte changes in the peripheral blood following splenectomy and/or the asplenic state have direct relationship to the above functions:

Morphologic Findings in the Hyposplenic State:

1. Prolonged persistence of reticulocytes in the circulation with a near doubling of normal reticulocyte count.

2. Presence of cells with increased surface area with normal volume producing thin cells with resultant "targeting".

3. Evidence of variation in size (persistence of spherocytes) and shape (cells with "spicules").

4. Presence of intracellular inclusions (Howell-Jolly bodies and siderotic granules).

3.) Rous, P. and Robertson, O. H.: The normal fate of erythrocytes. I. The findings in healthy animals. J. Exp. Med. 25:651, 1917.

4.) Singer, K. and Weisz, L.: The life cycle of the erythrocyte after splenectomy and the problems of splenic hemolysis and target cell formation. Amer. J. Med. Sci. 210:301, 1945.

5.) Crosby, William H.: The pathogenesis of spherocytes and leptocytes (target cells). Blood 7:261, 1952.

Of these observations two have served to focus new attention to the red cell membrane and provide a stimulus for recent studies which have expanded our understanding of the morphologic characteristics of the red cell and have helped to clarify the mechanisms of accelerated (hemolysis) destruction of the erythrocyte:

I. Maturation of the Macroreticulocyte:

A. The following changes have now been well characterized:

(Macro) Reticulocyte	Adult Erythrocyte
1. Contain mitochondria (largely lost in first 2 days of maturation)	1. Absent
2. Contain free ribosomes	2. Absent
3. High cholesterol content (2.5 X that of adult cell)	3. Maturation results in 44% loss of cholesterol
4. High phospholipid content (4 X that of adult)	4. Maturation results in 67% loss of phospholipid.
5. High MCV	5. Loss of 29% of volume
6. High MCH	6. Loss of 26%
7. Normal life span	7. -

- An important source of the lipid loss is due to the loss of the mitochondria which accounts for 19% of the cholesterol and 50% of the phospholipid.

- However plasma membrane lipid loss also occurs:

cholesterol: 33% loss (from 25 → 17 $\mu g/10^8$)
phospholipid: 36% loss (from 59 → 38 $\mu g/10^8$)

(the phospholipid partition - lysolecithin, sphingomyelin, lecithin, phosphatidylserine, phosphatidylethanolamine - is the same in the reticulocyte and the adult cell).

- Since the surface area of the cell is related to lipid content (which accounts for 40% of the red cell membrane by weight) a decrease in lipid by 1/3 would be expected to result in a similar decrease in surface area.

B. Effect of splenectomy on maturation:

Splenectomy results in a decreased rate and extent of lipid loss resulting in mature red cells with increased lipid content (approximately 25% greater than normal). As we'll discuss subsequently this lipid loading appears to be the basis for the "targeting" which is one of the cytologic findings in the peripheral smear in the asplenic state.

6.) Winterboune, C. C. and R. D. Bott: Lipid composition of human red cells of different ages. Biochim. Biophys. Acta 202:1, 1970.

7.) Ganzoni, A., R. S. Hellman and C. A. Finch. Maturation of the macroreticulocyte. Brit. J. Haemat. 16:119, 1969.

8.) Shattel, S. J. and R. A. Cooper. Maturation of macroreticulocyte membranes in vivo. J. Lab. & Clin. Med. 79:215, 1972.

II. Structural Changes After Splenectomy: "Spiculed Erythrocytes":

The presence of occasional spiculed erythrocytes in the post splenectomy state has been frequently commented on in the past. Recent studies by Bucher et al. (9) have characterized these changes further:

- 12 patients were serially evaluated 6 to 66 months post splenectomy. They demonstrated that:

a.) All of the patients had intracellular inclusions - Howell-Jolly bodies - but that the percentage of such inclusions was quite variable. Three had less than 0.25% of the erythrocytes which contained Howell-Jolly bodies.

b.) Spiculed erythrocytes were seen in 9 of the 12. Such cells were always less numerous than red cells containing Howell-Jolly bodies. In general those patients with higher levels of Howell-Jolly body containing red cells had higher levels of spiculed cells. The spiculed cells are classical acanthocytes (see below).

c.) There was no correlation between the levels of Howell-Jolly bodies or acanthocytes and the interval since splenectomy. The levels of each appear to increase in the first few weeks post splenectomy and then stabilize at that level for the life of the patient.

d.) Acanthocytes can be first identified one hour post splenectomy.

They concluded that the acanthocyte is normally produced but that the presence of a functional spleen promptly eliminates them.

9.) Brecher, G., J. E. Haley and R. O. Wallerstein: Spiculed erythrocytes after splenectomy. Acanthocytes or non-specific poikilocytes? Nouv. Rev. Franc. d'Hemat. 12:751, 1972.

Red Cell Shapes:

Very little information has been available on the mechanisms by which alterations in red cell shape (poikilocytosis) occur. Although Eric Ponder's monumental studies of the relationships of cell shapes to erythrocyte survival (10, 11) provided evidence of a normal pattern of change from classical disc to sphere, little attention had been focused upon such alterations. Two recent series of observations have provided new data concerning the shape of the red cell:

1.) The recent availability of the scanning electron microscope has provided markedly improved resolution of the nature of the red cell membrane. Its application has resulted in the need for a new classification of various red cell shapes which now have the potential of providing the clinician helpful diagnostic data.

2.) Correlative studies of red cell membrane constituents and metabolic status with the observed shape changes have provided important data on mechanisms of hemolysis and hemolytic states not previously recognized.

The Current Classification of Red Cell Shapes:

The optical microscope has a limiting resolution of 0.2 micron. The scanning electron microscope (SEM) provides details of red cell shape with at least a tenfold improvement. Since the depth is very large (1600 μm at 100 X magnification) and the emission is collected at an angle to the incident beam, a striking 3-dimensional view is obtained as though the cell has been illuminated from the side (12). Conventional SEM provides resolution in the range of 200-100 Å and the more recent Crewe-type scopes provide resolution at the level of 5 Å.

The following changes have now been characterized:

1. Reversible Discocyte-Echinocyte Transformation:

It is now evident that a series of membrane changes occur, easily demonstrable in vitro and with in vivo counterparts, in the history of the erythrocyte as it changes from a normal disc to its pre-lytic state, the sphere (13, 14, 15). These changes occur without a change in cell volume or viability, and are largely if not completely reversible. The changes are primarily those of the development of crenations or spicules on the surface which led Bessis to the use of the Greek term echinocyte (sea urchin) to describe the cells (13, 14, 15).

