Studies on the Mechanical Fragility of Erythrocytes

I. Normal Values for Infants and Children

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IN ORDER to obtain a better understanding of the factors causing destruction of human erythrocytes in vivo, a number of methods have been devised to test their susceptibility to destruction by various forces applied in vitro. By manipulation of temperature, pH, osmotic equilibrium and by the addition of hemolysins, it has been possible consistently to detect abnormalities in certain disease states.

Although the use of mechanical shaking in the destruction of erythrocytes was first suggested by Meltzer[1] in 1884, it was Rous[2] who stated that "resistance to hypotonic solutions is in no real sense an index to fragility of red blood cells. A clinical investigation of this fragility as determined by shaking experiments might not be without importance."

Shen[3] demonstrated that the fragility of erythrocytes subjected to the measured mechanical trauma of rolling glass beads stays within well defined limits in normal persons and shows significant increase in conditions such as congenital and acquired hemolytic anemias. Young[4–6] has felt that the process of mechanical fragility simulates most closely the physiologic conditions in the blood stream. Both Young and Shen[3] have found that in general mechanical fragility is nearly always increased when osmotic fragility is increased, but in some instances mechanical fragility may be increased when osmotic fragility is normal or actually decreased.

By the use of cells tagged with radioactive iron, Stewart and his co-workers[7] have shown that young erythrocytes in dogs have a mechanical fragility less than that of the general red cell population, while the osmotic fragility is relatively increased. They also showed that with aging of the red cell, the mechanical fragility finally exceeds that of the general red cell population. They point out that although the "wear and tear" imposed on the erythrocyte in the laboratory test is more severe than that which may occur in vivo because of the necessity for testing over a short period of time, the life span of the cell may be partly limited by factors which increase its mechanical fragility in vivo.

The present study was undertaken to establish the range of values of mechanical fragility of erythrocytes in normal infants and children, using a modified and relatively inexpensive rotator.

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Methods and Materials

The mechanical fragility of red cells was determined in 40 newborn infants between 1 and 6 days of age, in 43 children between the ages of 6 months and 13 years and in 25 adults, all of whom were normal.

The mechanical fragility was determined by the method of Shen, Castle and Fleming[3] with modifications suggested by Young[6] and Gardner.[8] Defibrination was performed under aseptic precautions in 50 cc. Erlenmeyer flasks containing glass beads. The fibrin clot was then removed with sterile applicators. Part of the specimen was used immediately for the determination of mechanical fragility, and the rest was incubated for 24 hours at 37 C. before testing.

A photograph of the rotator* used in our laboratory for subjecting red cells to the trauma of rolling glass beads is shown in figure 1. This apparatus has the advantages of being simple, portable and inexpensive. It is identical in principle with the machines in use in other laboratories.

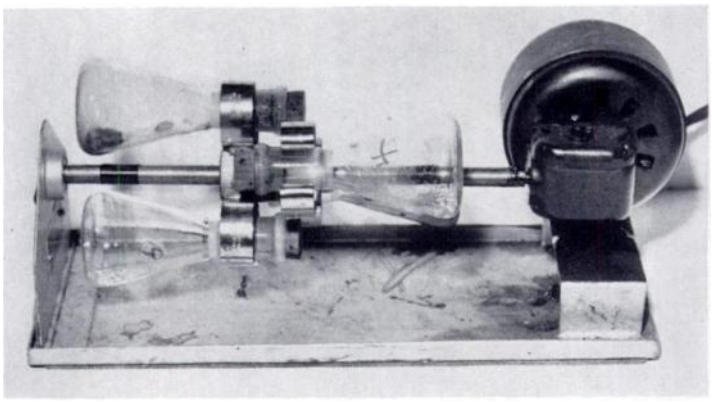

Fig. 1.—Photograph of mechanical rotator used in the study of mechanical fragility.

The hematocrit of the specimen was adjusted to 35 per cent by adding or removing plasma and 0.5 cc. of the corrected specimen was placed in the apparatus and rotated for 60 minutes at 100 r.p.m. in a 50 cc. Erlenmeyer flask containing 8 glass beads of 4.0 mm. diameter, with the center of the flask 3.25 cm. from the center of the wheel. Frothing was not a significant problem probably because of the small radius of the wheel.

