THE DISTRIBUTION CURVE OF ERYTHROCYTE FRAGILITY

A Different Method of Presentation of Fragility of Erythrocytes to Hypotonic Saline, with Preliminary Remarks on the Function of Reticulocytes

By J. H. Bolton M.D.

INTRODUCTION

Following Haden's demonstration of the close correlation between spheroctosis and the fragility of erythrocytes to hypotonic saline, Dameshek and Miller postulated that spheroctosis was a preliminary stage in the destruction of the red cell; i.e., partial hemolysis. They were able to show experimentally that the degree of spheroctosis was related to the concentration of hemolysins in the blood and that this was also correlated with fragility. They also described cases of acquired hemolytic anemias with spheroctosis and increased fragility, thus emphasising the danger of using spheroctosis and increased fragility as criteria for the diagnosis of familial acholuric jaundice. In addition, they demonstrated the presence of hemolysins in a number of cases of hemolytic anemia.

The chain of reasoning is that hemolysins may lead to partial damage to the cell; this is followed by entry of fluid and loss of the biconcave disc form with approximation to a spherical shape. Fluid can enter a cell to a certain maximal degree, but if this is exceeded, the cell will rupture. Hence, if a cell is already partially spherical, it will rupture with a smaller entry of fluid than if it possesses the biconcave disc form. As the cell acts as an osmometer, the amount of fluid entering the cell will depend on the concentration of electrolytes on either side of the membrane, and this forms the basis of the fragility test with hypotonic saline.

METHOD

As usually presented, the fragility curve is sigmoid in shape and alteration of form is difficult to assess. But this sigmoid shape is due to the fact that the curve is a composite one and at each decreasing concentration of saline the percentage hemolysis at any particular point is the sum of all the hemolysis which has occurred at higher concentrations of saline, plus the actual hemolysis occurring at that point. The curve is, in fact, a cumulative curve—known statistically as an ogive.

This being so, we can readily convert our findings in any case to indicate what degree of hemolysis occurs at any particular saline concentration.

All that is necessary is to deduct from the percentage hemolysis occurring at any particular level, that which occurred at the immediately higher concentration, and this will give the percentage hemolysis occurring in the range of saline concentration between these two points.

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The normal range of fragility as given by Creed is shown in table 1 with its conversion to the derived curve. This is shown graphically in figure 1.

| Table 1 |
|------------------|------------------|------------------|------------------|------------------|
| Percentage of saline | 0.28 | 0.32 | 0.36 | 0.40 | 0.44 |
| Total maximal percentage of cells hemolyzed | 100 | 98 | 90 | 46 | 10 |
| Total minimal percentage of cells hemolyzed | 98 | 90 | 45 | 10 | 0 |
| Average saline concentration | 0.16 | 0.30 | 0.34 | 0.38 | 0.42 | 0.46 |
| Maximal percentage of cells hemolyzed | 0 | 2 | 8 | 44 | 36 | 10 |
| Minimal percentage of cells hemolyzed | 2 | 8 | 45 | 35 | 10 | 0 |

Fig. 1. Usual representation of normal range of fragility as sigmoid curves and the derived curves (dotted) of the distribution of red cell fragility.

The grouping is rather coarse but it will be seen that the two derived curves are very similar in form to that found in a Price-Jones curve.

If fragility were directly related to spherocytosis and spherocytosis only, this derived curve could be considered to be that of the distribution of cells in terms of their degree of spherocytosis; i.e., a curve directly comparable with a Price-Jones curve of diameter. Unfortunately, as pointed out by Ponder, the red cell does
not act as a perfect osmometer, its degree of perfection in this respect being reduced by decrease in the tonicity of its environment. This is the probable explanation of the skewness of the curves shown for the normal distribution of hemolysis.

**Fig. 1.** Fragility distribution in a case of familial acholuric jaundice before and after splenectomy compared with the normal (dotted).

