The contact phase of coagulation in the presence of heparin

A. L. BLOOM

From the Institute of Pathology, Royal Infirmary, Cardiff

SYNOPSIS  The effect of heparin on the contact phase of coagulation has been investigated by a technique utilizing a solution of toluidine blue in calcium chloride. Heparin in a concentration of 7 units/ml. of plasma does not inhibit contact activation by glass. It is suggested that heparin does not prevent the activation of factor XII (Hageman factor) by glass or the subsequent formation of active factor XI (plasma thromboplastin antecedent).

The antithrombin effect of heparin has been known for many years. Biggs, Douglas, and Macfarlane (1953) have shown that heparin also inhibits the formation of plasma thromboplastin, and O'Brien (1958 and 1960) considers that this is due to its effect on factor IX (Christmas factor). The earlier stages of the coagulation mechanism in vitro involve factor XI and factor XII. According to Hardisty and Margolis (1959) and Soulier and Prou-Wartelle (1960), contact of plasma with glass or other forms of silica results in the adsorption and activation of factor XII. Activated factor XII then reacts with factor XI which in turn is activated and in the presence of calcium initiates further stages of thromboplastin generation. The reactions leading to the formation of active factor XI are referred to as the contact phase of coagulation. Contact with glass normally plays no part in the establishment of haemostasis in vivo and presumably these early stages of coagulation are effected by some other means, possibly associated with tissue fluids (Biggs and Nossel, 1961). When an extracorporeal circulation, such as the heart-lung machine or artificial kidney, is used, however, blood is brought into contact with 'foreign' surfaces and recirculated in the patient. The extracorporeal circuit, in fact, temporarily becomes part of the patient's circulation. Heparin has been widely used in high concentration to prevent coagulation occurring during these procedures. A knowledge of the effect of heparin on all stages of the coagulation mechanism, including the contact phase, may therefore be of considerable practical importance. The investigations reported here show that a high concentration of heparin in citrated plasma does not inhibit contact activation of the coagulation mechanism.

Received for publication 5 June 1962.
The contact phase of coagulation in the presence of heparin

tested immediately. Plasma from the same donor was used for each experiment.

CEPHALIN A chloroform extract of brain was used as a substitute for platelet extract. This was prepared by the method of Bell and Alton (Biggs and Macfarlane, 1957) and was used at the optimum concentration of 1:100 in normal saline.

METHODS

TEST FOR CONTACT ACTIVATION Margolis' indirect method (Margolis, 1957) was used modified to allow for testing in the presence of heparin and using brain cephalin instead of platelet extract. Rows of tubes were placed in a bath of melting ice. In them a series of five threefold dilutions of contacted normal or heparinized plasma were made in intact normal plasma which had been diluted with one-tenth volume of saline. The contacted plasma samples also contained this proportion of saline or heparin solution. The range of concentrations of contacted in intact plasma was then 1 in 3 to 1 in 243. To 0.2 ml. of each dilution was added 0.05 ml. of cephalin. A row of tubes was transferred to a water bath at 37°C and after three minutes 0.1 ml. of the toluidine blue-calcium chloride reagent was added to each tube in turn. The recalcification times were determined using a bank of stop watches. Delay in testing and prolonged incubation of the mixtures at 37°C were thus avoided. In some experiments recalcification was performed with M/20 calcium chloride alone. The beads used to prepare contacted plasma were the only unsiliconed glass to come into contact with the plasma at any stage in this test.

Preliminary experiments were performed to determine if toluidine blue is a suitable heparin antagonist for this study. Details are given below.

RESULTS

EFFECT OF TOLUIDINE BLUE IN NEUTRALIZING HEPARIN O'Brien (1958) stated that toluidine blue failed to correct the abnormal thromboplastin generation produced by heparin. The effect of toluidine blue in correcting the calcium-cephalin clotting time of heparinized plasma was therefore investigated. Contacted normal plasma was prepared to contain concentrations of heparin similar to those obtained in the indirect test described above, i.e. 2-3 units to 0.029 unit per ml. To 0.2 ml. of each sample in siliconed tubes was added 0.05 ml. of cephalin. The tubes were transferred to a water bath at 37°C and after three minutes 0.1 ml. of the toluidine blue-calcium chloride reagent was added and the clotting times determined. The experiment was repeated recalculating with M/20 calcium chloride alone. Table I shows that the addition of toluidine blue with the calcium chloride satisfactorily neutralizes heparin. Toluidine blue prolongs the calcium-cephalin clotting time, but this effect is independent of the concentration of heparin present provided the latter is completely neutralized. Protamine sulphate and Polybrene (hexadimethrine bromide) are other substances which neutralize heparin. In excess they also prolong the calcium clotting time but this effect varies with the concentration of heparin. In the indirect test for contact activation used in this investigation the concentration of heparin is varied by dilution. For this reason toluidine blue has been used as the antiheparin agent of choice. Incubation of plasma with toluidine blue causes a further prolongation of the calcium-cephalin clotting time and for this reason the combined toluidine blue-calcium chloride reagent was used.

