The Osmotic Resistance of Leukocytes in Peripheral Blood and Splenic Circulation of the Rabbit

By E. STORTI, L. BELLESIA AND E. LUSVARGHI

IT HAS BEEN suggested by many investigators that at least in some conditions the spleen is one of the main sites of sequestration and removal of leukocytes. Little is known of the mechanisms of such splenic activity. In the hope of gathering useful information on this topic we have measured the osmotic resistance of leukocytes of peripheral blood, splenic venous and splenic arterial blood of the rabbit. The test of leukocyte resistance for evaluating the degree of leukocytic osmotic fragility as introduced by Storti and Pederzini and used by others gives information on at least one of the important qualities of leukocytes.

METHODS

Blood from the fleshy end of the finger is drawn into a counting pipet to the mark 1. This is followed immediately by taking up 0.20% NaCl, bringing the contents to the mark 11, and mixing. For studies of human leukocytes 0.20% NaCl solution is used but, for studies of the more fragile rabbit leukocytes 0.33% NaCl solution is used. Immediately after the blood is withdrawn and mixed and at intervals of 30, 60, 120, 180 minutes counts of leukocytes and observations of their morphology are made in a Bürker chamber. The pipet must be agitated 10 to 15 times to homogenize its contents before each count. Observations are made in dark field or direct light. In the latter case the cell suspension is stained lightly to make morphologic features more readily visible. A tiny drop of Giemsa stain (not diluted but filtered several times to eliminate all traces of precipitate) is placed on the border of the Bürker chamber. A drop of the contents of the pipet is allowed to fall on the stain and is mixed slowly with the aid of the pipet. The mixture is pushed to the border of the Bürker chamber so that the counting area is filled. The pipet is maintained at room temperature (20-25 C.).

Cells presenting evident cytoplasmic and nuclear modifications (breakage of cell membranes with loss of cytoplasm or nucleus, shrinkage, destruction of the cell) are excluded in counts following the first one (fig. 1).

The amount of fall of leukocyte counts carried out after 30, 60, 120, 180 minutes measures the leukocyte resistance to the hypotonic saline solution. In order to evaluate the changes with more clarity the data are plotted. The percentage decrease of counts is plotted on the ordinate axis and time on the abscissa. Curves with rising slopes are obtained. From a qualitative viewpoint the process affects granulocytes, monocytes and lymphocytes in the same manner. Quantitatively the phenomena are different for granulocytes as compared with other cells.

The area between the curve and the time axis is referred to as the index of leukocyte resistance (LRI) and it may be calculated by using the formula: $A = \int_0^x ydx$ where $y$ is the interpolative function and $x$ is the unit time, fixed at 30 minutes in the graph.

MATERIAL

Studies were carried out on 15 healthy rabbits of both sexes, each weighing about 3 Kg. Blood samples were withdrawn simultaneously from splenic artery and vein following a small abdominal incision, and from the marginal vein of the ear.

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Alterations of granulocytes and mononuclear cells (monocytes and lymphocytes) observed in hypotonic saline solution. Leukocytes exhibiting the morphologic changes shown are excluded in counts following the initial count.

**THE RESISTANCE OF THE WHITE BLOOD CELLS IN SPLENIC CIRCULATION AND PERIPHERAL BLOOD OF THE RABBIT**

Curves and index of the resistance of the poly- and mononucleates. Averaged of the values obtained in 15 rabbits.

Fig. 1.—Alterations of granulocytes and mononuclear cells (monocytes and lymphocytes) observed in hypotonic saline solution. Leukocytes exhibiting the morphologic changes shown are excluded in counts following the initial count.

Fig. 2.—Percentage decreases of counts of granulocytes and mononucleates (monocytes and lymphocytes) in hypotonic saline solution at various time intervals (left and middle boxes), and the leukocyte resistance indices of leukocytes from various sources (right box.)
TABLE 1.—Mean Values of L.R.I. (Leukocyte Resistance Index), Differences between L.R.I. of Cells from Various Sites, and Results of Statistical Analyses of Data