The New Nomenclature for Red Cell Shape Change (14):

Name	Greek meaning	Description
Discocyte	disc	normal biconcave erythrocyte
Echinocyte I	sea urchin	an irregularly contoured disc
Echinocyte II	" "	a flat cell with spicules
Echinocyte III	" "	an ovoid or spherical cell with 10-30 spicules evenly distributed over its surface
Sphero-echinocyte I	———	distinctly spherical cells whose spicules have become fine, needle like projections
Sphero-echinocyte II	———	a spherical cell with very fine tiny spicules
Spherocyte	sphere	spherical shape without a change in volume

This classification deletes two commonly misused and poorly defined terms ("crenated" and "burr") to describe red cell changes.

Experimental studies have now shown that visual resolution of the sphero-echinocyte stage may be very difficult by light microscopy; many of these cells appearing to be classical spherocytes of the "pre-lytic" type. Thus incubation of cells in 15-30 mM oleic acid produces sphero-echinocytes (13). The differentiating point is that these cells have a normal or increased volume in contrast to "pre-lytic" spherocytes that have a reduced volume (i.e. microspherocytes).

Although inconclusive, the data suggests that the above changes are largely reversible and may not be associated with shortened red cell survival.

10.) Ponder, E. Hemolysis and Related Phenomena. Grune & Stratton, N. Y. pp. 26-50, 1948.

11.) Ponder, E. Red Cell Structure and Its Breakdown. Springer-Verlag, Vienna, 1955.

12.) Parsons, D. F. and J. R. Subjeck. The morphology of the polysaccharide cost of mammalian cells. Biochim. et Biophys. Acta 265:85, 1972.

13.) Brecher, G. and M. Bessis. Present status of spiculed red cells and their relationship to the discocyte-echinocyte transformation: A critical review. Blood 40:333, 1972.

14.) Bessis, M. Red cell shapes. An illustrated classification and its rationale. Nouv. Rev. Franc. d'Hemat. 12:721, 1972.

15.) Brecher, G. Nomenclature of red cell shapes. Nouv. Rev. Franc. d'Hemat. 12:747, 1972.

Echinocytogenic Agents:

The isovolemic changes of erythrocytes from disc to sphere are now known to be produced by a variety of in vitro and in vivo agents. Two classical methods to demonstrate these changes are: 1.) Red cells washed with isotonic saline and examined between two glass surfaces. (Thus shape changes must be cautiously interpreted, and the best appraisal of a true change is when cells are studied under plastic cover slips.

2.) Incubation of red cells in plasma at 37° C. (As will be noted below many "spur" cell states have been alleged based upon similar incubations of a given patient's plasma with normal red cells.) The data suggests this is a lysolecithin effect.

Echinocytogenic Agents (16, 17)

Fatty Acids (Oleate)
Alkysulfonates
Dihydrobenzenes
Bile Acids
Ethacrynic Acid
2,4 Dinitrophenol
Lysolecithin
Ethanol
Butanol
Substituted Benzoates
  Salicylate
  Gentisate
Dioxypyrazolidines
  Phenylbutazone
  Phenopyrazone
Indomethacin
Furosemide
Barbiturates
Phloretin
Phloridzin
Tannic Acid
Thiosemicarbazone
Dipyridamole
Alkylpyridinium Chlorides
Uranyl salts

These all cause shape change independent of volume change. These changes are largely reversible, although prolonged contact and/or high concentrations of the agents may lead to irreversible membrane alteration.

Recent studies by Weed et al. (17, 18) have demonstrated that high external (to the erythrocyte) calcium concentrations in the presence of an increased pH promptly lead to echinocyte formation and enhances potency of echinogenic agents. The postulate from the data is that at least this model is consistent with the possibility that echinocytogenic agents may cause a repartition of intramembrane calcium such as to induce conformational change in a membrane protein thus inducing a type of "contraction".

16.) Deuticke, B. Transformation and restoration of biconcave shape of human erythrocytes induced by amphiphilic agents and changes of ionic environment. Biochem. Biophys. Acta 163:494, 1968.

17.) Weed, R. I. and B. Chailley. Calcium-pH interactions in the production of shape change in erythrocytes. Nouv. Rev. Franc. d'Hematol. 12:775, 1972.

18.) Lichtman, M. A. and R. I. Weed. Divalent cation content of normal and ATP-depleted erythrocytes and erythrocyte membranes. Nouv. Rev. Franc. d'Hemat. 12:799, 1972.

Some Clinical Events Associated With Altered Erythrocyte Membranes:

"Targeted" Erythrocytes In Liver Disease:

Target cells (flat, thin red cells with central accentuation; i.e. "bull's eye") are commonly seen in both obstructive and hepatocellular liver disease (19).

19.) Jandl, J. H.: The anemia of liver disease: Observations on its mechanisms. JCI 34:390, 1955.

Features: a) In the absence of splenomegaly (with the liver disease) - this is a "silent" abnormality in that the red cell survival is normal.

b) Decreased osmotic fragility (i.e. cells more resistant to osmotic lysis) characterizes the "target" cell.

The basis for the changes in liver disease is largely due to studies of Murphy (20) who demonstrated that:

- incubated normal erythrocytes had increased osmotic fragility due to loss of cholesterol from the membrane.

- the loss occurs during incubation because the level of free cholesterol in the serum medium falls due to esterification by the enzyme cholesterol acyl transferase.

- which lead to the established evidence that the surface area of the red cell was reasonably measured by osmotic lysis

and

- that surface area was influenced by the equilibrium between membrane cholesterol and the free cholesterol in the serum.

20.) Murphy, J. R. Erythrocyte metabolism. III. Relationship of energy metabolism and serum factors to osmotic fragility after incubation. J. Lab. & Clin. Med. 60:86, 1962.

Thus, the opposite effect [1.) increased membrane cholesterol
2.) increased surface area of red cell
and 3.) resultant decreased osmotic fragility]
could be expected where serum free cholesterol rises.

Congenital Deficiency of Enzyme Cholesterol Acyl Transferase (21, 22):

- provided clinical corroboration of the Murphy model. Here normal esterification of free cholesterol is not possible due to the enzymatic defect. These patients have: increased red cell cholesterol; decreased osmotic fragility and target cells.

