Activation of coagulation and fibrinogen loss after using an extracorporeal circulation

A. L. BLOOM

From the Institute of Pathology, Royal Infirmary, Cardiff

SYNOPSIS Alterations of the coagulation potential of heparinized blood after using an extracorporeal circulation have been studied by means of a toluidine blue-calcium chloride reagent. This technique was originally used to detect the effect of activation by contact on the coagulation mechanism in heparinized blood. It has been shown that it also detects, in the presence of heparin, the clot potentiating effect of blood cell contents liberated in vitro by mechanical trauma to blood.

Variable destruction of platelets, red cells, and white cells occurred in heparinized sheep blood recirculated in a heart-lung machine in vitro. This was accompanied by increased clotting potential. Complete coagulation was prevented by heparin and fibrinogen levels remained unaltered.

Similar enhancement of the coagulation potential and destruction of blood cells were detected in the blood of heparinized patients and sheep after perfusion for open-heart surgery. The coagulation changes were usually transient, and impaired coagulation associated with significant fibrinogen loss was detected in most samples taken after the neutralization of heparin.

It is suggested that the coagulation changes are due to contact of the coagulation mechanism during perfusion and to the clot-accelerating effect of blood cell contents. The results support the hypothesis that coagulation defects and fibrinogen loss after using an extracorporeal circulation are due, at least in part, to intravascular coagulation. This is thought to occur, especially during neutralization of heparin, while the coagulation mechanism is hyperactive.

Abnormal bleeding is one of the most important complications of the use of heart-lung machines for open-heart surgery. Although the use of heparin and its antagonists poses its own special problems other important defects may occur in the haemostatic mechanisms. These include thrombocytopenia (Perkins, Osborn, and Gerbode, 1958; Sharp, Excell, Salzman, and Thorup, 1961; Bloom, 1961), deficiency of factor V, factor VIII, and prothrombin (Nilsson and Swedberg, 1959; Fantl and Ward, 1960; Holemans, Amery, and Verstraete, 1960; Bloom, 1961), and the appearance of a circulating anticoagulant active in the thrombin-fibrinogen reaction (Von Kaulla and Swan, 1958; Hougie, Garrard, Warren, Dammann, and Muller, 1959). Several workers have reported fibrinogen loss after perfusion ranging up to 50% or so of the pre-perfusion levels (Rothnie, Norman, Steele, and Kinmonth, 1960; Bloom, 1961), and cases of severe fibrinogen depletion with excessive bleeding have been reported (Du Bourg, Trarieux, Fontan, Moulinier, Bricaud, and Broustet, 1960; Rothnie et al., 1960; Samama, 1961).

Two main theories have been put forward to explain the loss of fibrinogen and other coagulation factors after perfusion. Douglas (1961) favours the view that activation of the fibrinolytic system leads to proteolytic digestion of these factors, whilst Osborn, Mackenzie, Shaw, Perkins, Hurt, and Gerbode (1956), Wright, Darte, and Mustard (1958), Hoekema, Mustard, and Mustard (1959), and Gans Siegal, Lillehei, and Krivit (1962), amongst others, favour the view that activation of coagulation by the extracorporeal circuit, perhaps accelerated by products of destroyed blood cells, results in consumption of coagulation factors in vivo and fibrinolysis.

There is little doubt that increased fibrinolysis frequently occurs after perfusion, for it has been detected by several workers (Von Kaulla and Swan, 1958; Hougie et al., 1959; Sharp et al., 1961; Bloom, 1961; Gans, Lillehei, and Krivit, 1961). In fact the observation by Bloom (1961) that fibrinolysis was consistently decreased on the day after operation suggests that activation of this system with subsequent depletion of its components occurs in all cases.

The demonstration of activation of coagulation...
Activation of coagulation and fibrinogen loss after using an extracorporeal circulation

559

after perfusion is more difficult. It is reasonable to assume such activation when fibrin deposits are present in the circuit. The tendency for these to form on glass surfaces or at points in the circuit where blood is subjected to trauma was noted by several earlier workers (Stokes and Gibbon, 1950; Dennis, Karlson, Eder, Nelson, Eddy, and Sanderson, 1951; Clowes, Neville, Hopkins, Anzola, and Simeone, 1954). With improved apparatus and technique such deposits are now uncommon and histological evidence of fibrin emboli is seldom obtained. Nevertheless coagulation defects still occur and have been reported even in the absence of detectable fibrinogen loss (Fanti and Ward, 1960).