**Fig. 3.** Fragility distribution in a case of familial acholuric jaundice reported by Whitby and Hynes.

**APPLICATION TO CASES OF ANEMIA**

Derived curves were calculated for various types of anemia. Figure 2 shows the findings in a case of familial acholuric jaundice before and after splenectomy. It will be noted that after splenectomy a smooth curve is obtained, abnormal in that its single mode occurs at a higher concentration of saline than normal. Before splenectomy the curve is not completely smooth but shows a second mode at a saline concentration of 0.65 per cent. This bimodal curve is seen again in a case described by Whitby and Hynes¹⁹ (fig. 3) and suggests
that the cells are not homogenous but are composed of two populations of differing susceptibility to hypotonic saline.

This aspect is further emphasised in a case of hypersplenism (fig. 4) where we find, as might be expected, a maximal normal mode, but in addition, a marked secondary mode at a concentration of 0.45 per cent saline—a decidedly bimodal curve. Further examination of this curve shows that the abnormal peak involves some 30 per cent of the cells and this corresponds to the reticulocyte level at that time.

Examination of other biphasic curves shows a similar correlation between the height of the secondary mode and the reticulocyte level. The correlation is even closer if, instead of taking the height of the secondary mode, we calculate the percentage of cells involved in that mode. This can be done only approximately, but gives a rank correlation of 0.67 per cent.

A case of acute hemolytic anemia reported by Ross and Paegel16 is interesting.

<table>
<thead>
<tr>
<th>Case</th>
<th>Hemolysis due to secondary mode (%)</th>
<th>Reticulocyte (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. &amp; P</td>
<td>8</td>
<td>5.4</td>
</tr>
<tr>
<td>D. 1</td>
<td>7</td>
<td>16.0</td>
</tr>
<tr>
<td>D. 2</td>
<td>5</td>
<td>9.6</td>
</tr>
<tr>
<td>R. 2</td>
<td>12</td>
<td>16.0</td>
</tr>
<tr>
<td>M. 1</td>
<td>6</td>
<td>13.4</td>
</tr>
<tr>
<td>D. 1</td>
<td>10</td>
<td>11.0</td>
</tr>
<tr>
<td>B. 1</td>
<td>10</td>
<td>30.0</td>
</tr>
<tr>
<td>B. U</td>
<td>0</td>
<td>6.0</td>
</tr>
<tr>
<td>C. 1</td>
<td>10</td>
<td>9.6</td>
</tr>
<tr>
<td>C. 2</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>R. 1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>N.</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
in that it shows three modes (fig. 5). Reticulocyte counts and spherocyte counts were obtained on the same day, and their percentage corresponds roughly with the percentage of hemolysis involved in each particular modal area.

A similar curve can be derived from the results of Goldbloom and Gottlieb, who were investigating normal umbilical cord blood. By examination of the residual cells after hemolysis, they showed that the early peak corresponded to disappearance of reticulocytes and the middle peak to destruction of nucleated red cells.

![Distribution Curve of Erythrocyte Fragility](image)

**Fig. 5.** Fragility distribution in a case of acute haemolytic anaemia following sulphadiazine sensitization reported by Ross and Paegel. Note the three separate modes during the attack and the single mode on recovery.

**Table 3**

<table>
<thead>
<tr>
<th>Test</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulocytes</td>
<td>5.4% Early mode</td>
</tr>
<tr>
<td>Spherocytes</td>
<td>46.8% Middle mode</td>
</tr>
</tbody>
</table>

**Discussion**

Erythrocyte fragility has been expressed in different ways by different authors. It may be expressed as the range of saline concentration within which hemolysis occurs and notice may be taken particularly of the point where hemolysis is first seen—"maximal fragility"—or the point where hemolysis is complete—"minimal fragility." More commonly it is shown by means of a diagram relating percentage hemolysis to percentage of saline, as previously described. All these methods are open to the criticism that they deal only with the extreme variability of the phenomena and pay little attention to the form of variation.