21.) Norum, K. R. and E. Gjone. Familial plasma: Lecithin acyl transferase deficiency. Biochemical study of a new inborn error of metabolism. Scand. J. C. I. 20: 231, 1967.

22.) Gjone, E., H. Torsvik and K. R. Norum. Familial plasma cholesterol ester deficiency. A study of erythrocytes. Scand. J. C. I. 21:237, 1968.

Studies then demonstrated the same findings in liver disease:

23.) Cooper, R. A. and J. H. Jandl. Bile salts and cholesterol in the pathogenesis of target cells in obstructive jaundice. JCI 47:809, 1968.

24.) Neerhout, R. C. Abnormalities of erythrocyte stromal lipids in hepatic disease. J. Lab. & Clin. Med. 71:438, 1968.

The mechanisms of lipid acquisition in erythrocytes in liver disease are not clear. Actually the changes seen in the red cells are

- increased membrane cholesterol (↑ 25-50%)
- increased phospholipid (↑ 12-25%)
- resultant ↑ chol:PL ratio
- because of all the phospholipids, lecithin exchange is greatest resulting in a large fractional percentage membrane increase. The relationship can be expressed by chol:lecithin ratio which thus is <u>decreased</u>.

Some of the mechanistic proposals for this lipid loaded erythrocyte in liver disease:

- decreased lecithin:cholesterol transferase (LCAT)

25.) Turner, K. B., G. H. McCormack and A. Richards. The cholesterol-esterifying enzyme of human serum. I. In liver disease. JCI 32:801, 1953.

but:

26.) d'Hollander, F. and F. Chevallier. Movement of cholesterol in vitro in rat blood and quantitation of the exchange of free cholesterol between plasma and erythrocytes. J. Lipid Res. 13:733, 1972.

- showed only ? relationship between esterification and red cell chol.

- elevated bile salts which may depress LCAT activity. Ref. 23 and

27.) Cooper, R. A. Lipids of human red cell membranes: Normal composition and variability in disease. Semin. in Heme 7:296, 1970.

but:

28.) Kepkay, D. L., R. Poon and J. B. Simon. Lecithin-cholesterol acyl-transferase and serum cholesterol esterification in obstructive jaundice. J. Lab. & Clin. Med. 81:172, 1973.

- demonstrated a stimulated LCAT reaction.

- presence of lipid soluble compounds that result in an expanded membrane.

29.) Seeman, P. et al. in a series of papers in

Biochim. Biophys. Acta 183:490, 1969
" " " 183:499, 1969
" " " 183:512, 1969
" " " 183:520, 1969

but this appears to be a too selective mechanism.

Finally in the new nomenclature (14) the target cell has now been re-named:

Codocyte (from Greek meaning bell) - because of its SEM architecture.

Identifiable physiologic sequelae of membrane injury (i.e. shortened red cell survival - a hemolytic state) may not result from such minimal membrane alterations. The variability has led to considerable recent interest in the "minimal membrane lesion" - the circumstances in which membrane alterations result in a variable degree of hemolysis (30-35). These alterations can be separated into classifiable groups:

a.) Physical-chemical changes in membrane
b.) Alterations in hemoglobin (i.e. the hemoglobinopathies leading to secondary membrane change
c.) Alterations in metabolic erythrocyte machinery (i.e. enzymatic defects) leading to secondary membrane change

30.) Weed, R. I. and C. F. Reed. Membrane alterations leading to red cell destruction. Amer. J. Med. 41:681, 1966.

31.) Dourmoshken, R. R. and W. F. Rosse. Morphologic changes in the membranes of red blood cells undergoing hemolysis. Amer. J. Med. 41:699, 1966.

32.) Weed, R. I., P. L. LaCelle and E. W. Merrell. Metabolic dependence of red cell deformability. JCI 48:795, 1969.

33.) Cooper, R. A. and S. J. Shattil. Mechanisms of hemolysis - the minimal red cell defect. NEJM 285:1512, 1971.

34.) Shohet, S. B. Hemolysis and changes in erythrocyte membrane lipids. NEJM 286:577 and 638, 1972.

35.) Cooper, R. A. and S. J. Shattil. The red cell membrane in hemolytic anemia. Mod. Treatment 8:329, 1971.

Some Erythrocyte Characteristics For Normal Survival

1. Red cell deformability and plasticity:

	Characteristics	Factors
a.)	Thin flexible viscoelastic membrane capable of isochoric (i.e. no volume change) bending: thus, the seemingly rigid disc of mean diameter of 8 μm can traverse capillary channels ½ the diameter of the cell. (36-38)	Membrane lipid. ?Membrane protein. Calcium.
b.)	Advantageous surface area to volume relationship permitting deformability (39,40)	ATP adequacy.
c.)	Fluidity of its contents (40)	ATP adequacy.
d.)	Advantageous discocyte configuration for conformation to altered aperture (41)	?Lysolecithin ?Fatty acids

2. Ability to re-seal membrane after injury (3).

3. High ultimate tensile strength (37).

4. Translational mobility within the surface (37).

36.) Rand, R. F. The structure of a model membrane in relation to its viscoelastic properties of red cell membranes. J. Gen. Physiol. 52:173, 1968.

37.) Bull, B. Red cell biconcavity and deformability. Nouv. Rev. Franc. d'Heme 12:835, 1972.

38.) Shohet, S. B. and J. E. Haley. Red cell membrane shape and stability: Relation to cell lipid renewal pathways and cell ATP. Nouv. Rev. Franc. d'Heme 12:761, 1972.

39.) Rand, R. P. and A. C. Burton. Mechanical properties of the red cell membrane. I. Membrane stiffness and intracellular pressure. Biophys. J. 4:115, 1964.

40.) Rand, R. F. Some biophysical considerations of the red cell membrane. Fed. Proc. 26:1780, 1967.

41.) LaCelle, P. L., F. H. Kirkpatrick, M. P. Udkow and B. Arkin. Nouv. Rev. Franc. d'Hematol. 12:789, 1972.

42.) Weed, R. I. The importance of erythrocyte deformability. Amer. J. Med. 49:147, 1970.

43.) Rifkind, R. A. Destruction of injured red cells in vivo. Amer. J. Med. 41:711, 1966.