The demonstration of activation of coagulation in these circumstances depends upon laboratory tests. The presence of heparin in samples to be tested makes orthodox methods impracticable and most of the support for activation of coagulation after perfusion is derived from indirect evidence.

The object of this paper is to describe the application of a toluidine blue-calcium chloride reagent (Bloom, 1962) to the study of coagulation activity in heparinized blood. Evidence will be presented to show the development of increased coagulation potential in heparinized blood recirculated in a heart-lung machine in vitro and after experimental and clinical perfusion for open-heart surgery, and an attempt will be made to relate the changes to the development of fibrinogen loss. The evidence supports the hypothesis that activation of the coagulation mechanism during perfusion plays a part in the development of coagulation defects.

MATERIALS AND STANDARD METHODS

COLLECTION OF SPECIMENS Blood was collected using siliconed syringes and containers. For coagulation studies it was taken into 3.8% w/v trisodium citrate in the ratio of nine volumes of blood to one of citrate. Platelet-poor plasma was obtained by centrifuging at 3,000 r.p.m. for 30 minutes. Blood for cell counts and plasma haemoglobin estimations was taken into the dipotassium salt of ethylene diamine tetra-acetic acid (E.D.T.A.). For plasma fibrinogen estimation blood was taken into dry potassium oxalate (2.5 mg/ml.) and plasma obtained by centrifuging at 3,000 r.p.m. for 10 minutes.

HEPARIN SOLUTION, INTACT PLASMA, CEPHALIN, AND GLASSWARE These were prepared as described by Bloom (1962).

PLASMA HAEMOGLOBIN This was estimated by the method of Fielding and Langley (1958).

CELL COUNTS White cell counts were performed as described by Whitby and Britton (1953). Platelet counts were performed by the method of Baar (1948).

PLASMA FIBRINOGEN This was estimated by the method described by Varley (1960) modified as follows: Plasma, 0.1 ml., was added to 0.25 ml. of normal saline, and to the mixture was added 0.1 ml. of a solution of polybrene, 10 mg. % in normal saline, and 0.1 ml. of a 1% solution of epsilon amino-caproic acid (E.A.C.A.). The plasma was clotted at 37°C. with calcium chloride and subsequently the fibrin was estimated as tyrosine as described by Varley. Polybrene was added to neutralize heparin, and E.A.C.A. to minimize possible fibrinolysis during the performance of the test. Epsilon amino-caproic acid is an inhibitor of fibrinolytic activation (Ablondi, Hagan, Philips, and De Renzo, 1959). Preliminary studies were performed to ensure that the addition of these substances to plasma did not affect the fibrinogen estimation.

Fibrinogen estimations were performed on 0.1 ml. volumes of eight different normal plasma samples adding 0.1 ml. of heparin, 4 units/ml., and 0.1 ml. of polybrene and E.A.C.A. as described above. Simultaneous estimations were performed on aliquots of the same plasma substituting equal volumes of saline for the heparin, polybrene, and epsilon amino-caproic acid. The results are shown in Table I. The addition of heparin, polybrene, and E.A.C.A. to plasma does not affect the fibrinogen estimation.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Fibrinogen Level (mg. %) Recorded in Plasma + Saline</th>
<th>Fibrinogen Level (mg. %) Recorded in Plasma + Heparin, Polybrene, and E.A.C.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>273</td>
<td>273</td>
</tr>
<tr>
<td>2</td>
<td>280</td>
<td>273</td>
</tr>
<tr>
<td>3</td>
<td>237</td>
<td>245</td>
</tr>
<tr>
<td>4</td>
<td>469</td>
<td>480</td>
</tr>
<tr>
<td>5</td>
<td>225</td>
<td>225</td>
</tr>
<tr>
<td>6</td>
<td>175</td>
<td>180</td>
</tr>
<tr>
<td>7</td>
<td>320</td>
<td>308</td>
</tr>
<tr>
<td>8</td>
<td>329</td>
<td>343</td>
</tr>
<tr>
<td>Mean</td>
<td>288</td>
<td>291</td>
</tr>
</tbody>
</table>

TOLUIDINE BLUE-CALCIUM CHLORIDE This reagent was prepared as described by Bloom (1962). It has been found that the anticoagulant activity of toluidine blue may vary from batch to batch. The reagent was prepared so that 0.1 ml. clotted a mixture of 0.2 ml. glass contacted normal plasma (Bloom, 1962) containing 0.05 ml. cephalin in 160 to 200 seconds at 37°C. The concentration of toluidine blue needed varies from 80 mg. to 100 mg. per 100 ml. of M/20 calcium chloride.

