Factor VIII and antibody levels in plasma fractions prepared by cryoprecipitation

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SYNOPSIS Most of the antibody activity of plasma was found to remain in the supernatant fraction following the preparation of factor-VIII-rich cryoprecipitates. The low level of antibody remaining in these cryoprecipitates indicated that they could safely be used as a source of factor VIII for replacement therapy. Washing the cryoprecipitates further reduced their antibody titres without materially affecting their factor VIII content.

Following recent reports of success in preventing Rhesus isoimmunization in the early puerperium, by the injection of a high titre anti-D antibody (Woodrow, Clarke, Donohoe, Finn, McConnell, Sheppard, Lehane, Russell, Kulbe, and Durkin, 1965) it appears likely that this will become a standard procedure. Large amounts of high titre anti-D (anti-Rh) gamma globulin will be required and these will have to be obtained from deliberately immunized male volunteers.

This study was undertaken to determine whether plasma obtained from Rh-sensitized individuals could be used to prepare an antihaemophilic globulin-rich cryoprecipitate without detracting from its usefulness as a source of anti-D.

MATERIALS AND METHODS

Two 20 ml aliquots of blood were taken from Rh-sensitized women using disposable plastic syringes. These were each added to 5 ml acid citrate dextrose (ACD) in glass Universal containers and immediately centrifuged for 15 minutes at 3,000 r.p.m. at 4°C in an MSE Mistral centrifuge. The plasma was pooled and two 10 ml aliquots were placed in Universal containers labelled 'cryoprecipitate' and 'cryoprecipitate (washed)', the remainder being labelled 'whole plasma'. The three samples were frozen at −20°C for several hours after which the first two aliquots were transferred to a refrigerator and allowed to thaw out overnight at 4°C. These samples were centrifuged at 4°C for 15 min at 3,000 r.p.m. and the supernatant plasma was decanted, pooled, and refrozen. One ml citrate-saline at 4°C (1 part 3·8% trisodium citrate and 9 parts 0·9% NaCl) was added to the 'cryo-

precipitate' specimen which was immediately refrozen at −20°C while 10 ml citrate saline was added to the 'cryo-precipitate (washed)' specimen. This was mixed by gentle inversion without the precipitate being allowed to dissolve and centrifuged at 4°C and 3,000 r.p.m. for 15 minutes. The supernatant was discarded, a further 1 ml citrate saline added, and the specimen refrozen. Within a week of preparation, the samples were thawed out at 37°C and factor VIII levels in both the cryoprecipitate specimens measured by a one-stage method (Hardisty and Macpherson, 1962). A pool of blood from at least five haematologically normal staff was taken as 100% normal. Antibody titres were then measured in all four specimens by the indirect antiglobulin (Coombs) test except where otherwise stated.

RESULTS

There was no reduction in antibody titre in the supernatant plasma after removal of the cryoprecipitate as compared with whole plasma (Table I).

The cryoprecipitates showed on average a four to eightfold drop in titre from the original plasma, and washing of the cryoprecipitate resulted in a further halving of the antibody level. These results were not corrected for volume changes, and as the cryoprecipitate had been reduced to one tenth of the volume of the original plasma it would appear that only about 1% of the antibody remained in the washed cryoprecipitate.

Factor VIII levels in the cryoprecipitate averaged 920% of normal, ie, equivalent to 92% in the volume of the original plasma. Washing the cryoprecipitate had little effect on the factor VIII level except in one instance (case 8).
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TABLE I

RESULTS FOR ANTIBODY TITRES AND FACTOR VIII

<table>
<thead>
<tr>
<th>Patient</th>
<th>Antibody</th>
<th>Antibody Titres</th>
<th>Factor VIII (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole Plasma</td>
<td>Supernatant</td>
</tr>
<tr>
<td>1</td>
<td>Anti-D</td>
<td>1 in 128</td>
<td>1 in 256</td>
</tr>
<tr>
<td></td>
<td>Anti-D (papain)</td>
<td>1 in 64</td>
<td>1 in 64</td>
</tr>
<tr>
<td>2</td>
<td>Anti D (papain)</td>
<td>1 in 64</td>
<td>1 in 64</td>
</tr>
<tr>
<td>3</td>
<td>Anti D (papain)</td>
<td>1 in 8</td>
<td>1 in 4</td>
</tr>
<tr>
<td></td>
<td>Anti D (papain)</td>
<td>1 in 4</td>
<td>1 in 4</td>
</tr>
<tr>
<td>5</td>
<td>Anti D</td>
<td>1 in 4</td>
<td>1 in 2</td>
</tr>
<tr>
<td>6</td>
<td>Anti D</td>
<td>1 in 64</td>
<td>1 in 64</td>
</tr>
<tr>
<td>7</td>
<td>Anti D</td>
<td>1 in 32</td>
<td>1 in 32</td>
</tr>
<tr>
<td>8</td>
<td>Anti Fy (a)</td>
<td>1 in 2</td>
<td>1 in 2</td>
</tr>
<tr>
<td>9</td>
<td>Anti D</td>
<td>1 in 128</td>
<td>1 in 256</td>
</tr>
<tr>
<td>10</td>
<td>Anti D</td>
<td>1 in 16</td>
<td>1 in 16</td>
</tr>
<tr>
<td>11</td>
<td>Anti D</td>
<td>1 in 16</td>
<td>1 in 32</td>
</tr>
<tr>
<td>12</td>
<td>Anti D</td>
<td>1 in 16</td>
<td>1 in 8</td>
</tr>
</tbody>
</table>

DISCUSSION

The experimental work of Finn, Clarke, Donohoe, McConnell, Sheppard, Lehane, and Kulke (1961) and Freda and Gorman (1962) showed that Rh (D)-positive red cells could be injected into a D-negative recipient without producing sensitization if the recipient was protected by passive immunization with plasma containing a high titre of incomplete anti-D. Subsequent reports by these groups (Woodrow et al., 1965; Freda, Gorman, and Pollack 1965) have confirmed this work and shown that a high degree of clinical success can be achieved in preventing Rh sensitization after pregnancy. Gorman, Freda, and Pollack (1965) and Clarke, Finn, Lehane, McConnell, Sheppard, and Woodrow (1966) have emphasized the superiority of gamma globulin, which can be given intramuscularly in small doses, to high titre whole plasma. If this approach is to be successful, large amounts of immune anti-D gamma globulin will be required and the only practical method of obtaining this is by plasmapheresis from deliberately immunized male volunteers, as the supply from Rh-sensitized mothers will soon dry up if the treatment proves to be successful. A large amount of plasma will thus be available, and the complete utilization of this material is desirable.

The above results show that a factor-VIII-rich cryoprecipitate can be easily prepared from plasma without detracting from its value as a source of antibody, and that this can provide a valuable additional source of antiahaemophilic globulin.

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REFERENCES

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Reports and Bulletins prepared by the Association of Clinical Biochemists

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