Case 2. "Acanthocytosis" (44)

44 year old WM with long history of known alcoholic cirrhosis was admitted with a history of progressive pedal edema and increasing abdominal girth over previous week. A portacaval shunt had been done 3 years earlier in California where he had been seen with hematemesis secondary to bleeding esophageal varices. His interval history was unremarkable except for continued alcoholic excess.

Physical examination revealed a chronically ill icteric WM with scattered "spider" angiomata, gynecomastia and small testes. Ascites was evident and the liver was palpable 6 cm below the RCM. The spleen was not palpable and no percussion dullness was noted. There was ++++ edema of lower extremities extending up to and involving the scrotum.

Laboratory evaluation revealed a hemoglobin of 8 gm%, hematocrit of 23 vol% and a reticulocyte count of 5%. Examination of the peripheral smear revealed that 30-40% of the red cells were acanthocytic in form.

Other studies included:

- wet prep of red cells documented the acanthocytosis
- serial wet prep study demonstrated that instead of the normal rouleaux, the cells formed large clumps with interlocking of their spiny projections
- time lapse microphotocinematography documented rigid red cell membranes
- acanthocytic change was increased by mixing equal parts of patient's blood with normal saline
- disappearance of acanthocytosis and conversion to spheres occurred with incubation with Tween 80, glycerol, acid phosphate buffer, ACD, acetyl phenylhydrazine or sodium metabisulfate
- the acanthocytic change was reduced toward disc form by addition of bovine and/or human serum albumin
- the patient's plasma (fresh) failed to induce acanthocytic change in normal red cells
- red cell survival with ^{51}Cr tagged patient's cells was 10 days (normal 27-32 d) with splenic sequestration providing a S:L ratio of 3.2-3.6 (normal 1).
  - ^{51}Cr tagged patient cells into a normal volunteer had $T_{\frac{1}{2}}$ of 12 days with splenic sequestration
  - ^{51}Cr tagged patient cells into an asplenic normal revealed a normal $T_{\frac{1}{2}}$ of 30 days
- serum lipids were normal

Clinical course: Patient demonstrated marked protein intolerance and deteriorated over the next 3 months and died with evidence of hepatic encephalopathy.

44.) Douglass, C. C., M. S. McCall and E. P. Frenkel. The acanthocyte in cirrhosis with hemolytic anemia. Ann. Int. Med. 68:390, 1968.

The first description of the development in patients with severe liver disease of a hemolytic anemia characterized by the presence of erythrocytes with numerous thorny projections over their surface was:

45.) Smith, J. A., E. T. Lonegan, and K. Sterling. Spur cell anemia: Hemolytic anemia with red cells resembling acanthocytes in alcoholic cirrhosis. NEJM 271: 396, 1964.

Although the description of these cells has suffered from a plethora of names (spur cells, burr cells, etc.) the prototype of the cell now acceptably termed Acanthocyte (14) was first described in the circumstance of congenital abetalipoproteinemia (46-47). The cells can be characterized as: having 5-10 spicules of varying length irregularly distributed over the red cell surface (48). The individual spicules have knobby ends.

Case 3. Congenital Abetalipoproteinemia

[redacted] a 34 year old WF, was seen because of a lifelong history of recurrent diarrhea and fat intolerance. She was the product of nonconsanguineous parents and had a normal birth. From birth she had failed to thrive and had recurrent diarrhea.

Physical examination revealed that she was 4' 7" tall and weighed 70 lbs. Her gait was unsteady and waddling in nature. She had evident wasting with thin skin folds and poor muscle mass. She had no organomegaly. Deep tendon reflexes were absent. She had decreased visual acuity and low I.Q. Fundi revealed silvery spots in macular region.

Other studies: - Her hemoglobin was 11 gm% with a hematocrit of 32 vol%. Her red cells were acanthocytic.

- Osmotic fragility was normal.

- Mechanical fragility was increased.

- ^{51}Cr tagged red cell survival was 18 d. with modest splenic sequestration.

- Electrophoresis showed no beta-lipoprotein and a depletion of the alpha fraction. Absence of beta-lipoproteins was confirmed by ultra-centrifugation.

Total lipids	123	(normals 600-800 mgm%)
Cholesterol	36	
Esterified cholesterol	25	
Triglycerides	Trace	
Phospholipids	40	

Acanthocytosis is now well described in a variety of circumstances:

Acanthocytosis

1) Congenital abetalipoproteinemia (46, 47)
2) Severe liver disease (44, 45)
3) Post splenectomy (9)
4) Hypothyroidism [in 2-5% of cells] (9)
5) Uremia (9)
6) Hereditary variant (49)

The presence and degree of anemia have been variable in the acanthocytic states. Evidence of reversibility of the changes and variability of their degree is now clear (50). At least for the red cell in abetalipoproteinemia, the major mechanism for anemia appears to be related to an added membrane injury by lipid peroxidation due to associated vitamin E deficiency (51, 52). Erythrocyte phospholipid and cholesterol are present in normal amounts and ratios. PL partition reveals increased sphingomyelin and decreased phosphotidyl choline. The changes appear to be the result of the altered plasma lipid environment (53, 54).

One mechanism of the change in liver disease has been forcefully pressed by Cooper (55). He demonstrated that incubation of normal cells in serum from such a patient with acanthocytosis for 24 hours resulted in a 64% increase in cholesterol which was subsequently reversible in normal serum. The increase in cholesterol:PL ratio is greater than in "target" cells and the chol:lecithin ratio was markedly increased. Mechanistically he has related the etiology to increased lithocholic acid secondary to the liver disease.

The recent evidence (56) does suggest that the lipoprotein composition of the serum affects the lipoprotein concentration of the red cell.

Experimentally low serum FC:PL ratio of 0.1 → 60% ↓ membrane chol
high serum FC:PL ratio of 2.2 → 110% ↑ membrane chol

chol depleted red cells → appeared spheroidal.
chol rich cells → appeared acanthocytic.

In addition, a pre and post-splenectomy study of red cell lipids in one patient (57), supported our own previous evidence that the spleen is a site of membrane loss by acanthocytes.

However, the pathogenetic mechanisms are far from clear:

1.) Evidence that plasma incubations are echinocytogenetic (see above) (14) - casting some question on the documentation of some "spur" cell cases by this method.