Of the remaining portion of the corrected specimen, 0.1 cc. was added to 1 cc. of distilled water (complete osmotic lysis) in a centrifuge tube; another 0.1 cc. was added to 1 cc. of 1.25 per cent NaCl solution (no osmotic lysis) in a second tube. The amounts of free hemoglobin in the supernatant of these tubes were determined in an Evelyn photoelectric colorimeter, and the results designated H (complete lysis) and 0 (no lysis) respectively.

Two 0.1 cc. samples from the rotated specimen were added to each of two tubes containing 1 cc. of 1.25 per cent NaCl solution. These were similarly centrifuged, and the free hemoglobin in the supernatants determined. Results were designated S_1 and S_2 respectively.

The mechanical fragility of the sample was then determined by use of the formula:

$$MF = \frac{S \text{ minus } 0}{H \text{ minus } 0}$$

and expressed as a percentage value, where S is the average of S_1 and S_2.

* Manufactured by J. H. Emerson Company, 22 Cottage Park Avenue, Cambridge, Mass.

Results

The results of the determinations for each series of cases are shown graphically in figure 2 and summarized in table 1. In all cases tested we found excellent correlation between the duplicate rotated samples.

The most striking finding to be noted is that the mean mechanical fragility of nonincubated red cells in newborns (7.1 per cent) is nearly twice as great as that of older children and adults (4.0 per cent each). This difference appears much less striking in the results of mechanical fragility of incubated cells (table 1).

The mean mechanical fragility of red cells, both incubated and nonincubated, of children of 6 months to 13 years is noted to be essentially identical with the

TABLE 1.—*Summary of Normal Values for Mechanical Fragility of Erythrocytes for Infants, Children and Adults*

	Normal value for mechanical fragility							
	Unincubated				Incubated			
	No.	Mean	Standard error	Range	No.	Mean	Standard error	Range
1. Newborns	40	7.1%	±0.41%	1.1–11.5%	25	13.4%	±1.2%	3.7–21.6%
2. Children	43	4.0%	±0.25%	0–6.0%	36	10.5%	±0.73%	3.5–23.0%
3. Adults	25	4.0%	±0.30%	1.3–6.7%	15	10.8%	±1.35%	4.6–19.0%

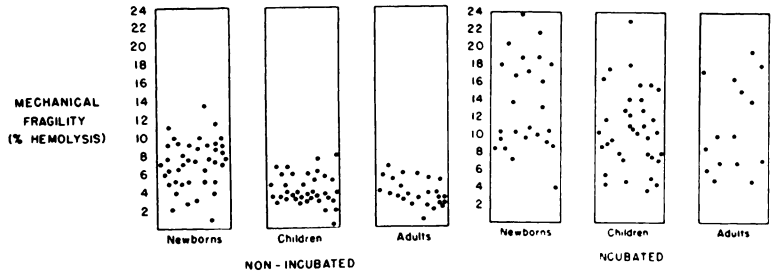

FIG. 2.—Results of mechanical fragility of erythrocytes of normal infants, children and adults.

values for normal adults. There appears to be no significant difference in the normal range for the children of different ages.

Discussion

The data show that this modified and simple rotator is satisfactory for determining mechanical fragility.

The mean value for normal adults as determined by this method is in the same general range as the few values presented by previous authors. Any differences might be due to technical variations, such as speed of rotation, differences in numbers of beads, and the shorter flask-to-axle distance in our rotator. Practically, the significant deviations from normal are usually sufficiently marked as to make these slight differences of little importance.

The significance of the increased mechanical fragility of erythrocytes in the newborn can only be inferred at this stage. Goldbloom and Gottlieb[9, 10] and Mollison[11] have demonstrated increased destruction of red cells in newborn infants. It is conceivable that the feature of decreased cellular resistance may be a factor in this hemolytic process, and may partially account for elevation of indirect bilirubin in the neonatal period. The importance of this factor might be further elucidated by serial studies over the first few days of life[12] and by correlation of results with serum bilirubin levels. It is noteworthy that none of the infants tested had erythroblastosis or marked physiologic icterus at the time of testing.