TESTS OF COAGULATION ACTIVITY Non-heparinized plasma was tested by the silicone calcium clotting time technique with and without cephalin. Plasma, 0.2 ml., was placed in siliconed tubes and 0.05 ml. of cephalin or normal saline added. The clotting time was determined at 37°C. on the addition of 0.1 ml. of M/20 calcium chloride. Heparinized plasma was tested similarly except that
the toluidine blue-calcium chloride reagent was used instead of M/20 calcium chloride. Experiments to determine if this technique is suitable to detect alterations in coagulation activity of heparinized plasma are described below.

DEMONSTRATION OF INCREASED COAGULATION
POTENTIAL IN HEPARINIZED BLOOD

The importance of contact with glass and other forms of silica and adsorbents in the activation of the coagulation mechanism has been demonstrated by the work of Margolis (1957), Ratnoff and Rosenblum (1958), and Soulier and Prou-Wattelle (1960), and substances active in this respect, such as stainless steel (Rose and Broida, 1954), are used in extracorporeal circuits.

In a previous paper Bloom (1962) described the use of the toluidine blue-calcium chloride reagent to demonstrate activation of the coagulation mechanism in heparinized plasma when in contact with glass. During extracorporeal circulation of blood, coagulation activity may be affected not only by contact with foreign surfaces but also by products liberated from destroyed blood cells. Substances active in coagulation are present in platelets (Van Creveld and Paulssen, 1952; Deutsch, Johnson, and Seegers, 1955), red cells (Quick, Georgatsos, and Hussey, 1954; Desmet, Verstraete, and Vandenburgoucke, 1957) and possibly in white cells (Gollub, 1953; O'Brien, 1959). Preliminary to studies with an extracorporeal circulation experiments were therefore carried out to demonstrate the effects of cellular damage on coagulation activity of normal blood and to determine if these could be detected in heparinized blood by using the toluidine blue-calcium chloride reagent.

CHANGES IN NORMAL BLOOD DAMAGED MECHANICALLY

Citrated blood, 2 ml., was delivered into each of two tubes. Fifteen siliconed glass beads, 2 mm. in diameter, were placed in one tube which was stoppered with a polythene-covered rubber bung and rotated on a Matburn mixer at 28 r.p.m. for about 15 minutes. The second tube was left unrotated. The blood from both tubes was transferred to fresh ones and samples removed for cell counts. The remainder was centrifuged at 3,000 r.p.m. for 30 minutes and the cell-poor plasma was removed. This was then tested by the silicone calcium clotting time technique with and without cephalin. To control the absence of contact activation by siliconed beads, intact normal plasma was rotated with such beads at the same time as the blood and subsequently tested. At no time did unsiliconed glass come into contact with the blood or plasma.

The results are shown in Table II. Trauma to blood by rotation with siliconed glass beads leads to release of haemoglobin and loss of platelets. The changes in the white cell counts were insignificant. These cellular changes were accompanied by acceleration of the plasma silicone clotting times. This occurred in the absence of contact with glass and was not observed to a significant extent when intact plasma was rotated with siliconed beads. It is concluded that the accelerated clotting times are due to substances liberated from blood cells. The addition of cephalin to the test system accelerated clotting but the effects of cellular products could still be detected.

DETECTION OF CHANGES IN HEPARINIZED BLOOD DAMAGED MECHANICALLY

Heparinized blood was prepared by adding 0.1 ml. of a solution of heparin, 40 units/ml., to 1 ml. of citrated venous blood. The final concentration of heparin was thus approximately 1 unit/ml., i.e., about 22 units/ml in plasma. This concentration is completely neutralized by the toluidine blue-calcium chloride reagent (Bloom, 1962). Non-heparinized blood was prepared by substituting normalsaline for the heparin. Samples of each were subjected to trauma with siliconed glass beads as described above, and the cell-poor plasma was tested using both M/20 calcium chloride and the toluidine blue-calcium chloride reagent.