2.) Lack of evidence of the bile salt changes proposed (58-60).

3.) Important recent evidence that lysophosphatide content critically affects red cell membrane stability, and in the metabolically (ATP) deprived cell a potent echinocytogenic circumstance exists (38, 61, 62).

4.) In addition, 2 recent forms of a familial non-spherocytic hemolytic anemia with altered membrane lipids did not support the proposed model (63, 64 and 65).

5.) Finally, the physiologic vulnerability of these cells to destruction may actually be the result of a stiffened membrane due to loss of ATP and increased calcium (17, 18, 32).

46.) Bassen, F. A. and A. L. Kornzweig. Malformation of the erythrocytes in a case of atypical retinitis pigmentosa. Blood 5:381, 1950.

47.) Singer, K., B. Fisher and M. A. Perlstein. Acanthocytosis, A genetic malformation. Blood 7:577, 1952.

48.) Kayden, H. J. and M. Bessis. Morphology of normal erythrocytes and acanthocytes using Normarski optics and the scanning electron microscope. Blood 35:427, 1970.

49.) Estes, J. W., T. J. Morley, I. M. Levine and C. P. Emerson. A new hereditary acanthocytosis syndrome. Amer. J. Med. 42:868, 1967.

50.) Leblond, P. The discocyte-echinocyte transformation of the human red cell: deformability characteristics. Nouv. Rev. Franc. d'Hemat. 12:815, 1972.

51.) Kayden, H. J. and R. Silber. The role of vitamin E deficiency in the abnormal autohemolysis of acanthocytosis. Trans. Assoc. Am. Phy. 78:334, 1965.

52.) Dodge, J. T., G. Cohen, H. J. Kayden and G. B. Phillips. Perioxidative hemolysis of red blood cells from patients with abetalipoproteinemia (acanthocytosis). JCI 46:357, 1967.

53.) Ways, P., C. F. Reed and D. J. Hanahan. Red cell and plasma lipids in acanthocytosis. JCI 42:1248, 1963.

54.) Simon, E. R. and P. Ways. Incubation hemolysis and red cell metabolism in acanthocytosis. JCI 43:1311, 1964.

55.) Cooper, R. A. Anemia with spur cells: A red cell defect acquired in serum and modified in the circulation. JCI 48:1820, 1969.

56.) Cooper, R. A., E. C. Arner and J. S. Wiley. Serum lipoproteins alter red cell membrane structure. JCI 52:20a, 1973 [Abstr.].

57.) Cooper, R. A., J. Durocher and D. R. Kimball. The spleen is the site of membrane loss by spur cells. Clin. Res. 21:550, 1973 [Abstr.].

58.) Calandra, S., M. J. Martin and N. McIntyre. Plasma lecithin:cholesterol acyltransferase activity in liver disease. Europ. J. C. I. 1:352, 1971.

59.) Gjone, E. and J. P. Blomhoff. Plasma lecithin:cholesterol acyltransferase in obstructive jaundice. Scand. J. Gastro. 5:305, 1970.

60.) Murphy, G. M., A. Ross and B. H. Billing. Serum bile acids in primary biliary cirrhosis. Gut 13:210, 1972.

61.) Feo, C. The role of lysolecithin formed in plasma on the discocyte-echinocyte transformation. Nouv. Rev. Franc. d'Hemat. 12:757, 1972.

62.) Lichtman, M. A. and G. V. Marinetti. Erythrocyte shape alteration "in vitro": Relationship to plasma and cellular factors. Nouv. Rev. Franc. d'Hemat. 12:755, 1972.

63.) Jaffe, E. R. and E. L. Gottfried. Hereditary non-spherocytic hemolytic disease associated with an altered phospholipid composition of the erythrocytes. JCI 47:1375, 1968.

64.) Shohet, S. B., D. G. Nathan, B. M. Livermore, S. A. Feig and E. R. Jaffe. Hereditary hemolytic anemia associated with abnormal membrane lipid. II. Ion permeability and transport abnormalities. Blood 42:1, 1973.

65.) Arakawa, T., N. Kalsushima, and T. Fujiwara. A hemolytic anemia with abnormality of erythrocyte lipids and dwarfism - probably a new syndrome. Tohoku J. Exp. Med. 88:35, 1966.

Case 4. Stomatocytosis

42 year old WM was admitted with severe epigastric pain following a major alcoholic debauch. There was no history of vomiting, hematemesis, change in bowel habits or evidence of melena. His recent history included a heavy alcoholic intake for at least 3 years.

Physical examination was within normal limits except for mid epigastric tenderness and a liver edge palpable at the RCM.

Laboratory studies demonstrated a hemoglobin of 13.8 gm% and a hematocrit of 44 vol%. Reticulocyte count was 1%. Examination of the peripheral smear revealed that 25% of the cells were cup shaped or stomatocytes. Serial evaluation over the next two weeks failed to evidence any data in support of increased red cell destruction and the peripheral smear reverted to normal by the 10th day of follow-up.

Other studies confirmed a diagnosis of alcoholic gastritis.

In the new nomenclature the term Stomatocytosis (meaning mouth) is the accepted term for this cup shaped cell (14). On stained smear the cells have a slot like central pallor which provide the "mouth like" pattern. By phase, they appear dome-shaped.

Stomatocytosis has been recorded with a rare hereditary hemolytic anemia characterized by a marked increase in membrane permeability to sodium (66-68). Recently it was also reported in 4 alcoholics as a transient event, but associated with evidence of increased red cell destruction (69).

Current SEM evidence (14) and evaluation of experimental models (16, 17) have demonstrated that a variety of agents or a low pH may produce a disc-sphere transformation in which the disc first becomes cup-shaped, then hemispherical and ultimately a sphere with a small hilum.

Stomatocytogenic Agents (16, 17)

Alkylammonium chlorides	Phenylamine
Phenylthiazines	Verapamil
Chlorpromazine	Papaverine
Local anesthetics	Primaquine
Cinchocaine	Chloroquine
Tetracaine	Benzydamine
Procaine	Colchicine
Antihistamines	Vincristine
Pheniramine	Vinblastine
Biopheniramine	LSD
Bampine	Triton X
Propanolol	Tween 80
Hexobendine	Vitamin A or E

Furthermore, it has been shown that these stomatocytogenic agents and/or low pH are capable of antagonizing echinocytogenic agents. These agents appear to produce their effect by interfering with the calcium pump (17), and appear to displace calcium from ATPase. This induces a conformational change in the form of "contraction" (17). This potentially reversible change then is another pattern in the sequence of alterations in the erythrocyte membrane.

