Antihemophilic Globulin Consumption During Blood Coagulation

By A. S. Douglas

It has been shown previously that blood contains a system for the formation of a powerful thromboplastin. The components which interact to form this are antihemophilic globulin, factor V, platelets, the Christmas factor and possibly factor VII.1,2 The successful formation of blood thromboplastin results in prothrombin conversion to thrombin, the prothrombin being utilized or consumed. The demonstration of defective prothrombin utilization is a nonspecific indication of failure to form blood thromboplastin. The consumption during clotting of other components of the coagulation system has not been extensively studied. Graham, Penick and Brinkhous4 and Douglas and Biggs5 have reported on the consumption of antihemophilic globulin (AHG) during normal blood coagulation. The object of the experiments now to be reported was to extend the observations on the normal, and to study the consumption of AHG during abnormal blood coagulation.

Material and Methods

Centrifugation.—All centrifugation was carried out at 4 C in a refrigerated centrifuge.

Glassware.—All clotting times were carried out in tubes of $\frac{1}{2}$ inch diameter maintained at 37 C., with the exception of the whole blood coagulation time when tubes of $\frac{3}{4}$ inch diameter were used.

Silicone Glassware.—Silicone M441 prepared by Imperial Chemical Industries, Ltd., was used.

Preparation of Reagents.—Aluminium hydroxide, 3.8 per cent sodium citrate and M/40 calcium chloride were prepared as described by Biggs and Macfarlane.7

Whole Blood Coagulation Time.—This was carried out as described by Biggs and Macfarlane.2

One-Stage "Prothrombin" Time.—The modification of Quick's one-stage procedure was used as described by Biggs and Macfarlane.3 The tissue extract was from human brain as prepared by Brown and Douglas.6

Prothrombin Consumption Test.—The procedure described by Douglas and Biggs4 was employed.

Antihemophilic Globulin Assay.—The thromboplastin generation test of Biggs and Douglas4 was modified to give a measure of AHG as described by Douglas and Biggs.4 A supply of hemophilic plasma was maintained in a deep freeze at -20 C. In the technic the AHG content of the test specimen was assayed by its ability to correct the deficiency of alumina-treated hemophilic plasma in the thromboplastin generation test; a mixture was made of equal parts of the test specimen and of hemophilic plasma after both had been treated with alumina. These were used together with platelets and normal serum to generate thromboplastin. The assay is made by comparison with the thromboplastic activity developed by dilutions of normal in hemophilic plasma. 100 per cent of AHG was represented by a mix-

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Fig. 1.—Antihemophilic globulin consumption in the normal. The mean result of the ten observations on the normal is represented (●—●) while the range is represented (O—O).

ture of equal parts of normal and hemophilic adsorbed plasmas. The other levels of AHG were obtained by making doubling dilutions of the 100 per cent specimen in the hemophilic adsorbed plasma.

Collection of Specimens: Needles and syringes of identical size were used throughout. By venipuncture using a wide bore needle (S.W.G. 18), 30 ml. of blood was collected into a 30 ml. syringe, care being taken to avoid frothing. A mixture was made immediately of 4.5 ml. of blood with 0.5 ml. of 3.8 per cent citrate and further 4.5 ml. volumes of blood were delivered into each of 4 identical graduated centrifuge tubes. These tubes were placed in a water bath at 37 C. and at intervals of 15 minutes after collection 0.5 ml. of 3.8 per cent sodium citrate added and the contents of the tube mixed with a wooden applicator stick. In this way the process of clotting was arrested at 15 minute intervals after withdrawal of the blood. The tubes were left in the water bath for a further hour to allow for the neutralization of thrombin formed. After this the specimens were tested to determine the amounts of antihemophilic globulin present and thereby the progress of antihemophilic globulin consumption followed.

This study of antihemophilic globulin consumption was applied to normal blood and to blood with deficiencies of Christmas factor or platelets. Observations were made also on blood from patients under therapeutic doses of phenylindanedione or of heparin.

Normal:—Observations were made on 10 normal subjects.