The results are shown in Table III. The accelerating effects of trauma to whole blood on the plasma silicone clotting times is easily detected with the toluidine blue-calcium chloride reagent, although the presence of the dye prolongs the clotting times. When plasma from heparinized blood is tested with the reagent, the results are very similar to those obtained with non-heparinized blood. The slight differences observed are probably due to variations which occur in the extent of cellular destruction even when the same number of glass beads is used. It is concluded that the technique satisfactorily detects the effects of

<table>
<thead>
<tr>
<th>Sample Tested</th>
<th>Uncontacted Rotated Plasma</th>
<th>Uncontacted Undamaged Blood</th>
<th>Uncontacted Damaged Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (\text{CaCl}_2) clotting time (sec.)</td>
<td>509</td>
<td>520</td>
<td>288</td>
</tr>
<tr>
<td>Plasma (\text{CaCl}_2) clotting time (sec.) (cephalin added)</td>
<td>306</td>
<td>313</td>
<td>244</td>
</tr>
<tr>
<td>Plasma haemoglobin (mg. %)</td>
<td>6</td>
<td>6</td>
<td>74</td>
</tr>
<tr>
<td>Platelet count (c.mm.)</td>
<td>231,000</td>
<td>147,000</td>
<td></td>
</tr>
<tr>
<td>White cell count (c.mm.)</td>
<td>7,700</td>
<td>7,500</td>
<td></td>
</tr>
</tbody>
</table>
TABLE III
DETECTION OF COAGULATION CHANGES IN DAMAGED HEPARINIZED BLOOD USING TOLUIDINE BLUE-CALCIUM CHLORIDE REAGENT

<table>
<thead>
<tr>
<th>Sample Tested</th>
<th>Undamaged Blood</th>
<th>Damaged Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-heparinized</td>
<td>Heparinized</td>
</tr>
<tr>
<td>Plasma CaCl₂ clotting time (sec.)</td>
<td>625</td>
<td>2,000 +</td>
</tr>
<tr>
<td>Plasma toluidine blue-CaCl₂ clotting time (sec.)</td>
<td>1,000 +</td>
<td>1,000 +</td>
</tr>
<tr>
<td>Plasma CaCl₂ clotting time (sec.) (cephalin added)</td>
<td>336</td>
<td>2,000 +</td>
</tr>
<tr>
<td>Plasma toluidine blue-CaCl₂ clotting time (sec.) (cephalin added)</td>
<td>863</td>
<td>766</td>
</tr>
</tbody>
</table>

destruction of blood cells on plasma coagulation potential in the presence of heparin.

ACTIVATION OF THE COAGULATION MECHANISM DURING EXTRACORPOREAL CIRCULATION OF BLOOD

RECIRCULATION EXPERIMENTS The object of these experiments was to determine the effect of recirculation of heparinized blood in a Melrose heart-lung machine on the cellular elements and on the plasma toluidine blue-calcium chloride clotting times.

The priming blood was obtained from sheep on the morning of each experiment. In the first four experiments it was obtained by exsanguination of one animal and collected into glass bottles. In the last four experiments 1 pint of blood was taken from each of several donor animals into siliconed glass bottles. Heparin was used as anticoagulant at concentrations of 1,000 to 3,000 units to 500 ml. of blood. About 3 litres of blood were circulated intermittently in the closed circuit for 30 to 40 minutes with continuous oxygenation. This procedure was adopted for the purpose of other experiments in progress. Samples of blood were obtained before and after recirculation by aspiration from the oxygenator through wide plastic catheters into siliconed syringes and were kept at 4°C. until tested.

The toluidine blue-calcium chloride clotting times were determined in neat plasma and in plasma diluted 1 in 3 in non-heparinized intact plasma in order to exclude incomplete neutralization of heparin by the reagent. The results with neat and diluted plasma always showed identical trends so that only those of the former are reported.

RESULTS The results of clotting time determinations in individual experiments as well as mean values are shown in Fig. 1. A marked decrease in the clotting times without cephalin occurred in nearly all samples after recirculation. Only in experiment 6 was this less obvious. The addition of cephalin to the test accelerated clotting in all specimens, but increased activity after recirculation could still be detected. The collection of the priming blood in siliconed (experiments 5 to 8) or unsiliconed (experiments 1 to 4) glass bottles did not apparently affect the results.