66.) Lock, S. P., R. S. Smith and R. M. Hardisty. Stomatocytosis: A hereditary red cell anomaly associated with hemolytic anemia. Brit. J. Heme 7:303, 1961.

67.) Meadow, S. R. Stomatocytosis. Proc. Roy. Soc. Med. 60:13, 1967.

68.) Zarkowsky, H. S., F. A. Oski and R. Shaafi. Congenital hemolytic anemia with high sodium, los potassium red cells. NEJM 278:573, 1968 and 280:909, 1969.

69.) Douglass, C. C. and J. J. Twomey. Transient stomatocytosis with hemolysis: A previously unrecognized complication of alcoholism. Ann. Int. Med. 72:159, 1970.

Case 5. Hereditary Spherocytosis

This 38 year old WF was first seen because of ease of fatigue. She had been well until age 11 at which time she was noted to be icteric and have a palpable spleen and liver. A diagnosis of hepatitis was made and she was treated with bed rest for three months during which time her jaundice cleared and her spleen was said to be no longer palpable. At age 19 she again became icteric and her spleen was palpable 3 cm beneath the LCM. A diagnosis of recurrent hepatitis was made. Because

she failed to reduce her bilirubin below 2.5 mgm% after 4 months of bed rest she was referred to the Mayo Clinic for evaluation. When seen there her hemoglobin was 10 gms% and her reticulocytes were 12%. Microspherocytosis was identified on peripheral smear. Her bilirubin was primarily indirect reacting and no antibodies were noted. A diagnosis of Congenital Spherocytosis was made. Splenectomy was refused by the patient. She subsequently married and had 3 children and led an uneventful life. During the past decade she was chronically icteric (bilirubin 2.5-5) and had evident splenomegaly. She sought medical advice because of progressive ease of fatigue and inability to continue to play "championship golf".

Examination revealed evident icterus, a liver edge 2 cm beneath the RCM and a spleen palpable 6 cm beneath the LCM.

Studies revealed a hemoglobin of 9.5 gm% with a hematocrit of 30 vol% and 18% reticulocytes. The MCHC was 38.5. Her peripheral smear revealed 20% microspherocytosis. She had increased osmotic fragility. Autohemolysis was increased with near complete correction by added glucose and only partial correction by ATP.

At elective splenectomy she was noted to have multiple stones in gall bladder and common bile duct which were removed. Postoperatively she repaired her red cell values to normal, with the expected persistence of her microspherocytosis. Family studies revealed the same lesion in her children and her mother.

The identifiable cell in this classical picture of Hereditary Spherocytosis is the <u>micro</u>spherocyte.

The <u>pathophysiologic feature</u> of this sphere form is the evidence of loss of membrane mass producing a significant surface area to volume change. This is documented by the visual and measurable microcytic form, the evidence of increased osmotic fragility and the high MCHC. The microspherocyte is the <u>pre-lytic form</u>.

<u>The clinical occurrence</u> of microspherocytes has been well documented in circumstances where such membrane mass loss occurs:

a.) Hereditary Spherocytosis
b.) Autoimmune Hemolytic Anemia
c.) Transfusion of Senescent (Aged) RBC's
d.) Circumstances of In Vivo Mechanical Injury of RBC with membrane loss (red cell fragmentation syndromes, burns, etc.)

<u>Clinical features</u> of Hereditary Spherocytosis are:

- inherited as autosomal dominant character
- incidence 220/million
- jaundice
- splenomegaly
- chronic hemolytic anemia, which may be "compensated" for by increased red cell production
- pigmentary gall stones

Laboratory features of H. S. are:

- hemolytic anemia, which is usually mild to moderate with exacerbations in severity of the peripheral red cell values during periods of decreased production (infection, etc.)
- evidence of microspherocytosis
- reticulocytosis
- increased MCHC
- increased osmotic fragility (which in mild cases is demonstrable only by pre-incubation of the red cells) (70)
- increased autohemolysis which is corrected by addition of glucose during the incubation (71, 72)
- shortened red cell survival with evidence of splenic sequestration of ^{51}Cr tagged patient's cells into patient or normal. Evidence of normal survival of normal cells in patient.

Pathophysiologic Mechanisms:

Although the primary biochemical defect is not known, the following alterations have been documented:

- accelerated glycolytic rate (which may be compensatory for:)
- increased membrane permeability with resultant increased passive sodium influx

In vivo destruction of this microspherocyte is due to 2 major factors:

1.) Tendency for pooling of the cells in the spleen because the rigid spheroidal cells have difficulty passing the slit like stomata leading from the pulp to the splenic sinuses, this aperture having a mean diameter of 3 μ. (73).

2.) Inordinate susceptibility to further membrane damage under the adverse conditions existing in the splenic milieu of this metabolically demanding cell (74).

The result is further membrane loss with progressive spheroidal change (75, 76). This membrane loss is clearly secondary to the injury and not a primary mechanism (77, 78).

It is because of this sequence that splenectomy is the indicated treatment of choice.

Mechanism of the Defect:

Recent data has suggested that the primary defect may represent an alteration in membrane protein that makes up the "muscle-like microfilaments" - an actin-like material. Presumably this produces a defective membrane lattice with resultant increased permeability (79). Membrane vacuolar formation has also recently been shown in H. S. which further imparts rigidity to the wall (80).

70.) Emerson, C. P., S. C. Shen, T. H. Ham, E. M. Fleming and W. B. Castle. Studies on the destruction of red blood cells. IX. Quantative methods for determining the osmotic and mechanical fragility of red cells in the peripheral blood and splenic pulp; the mechanism of increased hemolysis in hereditary spherocytosis (congenital hemolytic jaundice) as related to the function of the spleen. Arch. Int. Med. 97:1, 1956.

71.) Selwyn, J. C. and J. V. Dacie. Autohemolysis and other changes resulting from the incubation in vitro of red cells from patients with congenital hemolytic anemia. Blood 9:414, 1954.

72.) Young, L. E., M. J. Izzo, K. I. Altman and S. N. Swisher. Studies on spontaneous in vitro autohemolysis in hemolytic disorders. Blood 11:977, 1956.