<table>
<thead>
<tr>
<th>L. R. I. (Mean Values)</th>
<th>Difference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polynucleates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenic artery: 15,326 cm$^2$</td>
<td>4,595 cm$^2$</td>
<td>Significant difference at 5% limit*</td>
</tr>
<tr>
<td>Splenic vein: 19,921 cm$^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenic artery: 15,326 cm$^2$</td>
<td>2,700 cm$^2$</td>
<td>Significant difference at 5% limit*</td>
</tr>
<tr>
<td>Peripheral blood: 18,026 cm$^2$</td>
<td>1,895 cm$^2$</td>
<td>Significant difference at 5% limit*</td>
</tr>
<tr>
<td>Splenic vein: 19,921 cm$^2$</td>
<td>2,981 cm$^2$</td>
<td>Significant difference at 5% limit*</td>
</tr>
<tr>
<td>Peripheral blood: 18,026 cm$^2$</td>
<td>174 cm$^2$</td>
<td>The difference is not significant</td>
</tr>
</tbody>
</table>

**Mononucleates**

| Splenic artery: 7,983 cm$^2$ | 2,807 cm$^2$ | Significant difference at 5% limit* |
| Splenic vein: 10,790 cm$^2$ |            |                  |
| Splenic artery: 7,983 cm$^2$ | 2,981 cm$^2$ | Significant difference at 5% limit* |
| Peripheral blood: 10,964 cm$^2$ | 174 cm$^2$  | The difference is not significant |
| Splenic vein: 10,790 cm$^2$ |            |                  |
| Peripheral blood: 10,964 cm$^2$ |            |                  |

* The statistical methods used were those described by Cochran and Cox. 

RESULTS

Figure 2 shows the resistance curves for granular leukocytes (polynucleates) and monocytes and lymphocytes (mononucleates) from splenic artery, splenic vein, and a peripheral region of the body. The LRI of cells from the various sites are shown in the box on the right. The curves and indices represent averages of values obtained in the series of 15 animals.

Table 1 shows mean values of the LRI for leukocytes from various sites, differences in mean values for LRI between selected sites, and information regarding the statistical significance of such differences.

Our findings indicate (1) leukocytes leaving the spleen are much less resistant to the hypotonic saline solution than is the case for leukocytes entering the spleen; (2) granulocytes in splenic venous blood are less resistant than those of the peripheral circulation. The data suggest that the spleen of the healthy rabbit modifies the leukocyte resistance to the hypotonic saline solution.

DISCUSSION

The results show that leukocytes passing through the spleen undergo a change resulting in the lowering of their resistance to hypotonic saline solution; that is, they become highly fragile. So far we are unable to explain the mechanism by which this change occurs. The biologic meaning of the change is also obscure. However it is not surprising that leukocytes passing through the spleen where they probably are detained for more or less definite periods of time may undergo some changes. Recent work indicates that the spleen has the faculty of sequestering and removing leukocytes from the circulating blood. It is also known that erythrocytes undergo changes in passing through the spleen in analogous fashion. Other recent studies we have carried out show that the leukocyte resistance
OSMOTIC RESISTANCE OF LEUKOCYTES

index of leukocytes leaving the lung is much less than that of leukocytes entering the lung. These findings are in excellent agreement with the conclusions of other investigators concerning the behavior of leukocytes in the pulmonary circulation.

With regard to the biologic significance of our findings, that is the meaning of the change affecting leukocytes passing through the spleen, we can but suppose that such a phenomenon is related to a leukocatheretic function of the spleen.

Some data we have gathered show that the degree of leukocyte resistance is correlated with their life span. Leukocytes in either physiologic or pathologic conditions which have an osmotic resistance of lower degree than that we consider to be normal have a life span of a markedly lower duration than normal. It seems highly probable that the change in resistance affecting leukocytes during their passage through the spleen is a manifestation of one of the mechanisms by which the spleen regulates the life span of leukocytes.

SUMMARY

By studying the behavior of the resistance of leukocytes to hypotonic saline solution we have found that leukocytes leaving the spleen are much less resistant than those entering it.

It is felt that the change in resistance affecting leukocytes on their passage through the spleen is in all probability the manifestation of one of the mechanisms by which the spleen regulates the life span of leukocytes.

SUMMARIO IN INTERLINGUA

Per studiar le resistencia de leucocytos a hypotonic solutiones salin, nos ha constatat que leucocytos que quita le splen es multo minus resistente que leucocytos que entra in illo.

Es opinate que le alteration del resistencia occurrente in leucocytos que passa per le splen es multo probabilmente le manifestation de un del mechanismos per que le splen regula le duration vital del leucocytos.

REFERENCES

STORTI, BELLESIA AND LUSVARGHI 833


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