EFFECT OF TOLUIDINE BLUE IN DETECTING CONTACT ACTIVATION Margolis' indirect test was performed as described on contacted normal plasma. The effect of recalcification with toluidine blue-calcium chloride reagent was compared with that of recalcification with M/20 calcium chloride alone. Table II shows the results. Although the presence of toluidine blue prolongs the clotting times, the effect of the contacted plasma on the intact plasma is easily detectable.

THE EFFECT OF HEPARIN ON CONTACT ACTIVATION The indirect test was performed on contacted normal

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFFECT OF TOLUIDINE BLUE IN NEUTRALIZING HEPARIN</td>
</tr>
<tr>
<td>Concentration of heparin per ml. of plasma (units)</td>
</tr>
<tr>
<td>Clotting times using M/20 CaCl₂ alone (sec.)</td>
</tr>
<tr>
<td>Clotting times using toluidine blue-CaCl₂ reagent (sec.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>DETECTION OF CONTACT ACTIVATION OF NORMAL PLASMA USING TOLUIDINE BLUE-CALCIUM CHLORIDE REAGENT</td>
</tr>
<tr>
<td>Dilution of contacted plasma in intact plasma</td>
</tr>
<tr>
<td>Clotting times on addition of M/20 CaCl₂ (sec.)</td>
</tr>
<tr>
<td>Clotting times on addition of toluidine blue-CaCl₂ reagent (sec.)</td>
</tr>
<tr>
<td>Cephalin, 0.05 ml., was added to 0.2 ml. of each dilution of contacted plasma. The clotting times were determined after the addition of 0.1 ml. of M/20 CaCl₂ or toluidine blue-CaCl₂ reagent.</td>
</tr>
</tbody>
</table>
plasma and contacted heparinized plasma using the
toluidine blue-calcium chloride reagent. Table III
gives the results of such an experiment. The clotting
times with heparinized plasma are very similar to
those with normal plasma and clearly show that
heparin has no inhibitory effect even on the minimal
contact activation produced by a small number of
glass beads.

**TABLE III**

**EFFECT OF HEPARIN ON CONTACT ACTIVATION OF NORMAL PLASMA**

<table>
<thead>
<tr>
<th>Dilution of contacted plasma</th>
<th>1:3</th>
<th>1:9</th>
<th>1:27</th>
<th>1:81</th>
<th>1:243</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting times (sec.) with</td>
<td>238</td>
<td>280</td>
<td>375</td>
<td>480</td>
<td>575</td>
</tr>
<tr>
<td>contacted normal plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clotting times (sec.) with</td>
<td>245</td>
<td>298</td>
<td>384</td>
<td>480</td>
<td>595</td>
</tr>
<tr>
<td>contacted heparinized plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cephalin, 0.05 ml., was added to 0.2 ml. of each dilution of contacted
plasma. The clotting times were determined after the addition of
0.1 ml. of toluidine blue-CaCl₂ reagent.

**DISCUSSION**

According to Hardisty and Margolis (1959) contact
of plasma with glass results in the adsorption and
activation of factor XII which in turn adsorbs and
activates factor XI. Activated factor XI then returns
into solution and initiates further stages in the for-
tmation of thromboplastin. Activated factor XI in
solution is presumably responsible for the activity
in the contacted citrated platelet-poor plasma used
in Margolis' indirect test. Heparin does not prevent
the formation of this soluble active agent and there-
fore if the theory of Hardisty and Margolis is correct
heparin does not prevent the formation of activated
factor XI or the reactions which precede it.

Little information has previously been available
on the effect of heparin on contact activation.
Conley, Hartmann, and Lalley (1950) showed that
contact with glass shortened the clotting time of
native platelet-poor plasma containing heparin to a
concentration of 0.075 unit/ml and that this effect
was proportional to the area of glass to which such
plasma was exposed. They could not, however,
demonstrate this effect with higher concentrations
of heparin because the plasma then became in-
coagulable. The results presented here support the
findings of these workers. O'Brien (1960) stated that
heparin does not affect the coagulant activity of
factor XII although his experiments were not
designed to detect its effect on the contact phase of
coaulation and the concentration of heparin used
was lower than that used in the present investigation.
Gormsen (1961), using celite, found that the heparin
resistance of contacted plasma is greater than that
of intact plasma but did not test the effect of heparin
on celite activation. The effect of heparin on the
interaction of activated factor XI with calcium and
other thromboplastic factors has not been tested
in the experiments described here, since at this stage
toluidine blue was added to the system.

Ollendorff, Storm, Rygg, and Arnfred (1961) were
able to demonstrate activation of the 'thromboplastic
system' when heparinized blood was circu-
lated through a heart-lung machine used as a closed
circuit or with a patient. They suggest that this might
be a foreign surface reaction involving factor XII
and factor XI, but admit that their technique did not
demonstrate the stages or factors involved in thromb-
oplastin generation which were effected. The results
presented in this paper suggest that activation of
factor XII and factor XI during extracorporeal
circulation of blood will not be prevented by heparin
if a suitable surface is present in the circuit.

**REFERENCES**

The contact phase of coagulation in the presence of heparin
A. L. Bloom

J Clin Pathol 1962 15: 508-510
doi: 10.1136/jcp.15.6.508

Updated information and services can be found at:
http://jcp.bmj.com/content/15/6/508

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/