Various attempts have been made to obtain a single expression for fragility and the most satisfactory discussion on the subject is that of Janet Vaughan. She used the median as her descriptive statistic and recognized that the sigmoid curve was an integral of cell fragility at specific levels. Unfortunately, she assumed that the curve was normal in type and on this basis attempted to express the variability as the slope (b) of the regression line of hemolysis (in terms of the standard deviation from the median) on saline concentration. Extreme values were neglected and,
not surprisingly, she found this second statistic of little practical importance. A similar approach was used by Hunter, who also realized that more than one maximum might be found, but was unable to explain this. His methods were used by Parpart et al., but uncritically in that a 'normal' curve was assumed.

From the previous results it will be seen that the curve of fragility is unlikely to be normal and in pathologic conditions frequently shows two or more modes. In the presence of such irregular curves the use of mean, median, or range, can lead to very erroneous conclusions and the advantage of the suggested method of presentation is that it permits the form of the distribution to be determined before using any statistic to describe position.

The apparent relationship between secondary modes and reticulocytes does not necessarily mean that reticulocytes are more fragile than normal. The presence of reticulocytes may only indicate increased marrow activity and this may be associated with the production of abnormally fragile cells quite apart from reticulocytes.

The literature on the subject of reticulocyte fragility is confusing, it being variously alleged that reticulocytes are less fragile, equally fragile and more fragile than normal.

That young cells are more fragile than normal appears to have been conclusively demonstrated by Cruz et al. They used dogs rendered anemic by bleeding and tagged transfused red cells with radioactive iron so that their age could be followed. A marked difference in the fragility between young and old cells was found and this difference was maintained for five days. Thereafter the fragility of the old and the new cells became virtually identical. Reticulocyte counts were not done, so that no further conclusions can be drawn with regard to these particular cells.

Key concluded from his work that reticulocytes were normally fragile. He used rabbits as an experimental animal and emphasized the difficulty in performing reticulocyte counts after partial hemolysis. He pointed out that 'ghost' cells would still retain their reticulum which would stain and cause confusion in counting and also noted the danger in counting only the sedimented cells at the bottom of the tube. If this were done, reticulocytes would appear to be more resistant than normal, but if samples were taken from the bottom of the tube and from the cells floating free in the plasma, no constant differences could be obtained. This technic is not well described, but, from the above description, appears to be inadequate.

Swjatskaja concluded that reticulocytes were less resistant than normal but his views were based on an apparent correlation between reticulocyte counts in the peripheral blood of anemic dogs and changes in the fragility of the cells. Residual cells were not examined for reticulocytes and his curves do not correlate accurately in time. The increase in resistance could have been due to the presence of target cells which, according to Bohrod, appear shortly after hemorrhage and are more resistant to hypotonic saline.

The most thorough examination of the problem was performed by Goldbloom and Gottlieb, who did reticulocyte counts and further fragility tests on the residual cells in each tube after a fragility estimation. Their work was performed on normal infant's blood from the umbilical cord and their conclusion was that reticulocytes were more fragile than normal.

Further work on this point is indicated.

In the application of these curves to actual cases of anemia, it would appear possible to decide whether an apparent increase in fragility was due to the result of an active bone marrow with the production of more fragile cells or to an intrinsic defect in some or all of the cells present. In this way the test should become enhanced in its diagnostic value.
DISTRIBUTION CURVE OF ERYTHROCYTE FRAGILITY

SUMMARY

1. A simple method of representing erythrocyte fragility as a distribution curve of actual cellular fragility is described.
2. The importance of deciding the form of a distribution before using summarizing statistics is emphasized.
3. The danger of concluding that abnormal fragility is present in the presence of increased marrow activity is pointed out.
4. Possible applications in diagnosis are suggested.

REFERENCES

THE DISTRIBUTION CURVE OF ERYTHROCYTE FRAGILITY: A DIFFERENT METHOD OF PRESENTATION OF FRAGILITY OF ERYTHROCYTES TO HYPOTONIC SALINE, WITH PRELIMINARY REMARKS ON THE FUNCTION OF RETICULOCYTES

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