73.) Bjorkman, S. E. The splenic circulation. Acta Med. Scand. Suppl. 191, 1947.

74.) Jacob, H. S. and J. H. Jandl. Increased cell membrane permeability in the pathogenesis of hereditary spherocytosis. JCI 43:1704, 1964.

75.) Griggs, R. C., R. Weisman and J. W. Harris. Alterations in osmotic and mechanical fragility related to in vivo erythrocyte aging and splenic sequestration in hereditary spherocytosis. JCI 39:89, 1960.

76.) Reed, C. F. and S. N. Swisher. Erythrocyte lipid loss in hereditary spherocytosis. JCI 45:777, 1966.

77.) Cooper, R. A. and J. H. Jandl. The role of membrane lipids in the survival of red cells in hereditary spherocytosis. JCI 48:736, 1969.

78.) Cooper, R. A. and J. H. Jandl. The selective and conjoint loss of red cell lipids. JCI 48:906, 1969.

79.) Jacob, H. S., T. Amsden and J. White. Etiology of hereditary spherocytosis: Role of defective membrane microfilaments. Amer. Soc. Heme 14:30, 1971 (Abstr.).

80.) Scrier, S., K. Bensch and I. Ben-Basset. Erythrocyte membrane vacuole formation in hereditary spherocytosis. Amer. Soc. Heme 15:49, 1972.

Case 6. Hereditary Elliptocytosis (Ovalocytosis)

[redacted], a 40 year old WF, was referred for evaluation because of anemia with abnormally shaped red cells on her peripheral smear that had been noted at the time of a D&C. She had had recent vaginal bleeding which led to the identification of an endometrial polyp for which the surgery was performed. She was otherwise asymptomatic.

Examination was within normal limits.

Laboratory evaluation revealed a hemoglobin of 8.2 gm%, hematocrit of 22 vol% and reticulocyte count of 1%. Her red cells were remarkably oval in shape and were slightly hypochromic. Serum iron was 12 µgm% and IBC was 420 µgm%. Bone marrow examination revealed slight erythroid hyperplasia (EG ratio of 1:2) with the absence of stainable iron.

Therapy with oral iron returned her red cell values to normal with a persistent reticulocyte count of 2%. Virtually all of her cells were ovalocytic. Further study revealed a red survival of 21 days. Corroboration of the defect was provided by similar cells in 2 children.

The identifiable cell is an oval cell. The accepted nomenclature is Ovalocytosis. The oval cell characteristically has an axial ratio of less than 0.78.

Clinical Features of Hereditary Ovalocytosis:

- autosomal dominant disease
- The clinical features are as noted above for H. S.
- In most patients anemia is not significant (Hb above 12 gm%). Approximately 15% of patients have evident anemia.
- Osmotic fragility and autohemolysis are normal.
- The primary site of cell loss is the spleen.

Special Features of H.O.

- The elliptical red cell is normal in birds, reptiles, camels and llamas.
- In man however the cell demonstrates cytoplasmic polarization (81) with membrane cholesterol concentrated at sites of greatest convexity (82). Although the red cell precursors are normal, prepared "ghosts" (with absent Hb) maintain elliptical shape (83).
- The inheritance has been linked to one of two genes, one of which is linked to the Rh gene (84). Hemolysis seems more severe in those patients without the Rh linkage (85).

Pathophysiologic Mechanisms:

In contrast to H. S., evidence of a definable metabolic abnormality as the basis for the increased destruction is not clear for the elliptocyte.

The clinical similarities to H. S. have not shown paralleled metabolic abnormalities. Only one study to date (86) has reported - in elliptocyte ghosts - a 40-50% increase in Na efflux suggesting a similar but very much milder membrane defect.

In our own studies (87) in intact cells from 8 patients with hereditary elliptocytosis we found:

Intracellular Na^+ conc.: 6.5-8 mEq/L (normal 6-12 mEq/L)
Na^+ influx rates: 3.0-3.6 mEq/L of RBC/hr (normal 2.6-3.8 mEq/L of RBC/hr)
(This is in contrast to H. S. where rates were 3.7-6.0 mEq/L of RBC/hr.)
Similarly erythrocyte K^+ conc. and K^+ flux rates were also normal.

Although none of these patients had the "severe" form of hereditary elliptocytosis, it is clear that, at least in those with the mild defect, the membrane does not possess the same characteristics as that seen in Hereditary Spherocytosis.

Whatever the basis however, splenic sequestration of these cells appears to be a significant factor in the anemia, since correction has been well documented to correct the circulating red cell numbers to normal.

81.) Rebuck, J. W. and E. J. van Slyck. An unexpected ultrastructural fault in human elliptocytes. Amer. J. Clin. Path. 49:19, 1968.

82.) Murphy, J. R. Erythrocyte metabolism. VI. Cell shape and the location of cholesterol in the erythrocyte membrane. J. Lab. & Clin. Med. 65:756, 1965.

83.) Rebuck, J. W., W. H. Appelhof and F. W. Meier. Electron microscopy of elliptocytes in man and llama. Anat. Rec. 130:362, 1958.

84.) Chalmers, J. N. M. and S. D. Lawler. Data on linkage in man: Elliptocytosis and blood groups. I. Families 1 and 2. Ann. Eugen. 17:267, 1952.

85.) Geerdink, R. A., P. W. Hellerman and M. C. Verloop. Hereditary elliptocytosis and hyperhemolysis. A comparative study of 6 families with 145 patients. Acta Med. Scand. 179:715, 1966.

86.) Peters, J. C., M. Rowland, L. G. Israels and A. Zipursky. Erythrocyte sodium transport in hereditary elliptocytosis. Canad. J. Physiol. Pharm. 44:817, 1966.

87.) Waterbury, L. and E. P. Frenkel. The mechanism of hemolysis in hereditary elliptocytosis. Clin. Res. 17:35, 1969 (Abstr.).

