THE PROPERDIN CONTENT OF MATERNAL AND INFANT BLOOD

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In the course of some work on immunity at birth (Edwards, Griffiths and Swift, 1958) it became necessary to investigate the role played by properdin. We were unable to duplicate the results obtained by Pillemer, Blum, Lepow, Wurz and Todd (1956) nor were we any more successful with the modifications introduced by Isliker (1956) or Soulier, Ménaché and Larrieu (1957). Analysis of our results showed that the discrepancies could only be due to very low levels of properdin in the population, and the original technique was not sufficiently sensitive. We therefore modified the method to overcome this defect.

Materials and Method

2. Fresh human serum stored at −25° C. until required.
3. Buffer: NaCl 85·00 g.
   Diethyl-barbituric acid 5·75 g.
   Barbitone sodium 3·75 g.
   MgCl₂ 5·00 g.
   CaCl₂ 1·50 g.

Dissolve in hot water and make up to 2 litres. Dilute with an equal amount of water for use.
4. 1% washed sheep cells sensitized with 10 units/ml haemolysin.
5. Zymosan 12 mg./ml. in saline.

The test is carried out in two stages. In the first, equal quantities of serum and R3 are mixed. This ensures that adequate amounts of C₁, C₂ and C₄ are present, and makes C₃ the limiting factor. A 1/40 dilution of the mixture was titrated according to the scheme shown in Table 1. The results are plotted, density against actual amount of serum used, and the 50% haemolytic dose calculated. This titration gives the C₃ content of the serum.

In the second stage equal amounts of serum and zymosan solution are mixed and incubated at 37° C. for one hour, inverting the tubes every 10 minutes. At this stage zymosan combines with any properdin present and the properdin-zymosan complex formed selectively removes C₃ from the serum.

At the end of the incubation period the mixture is centrifuged, and to 1 ml. supernatant is added 1 ml. R3 and 3 ml. buffer. This gives a 1/10 dilution of the original serum and is titrated as in Table 1, using the 1/10 dilution instead of the 1/40 dilution used in stage one. This titration gives the residual C₃. The difference between the first and second titrations showed the amount of C₃ removed by the properdin-zymosan complex in the proportion, according to Pillemer et al. (1956), of 1 unit properdin to 120 units C₃.

Results

The results are plotted in the Figure with the corresponding regression lines.

The mean properdin C₃ levels are collected in Table 2 with the corresponding correlation coefficients and the value of Fisher's Z. In each case Z is over nine times its standard error and is highly significant and indicates an interdependence between C₃ and properdin that is extremely close. This may be due to C₃ and properdin being so similar that our technique is unable to discriminate between them, or they may be present in amounts that bear

| Table 1 |
|---|---|---|---|---|---|---|---|---|---|---|
| Tubes |   |   |   |   |   |   |   |   |   |   |
| Serum 1/40 | . | . | . | . | . | . | . | . | . | . |
| Buffer | . | . | . | . | . | . | . | . | . | . |
| Sensitized sheep R.B.C. | . | . | . | . | . | . | . | . | . | . |

Incubate at 37° C. for half an hour, centrifuge and read in photometer at 550/W.
PROPERDIN CONTENT OF MATERNAL AND INFANT BLOOD

1.00

0.90

0.80

0.70

0.60

0.50

0.40

0.30

C3 LEVEL IN UNITS

Maternal blood

Cord blood

Fig.—Comparison of properdin and C3 levels.

a quantitative relation to each other. A third explanation is that the effects postulated as due to properdin are really due to C3.

The mean amount of C3 removed by the properdin-zymosan complex was 78.8% ± 2.3% for the mothers and 78.4% ± 2.6% for their infants. These are so close that it is difficult to believe that there are two independent systems present. Nelson (1958) has cast some doubt on the necessity for introducing a new immune system and our results based on different evidence are in accordance with his.

If properdin is a distinct immunological entity then the levels of Kentish mothers and their infants are far below those of their North American counterparts. Whatever the explanation we do not believe that properdin plays any part in protecting patients from infection. Our patients were completely free from any complications during the trial in spite of their properdin levels being a small fraction of those reported in similar areas in the United States.

Summary

A method is reported for the estimation of small amounts of properdin. Reasons are given for believing that its effects are really due to C3 and that there are insufficient grounds for postulating a new immune system.

Table 2

<table>
<thead>
<tr>
<th>Properdin/units</th>
<th>Mothers</th>
<th>Infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3/units</td>
<td>0.76</td>
<td>0.40</td>
</tr>
<tr>
<td>Correlation coefficient r</td>
<td>0.97</td>
<td>0.56</td>
</tr>
<tr>
<td>Fisher's Z</td>
<td>2.08</td>
<td>1.94</td>
</tr>
<tr>
<td>Standard error of Z</td>
<td>0.23</td>
<td>0.23</td>
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</tbody>
</table>

REFERENCES