Christmas Disease:—Three observations were made on a patient with Christmas disease, who had a whole blood clotting time of 20 minutes. The diagnosis of Christmas disease was
Table 1.—This table records the results of the thromboplastin generation on the observations on the normal. The figures represent the mean of the 10 observations.

<table>
<thead>
<tr>
<th>Time in minutes after addition of calcium chloride</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting times in seconds</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
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<td>---</td>
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<tr>
<td>100 per cent AHG</td>
<td>52</td>
<td>22</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>50 per cent AHG</td>
<td>54</td>
<td>30</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>25 per cent AHG</td>
<td>54</td>
<td>37</td>
<td>21</td>
<td>15</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>12 per cent AHG</td>
<td>58</td>
<td>45</td>
<td>27</td>
<td>17</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>6 per cent AHG</td>
<td>61</td>
<td>46</td>
<td>31</td>
<td>21</td>
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<td>19</td>
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<tr>
<td>3 per cent AHG</td>
<td>65</td>
<td>52</td>
<td>38</td>
<td>27</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>0 per cent AHG</td>
<td>60</td>
<td>48</td>
<td>42</td>
<td>35</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>1/4 hr. specimen</td>
<td>50</td>
<td>24</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>1/2 hr. specimen</td>
<td>62</td>
<td>43</td>
<td>26</td>
<td>17</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>3/4 hr. specimen</td>
<td>65</td>
<td>49</td>
<td>40</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>1 hr. specimen</td>
<td>60</td>
<td>50</td>
<td>45</td>
<td>35</td>
<td>36</td>
<td>36</td>
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Established by defective prothrombin consumption and deficiency of Christmas factor on thromboplastin generation.

Platelet Deficient Blood:—Three observations were made on one patient with chronic idiopathic thrombocytopenia; the patient's platelet count at the time was less than 5000 per cu.mm. In addition plasma was obtained, artificially freed from platelets, by high speed centrifugation of normal blood. This blood was obtained by venipuncture as described above, and delivered to silicone-treated tubes which had been cooled to 4 C. in a refrigerator. These tubes were spun immediately at 10,000 r.p.m. for 5 minutes in a high speed attachment maintained at 4 C. in a refrigerated centrifuge. 2.2 ml. of the platelet-free plasma was delivered before clotting into each of 4 identical graduated centrifuge tubes. At intervals of 15 minutes after delivery into the tubes 0.5 ml. of 3.8 per cent sodium citrate was added and the contents of the tube mixed using a wooden applicator stick as described previously for collections of whole blood. Three observations were carried out. On microscopic examination this high spun normal plasma was found to be free from platelets.

Phenyldindanedione Therapy:—Three observations were made on successive days on a patient during the third week of therapy with phenyldindanedione for myocardial infarction. The one-stage "prothrombin" times when the observations were made, were 52 seconds, 46 seconds, and 42 seconds respectively as compared with a control of 15 seconds.

Heparin Therapy:—Blood was collected immediately after the intravenous administration of 10,000 units of heparin. The technic employed involved the removal of heparin from the specimens prior to the assay of AHG and are described in greater detail elsewhere (Douglas, 1956). In this present series of experiments three observations were made.
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Results

Normal:—The 10 normal observations are shown in figure 1 which represents the mean of the 10 observations together with the range at each 15 minute interval up to 60 minutes after withdrawal. The results of the thromboplastin generation test are shown in table 1; the clotting times are expressed as a means of the 10 observations.

Christmas Disease: The mean of the results on each of these in comparison with the range of the normal are shown in figures 2 and 3.

Phenylindanedione Therapy: Platelet deficient blood: The three observations on the patient with idiopathic thrombocytopenia were all within the normal range. The mean of the results on the high spun normal blood in comparison with the range of the normal is shown in figure 2.

It is evident that there is markedly defective utilization of AHG in Christmas disease and in heparin therapy. In phenylindanedione therapy there is some impairment of utilization whereas in platelet deficiency the rate of consumption is normal.