Case 7. Red Cell Fragmentation Syndrome

This 21 yr NM had been followed at [redacted] since age 7 when admitted with a classical clinical picture of acute rheumatic fever. By age 14 evidence of mitral stenosis and insufficiency were noted. Because of progressive cardiac deterioration he was admitted at age 18 for mitral valve replacement. At the time of replacement, his hematocrit was 37 vol% and a serum iron on the chart was 40 µgm% with IBC of 650. Postoperatively his replaced valve functioned poorly and he developed evident tricuspid insufficiency in addition. Over the subsequent year his hematocrit fell

to 26 vol% and when his red cells were noted to be hypochromic and microcytic with associated fragmentation he was started on iron. His red cell values rose to 30 vol% but by history it was evident that he took his iron only intermittently. On re-evaluation 18 months postoperatively his hematocrit was 30 vol% and TSI 13 µgm% with IBC of 350. Serum haptoglobin was essentially absent and marrow revealed no iron stores. A 24 hour urine iron was 4.6 mgm. Examination of peripheral smear revealed red cell fragmentation. Oral iron therapy over the next 6 months resulted in an increase in hematocrit to 38 vol% although his peripheral smear continued to demonstrate classical red cell fragmentation.

Etiologic Mechanisms for Red Cell Fragmentation:

1. Mechanical Trauma with Fragmentation on an Altered Intravascular Surface in Area of High Velocity.

    a.) Prosthetic Heart Valves, Severe Aortic Calcific Stenosis, Intracardiac Defect Repairs.

- clearly evident that fragmentation and intravascular hemolysis occurs when blood is subjected to a shearing force of 3,000 dynes/cm^2 as may occur with gradients between aorta and left ventricle, around prosthetic heart valves, etc. (88). In addition to such shearing forces, cell-surface interaction of red cells with an abnormal surface (prosthesis) appears to also be a factor. In fact, the evidence by Blackshear et al. (89, 90) suggests that such cell-surface interaction is more important than shear force alone.

2. Direct Mechanical Injury.

    a.) March Hemoglobinuria

- clearly the effect of direct trauma by impact (91). It is even a potential hazard in karate (92).

3. Mechanical Fragmentation by Cells Propelled Through Fibrin Thrombi - Microangiopathic Hemolytic Anemia (93)

- This exceedingly common cause for red cell fragmentation is the result of disseminated intravascular coagulation regardless of cause. The pathophysiologic mechanisms have clearly been demonstrated to be due to the presence of residual fibrin thrombi in the arteriolar bed secondary to the DIC. The fragmentation is the result of arrest of an individual red cell on an obstruction of small dimension (i.e. fibrin strand) relative to the size of the red cell and the subsequent trauma to the arrested cell by the rapidly flowing stream (94, 95).

Its occurrence has been widely documented in every conceivable mechanism for endothelial damage in the arteriolar circuit.

Nomenclature of the Red Cell Fragments (14)

Fragmentation of the red cell results in a wide variety of structural changes that have been given many discriptive names (helmet or triangular cells [96] by one group and "burr" cells by another [97]) thus causing confusion.

The terms to describe the cells seen in red cell fragmentation are:

Keratocyte (meaning horn cell) - previously called "burr", helmet or triangular cell.
Schizocyte (meaning cut cell) - this term was previously used.
Knizocyte (meaning pinch cell) - this may rarely be seen as well.

88.) Marsh, G. W. and S. M. Lewis. Cardiac hemolytic anemia. Semin. In Heme 6:133, 1969.

89.) Blackshear, P. L., Jr., F. D. Dorman and J. H. Steinbach. Some mechanical effects that influence hemolysis. Trans. Amer. Soc. Artif. Int. Organs 11:112, 1965.

90.) Blackshear, P. L., Jr., F. D. Dorman, J. H. Steinbach, E. J. Mayback, A. Singh and R. E. Collingham. Shear, wall interaction and hemolysis. Trans. Amer. Soc. Artif. Int. Organs 12:113, 1966.

91.) Davidson, R. J. L. March or Exertional Hemoglobinuria. Semin. Heme 6:150, 1969.

92.) Streeton, J. A. Traumatic hemoglobinuria caused by karate exercises. Lancet 2:191, 1967.

93.) Brain, M. C., J. V. Dacie and D. O'B. Hourihane. Microangiopathic hemolytic anemia: The possible role of vascular lesions in pathogenesis. Brit. J. Heme 8:358, 1962.

94.) Rubenberg, M. L., B. S. Bull, E. Regoeczi, J. V. Dacie and M. C. Brain. Experimental production of microangiopathic hemolytic anemia in vivo. Lancet 2: 1121, 1967.

95.) Bull, B. S., M. L. Rubenberg, J. V. Dacie and M. C. Brain. Red-Blood-Cell fragmentation in microangiopathic hemolytic anemia: in vitro studies. Lancet 2: 1123, 1967.

96.) Adelson, E., E. J. Heitzman and J. F. Fennessy. Thrombohemolytic thrombocytopenic purpura. Arch. Int. Med. 94:42, 1954.

97.) Bell, R. E. The origin of "burr" erythrocytes. Brit. J. Heme 9:552, 1963.

Some Other Forms of Membrane Injury:

Secondary forms of membrane injury occur. One interesting sequence has related to those circumstances in which intraerythrocyte aggregates of denatured hemoglobin (Heinz-Ehrlich bodies) form. Such Heinz bodies occur in presence of hemoglobin abnormalities (i.e. defect in primary structure of the Hb as in Unstable Hb or defective rate of Hb chain synthesis as in thalassemia) or in enzymatic defects of the hexose monophosphate shunt (oxidative hemolysis). The formed Heinz bodies attach to the internal surface of the erythrocyte membrane producing a gross distortion of the membrane surface resulting in malfunction of the Na-K pump, increased membrane permeability, cation leak and ATP depletion (98, 99).

98.) Jacob, H. S. Mechanisms of Heinz body formation and attachment to red cell membrane. Semin. in Heme 7:341, 1970.

99.) Lessen, L. Membrane ultrastructure of normal, sickled and Heinz-body erythrocytes by freeze-etching. Nouv. Rev. Franc. d'Heme 12:871, 1972.

Other Changes in Nomenclature (14):

New Term	Meaning	Old Term	Comment
DACRYOCYTE	(tear drop)	tear drop, tailed poikilocyte, maiden's hand mirror, etc.	Seen in extramedullary hematopoiesis, thal., P.K. etc.
DREPANOCYTE	(sickle)	sickle cell	Seen in S Hb states.
MEGALOCYTE	(giant)	macrocyte	Seen in megaloblastic states.
TOROCYTE	(torus)	doughnut cell	Cells have a thickened outer rim; probably only exists as an artifact due to desiccation of the thick portion at the beginning of a smear.