Plasma fibrinogen and haemoglobin levels are shown in Figure 2. Plasma fibrinogen levels showed no significant alteration. In some experiments plasma haemoglobin levels were slightly raised at the start. The reason for this was not apparent but it may have been due to difficulties in obtaining the

![FIG. 1. Plasma toluidine blue-calcium chloride clotting times before and after recirculation of heparinized sheep blood in a heart-lung machine. The code number of each experiment is shown indicating the observed data.](http://jcp.bmj.com/)
FIG. 2. Plasma fibrinogen and haemoglobin levels before and after recirculation of heparinized sheep blood in a heart-lung machine. The code number of each experiment indicates the observed data.

FIG. 3. Platelet and white cell counts before and after recirculation of heparinized sheep blood in a heart-lung machine. The code number of each experiment indicates the observed data.
blood in some animals. Further increases in plasma haemoglobin occurred in all experiments after recirculation. These varied from 10 to 170 mg. %.

White cell and platelet counts are shown in Figure 3. Platelet counts were surprisingly little affected although clumping made accurate counting difficult. In only three experiments (1, 5, and 8) did appreciable loss of platelets occur. White cell counts were not greatly affected but a loss of over 10% was observed in four experiments (1, 2, 5 and 7). Acceleration of the clotting times was most marked in the two experiments (1 and 5) in which the greatest cellular destruction was detected.

ACTIVATION OF THE COAGULATION MECHANISM DURING PERFUSION

The object of these studies was to investigate the changes in the coagulation potential of heparinized blood during clinical and experimental perfusion for open-heart surgery.

Donor blood for priming the heart-lung machine was collected for patients on the day before operation into E.D.T.A. as anticoagulant and was stored overnight at 4°C. Before operation each unit of 500 ml. was heparinized with 2,500 units of heparin and recalcified with 1-8 ml. of 10% calcium chloride. Donor blood for experimental perfusion of sheep was collected on the morning of operation. One pint was collected from each animal and was taken into heparin (2,500 units/500 ml.) in siliconed glass bottles. The blood for these experiments was not that used for the recirculation experiments.

Patients and animals received heparin at a dosage of 250 units per kilogram body weight.

The perfusions were all short, i.e., up to 40 minutes' duration. After perfusion heparin was neutralized by intravenous infusion during 10 minutes of polybrene in a dose ratio of 0-75 to 1 mg. for sheep and 1-25 to 1-5 mg. for patients to each 100 units of heparin administered. Neutralization was checked by the thrombin clotting time and further graded doses of polybrene given if indicated. This was actually needed in only one instance.

Samples of blood were obtained from patients and animals before perfusion (after heparinization), at the time perfusion was just ending, and after the administration of polybrene. The mixed donor blood in the oxygenator was also sampled.

Plasma was tested with the toluidine blue-calcium chloride reagent with and without cephalin. In order to exclude incomplete neutralization of heparin by the reagent it was tested both neat and diluted one in three in non-heparinized intact plasma. As in the recirculation experiments the clotting times with neat and diluted plasma showed identical trends and only those of the neat samples are reported.

RESULTS

Samples were obtained during operations on five patients and 10 sheep. The results of the plasma toluidine blue-calcium chloride clotting times are shown in Figures 4 and 5. Figure 4 records the clotting times without cephalin and Fig. 5 those with cephalin.

The clotting times of the mixed donor blood tended to be shorter than those of the pre-perfusion samples of the subject. At the end of perfusion, however, clotting with and without cephalin was always accelerated, usually considerably so, and clotting times shorter than those of the mixed donor blood were always obtained. This increased clotting potential in the heparinized blood was usually short lived. Fifteen to 20 minutes after perfusion following the administration of polybrene only five samples, all from sheep, still showed accelerated clotting times. The human samples and those from the other sheep showed a greater or lesser degree of impaired coagulation.

Increased clotting potential in the heparinized blood at the end of perfusion was thus often replaced by impaired clotting by the time the circulating heparin had been neutralized.

The mean values for the platelet counts and plasma haemoglobin and fibrinogen levels are shown in Table IV.