Fig. 2.—Antihemophilic globulin consumption in Christmas disease and thrombocytopenia. The mean result of the three observations on hemophilia is represented (●—●) and on thrombocytopenia (X—X) as compared with the range of the normal which is represented (O—O).
Fig. 3.—Antihemophilic globulin consumption in phenylindanedione therapy and heparin therapy. The mean result of the three observations on phenylindanedione is represented (●—●) and on heparin (X—X) as compared with the range of the normal which is represented (O—O).

**DISCUSSION**

A powerful blood thromboplastin can be prepared by incubating together platelets, alumina-treated normal plasma and normal serum with calcium chloride. The alumina-treated normal plasma can be fractionated by ammonium sulphate precipitation; the AHG is contained in the fraction precipitated by 33 per cent saturation; factor V is contained in the fraction precipitated between 33 and 50 per cent saturation. When the alumina-treated normal plasma is replaced by either factor V or AHG separately thromboplastin formation is reduced and delayed. When the two are combined rapid thromboplastin formation is restored. Serum is also required in the interacting mixture forming thromboplastin and the active fraction of serum is removed by adsorption on alumina. This property of serum is due to the presence of the Christmas factor and possibly factor VII--coagulation components not consumed during clotting. Deficiency of AHG in hemophilia results in an inability to form blood thromboplastin and there is consequent failure in prothrombin consumption. The disappearance of AHG during the coagulation of normal blood is likely to be due to its utilization in the formation of blood thromboplastin.
As mentioned above the components which interact to form blood thromboplastin are AHG, factor V, platelets, the Christmas factor and possibly factor VII.\textsuperscript{1-4} Our knowledge of the interactions of these components in the formation of thromboplastin is incomplete. It has been suggested by Biggs\textsuperscript{5} that there are three stages of blood thromboplastin formation. The first stage is the effect of contact with a foreign surface where the nature of the reactions is unknown. In the second stage platelets, Christmas factor and AHG interact to form an intermediate product equivalent to tissue extract. In the third stage there is completion of thromboplastin formation with factors V and VII entering the reaction. This concept has received some support from experimental work.\textsuperscript{2, 3, 12, 13} It would be unwise to attach a final interpretation to the results of this present investigation. It is possible however, that they may represent evidence of the order of interaction of some of the components. The finding that AHG is not utilized in Christmas disease whereas it is in platelet deficiency may be evidence that, in the second stage of thromboplastin formation,\textsuperscript{11} the AHG and the Christmas factor enter the reaction prior to the platelet component. The failure of AHG to be consumed in the presence of heparin may indicate that heparin interferes at the earliest stages of blood thromboplastin formation.

The exact nature of the defect in blood thromboplastin produced by phenylindanedione therapy has not been finally established and the interpretation of the impaired utilization of AHG is rendered difficult; it may be that this again is a manifestation of Christmas factor deficiency.

**SUMMARY**

(1) Antihemophilic globulin (AHG) consumption has been studied in normal blood maintained at 37 \textdegree{}C. for one hour after collection. By the end of one hour the AHG was completely utilized.

(2) AHG consumption is defective completely in Christmas disease and heparin therapy, and to a lesser degree in phenylindanedione therapy. In platelet deficiency consumption of AHG is normal.

(3) A possible interpretation of these results is discussed in relation to the order of the interactions involved in blood thromboplastin formation.

**SUMMARIO IN INTERLINGUA**

1. Le consumption de globulina antihemophilic (GAH) esseva studiate in specimens de sanguine normal mantenite a 37 \textdegree{}C durante un hora post le tempore de collection. Al fin del hora le GAH esseva completamente utilitate.

2. In morbo de Christmas e sub therapia a heparina, le GAH es completamente defective. Illo es defective a grados minus complete sub therapia a phenylindanediona. In casos de carentia de plachettas le consumption de GAH es normal.

3. Un possibile interpretation de iste resultatos es discutite in relation al sequentia del interactiones que es involvite in le formation de thromboplastina sanguinee.

**REFERENCES**


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\textsuperscript{3} ——, —— AND ——: The action of thromboplastic substances. J. Physiol. 122: 554, 1953.
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