The determination of mechanical fragility of incubated erythrocytes was first made in studying the mechanisms of red cell destruction in patients with congenital hemolytic anemia. In this condition the red cells are considered to be trapped within the spleen and in vitro incubation is therefore carried out to mimic the changes that may occur in red cells during stagnation within the spleen. The determination of mechanical fragility of incubated erythrocytes is at best a rather crude test, and the results in this, as in other series, show a considerably wider range of scatter than do the results with freshly drawn red cells. This difference is undoubtedly due to the number and variability of factors which may affect the resistance of the red cell in the course of incubation. Such factors include: (1) minor degrees of bacterial contamination, (2) precipitation of water inside incubated flasks, (3) pH changes, (4) autohemolysins, (5) evaporation from flasks not thoroughly sealed during incubation, (6) variability in duration of incubation and (7) primary extracellular osmotic differences, e.g. serum proteins and blood sugar.

However, despite the possible influence of all these factors, the variation from the normal range is usually sufficiently and consistently marked in disease states to be distinctly abnormal.

Studies of mechanical fragility in a number of diseases characteristically associated with anemia are in progress, and will be presented in future publications.

Summary and Conclusions

1. The mechanical fragilities of incubated and nonincubated erythrocytes of normal newborn infants, children and adults have been determined through the use of a simplified rotator.

2. The mean mechanical fragility of nonincubated erythrocytes was 7.1 per cent for newborns, 4.0 per cent for children and adults; the mean mechanical fragility of incubated erythrocytes was 13.4 per cent for newborns, 10.5 per cent for children and 10.8 per cent for adults.

3. The possible relationship of increased mechanical fragility of erythrocytes in the newborn to increased hemolysis in the neonatal period is suggested.

4. Possible causes of variation in the determination of mechanical fragility of incubated erythrocytes are discussed.

References

[1] Meltzer, S. J. and Welch, W. H.: The behavior of the red blood corpuscles when shaken with indifferent substances. J. Physiol. *5:* 255, 1884.

[2] ROUS, P. AND TURNER, J. R.: The preservation of living red blood cells in vitro. J. Exper. Med. *23:* 219, 1916.

[3] SHEN, S. C., CASTLE, W. B. AND FLEMING, E. M.: Experimental and clinical observations on increased mechanical fragility of erythrocytes. Science *100:* 387, 1944.

[4] YOUNG, L. E. AND LAWRENCE, J. S.: Atypical hemolytic anemia. Arch. Int. Med. *77:* 151, 1946.

[5] —: Hemolytic disorders: Recent advances in diagnosis, prevention, and treatment. New York State J. Med. *47:* 1875, 1947.

[6] —, IZZO, M. J. AND PLATZER, R. F.: Hereditary spherocytosis. Blood *6:* 1073, 1951.

[7] STEWART, W. B., IZZO, M. J. AND YOUNG, L. E.: Age as affecting osmotic and mechanical fragility of dog erythrocytes tagged with radioactive iron. J. Exper. Med. *91:* 147, 1950.

[8] GARDNER, F. H., MCELFRESH, A. E., HARRIS, J. W. AND DIAMOND, L. K.: The effect of adrenocorticotrophic hormone in idiopathic acquired hemolytic anemia as related to the hemolytic mechanisms. J. Lab. & Clin. Med. *37:* 444, 1951.

[9] GOLDBLOOM, A. AND GOTTLIEB, R.: Studies on icterus neonatorum. J. Clin. Investigation *8:* 375, 1930.

[10] — AND —: Icterus neonatorum. Am. J. Dis. Child. *38:* 57, 1929.

[11] MOLLISON, P. L.: Physiological jaundice of the newborn. Lancet *1:* 513, 1948.

[12] HSIA, D. Y. Y., ALLEN, F. H., JR. AND GELLIS, S. S.: Serum bilirubin levels in newborn infants. To be published.