<table>
<thead>
<tr>
<th>TABLE IV</th>
<th>MEAN PLATELET COUNTS, PLASMA HAEMOGLOBIN AND FIBRINOGEN LEVELS DURING OPERATIONS USING AN EXTRACORPOREAL CIRCULATION (5 HUMAN AND 10 SHEEP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data Recorded</td>
<td>Operative Subject</td>
</tr>
<tr>
<td>Plasma haemoglobin (mg. %)</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
</tr>
<tr>
<td></td>
<td>Whole series</td>
</tr>
<tr>
<td>Platelet counts/c.mm. × 10⁶</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
</tr>
<tr>
<td></td>
<td>Whole series</td>
</tr>
<tr>
<td>Plasma fibrinogen (mg. %)</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
</tr>
<tr>
<td></td>
<td>Whole series</td>
</tr>
</tbody>
</table>

*Estimation performed on seven sheep only
Some destruction of red cells occurred during perfusion but plasma haemoglobin levels above 50 mg. % were not observed. The mean platelet loss in the human cases during perfusion was 149,000/c.mm. and in the animals 288,000/c.mm. Clumping of platelets was usually present in the pooled donor blood in the animal experiments and may account for the relatively low counts observed in this blood.

The mean loss of plasma fibrinogen in the human cases was 55 mg. % and in the animals 84 mg. %. This occurred partly during perfusion and partly during the administration of polybrene, when the mean loss in the patients was 27-4 mg. % (S.D. 5-4) and in the animals 41-4 mg. % (S.D. 11-7). These changes differ significantly from zero (P<0-01 for patients and sheep).

The changes occurring in the toluidine blue-calcium chloride clotting times and in the platelet counts, plasma haemoglobin and fibrinogen levels are summarized graphically in Figure 6. The changes occurring during human perfusions were similar to those observed in the animal experiments and the mean values for the combined series are shown in the figure.

Increased coagulation potential occurring in the heparinized blood during perfusion is associated with the destruction of red cells and platelets and with mild fibrinogen loss. Further fibrinogen loss occurs during neutralization of heparin is associated with prolongation of the clotting times. These findings suggest intravascular coagulation with consumption of coagulation factors. It was not possible, however, in this small series to correlate the extent of the coagulation changes in individual subjects with that of the changes in blood cells and plasma fibrinogen levels.

**DISCUSSION**

Support for the hypothesis that activation of the coagulation mechanism occurs during perfusion in the absence of fibrin deposits in the circuit is based because of the difficulty in testing heparinized blood, mainly on indirect evidence.
Osborn et al. (1956) perfused dogs without using heparin and detected shortened clotting times followed by incoagulability of the blood with fibrinogen depletion. The authors attributed the changes to activation of coagulation and exhaustion of coagulation factors. Wright et al. (1958) and Hoeksema et al. (1959) drew attention to the similarity of the changes occurring in coagulation factors during extracorporeal circulation to those occurring during coagulation of blood in vitro. Gans et al. (1962) demonstrated accelerated clotting and thrombin generation in citrated blood recirculated in a heart-lung machine, and Penick, Averette, Peters, and Brinkhous (1958) have shown that transfusion of such blood in dogs leads to thrombocytopenia and loss of factor VIII. They attributed these changes to activation of the coagulation mechanism although fibrinogen levels were unaltered.

The demonstration of such changes in heparinized blood is more difficult. Perkins, Osborn, and Gerbode (1959) claimed that clotting times were accelerated and prothrombin consumption increased in samples taken immediately after perfusion and tested after careful neutralization of heparin with protamine. Later samples showed impaired coagulation. These results would support the hypothesis that coagulation is activated during perfusion but details of their experiments were not given.

In the present study a toluidine blue-calcium chloride technique has been used to detect changes in the coagulation potential of heparinized blood. This technique not only detects contact activation of coagulation in such blood (Bloom, 1962) but also the clot-accelerating effect of blood cell contents. This was more obvious when cephalin was omitted from the test system, a finding in keeping with observations that substances present in platelets, red cells, and brain cephalin have similar activity in thromboplastin generation (Bell and Alton, 1954; Quick et al., 1954; Troup and Reed, 1958). Some acceleration of the plasma clotting times of damaged blood occurred, however, when tested even in the presence of cephalin. This suggests that blood cells also contain a different type of activity.

Increased coagulation potential developed in heparinized sheep blood during recirculation in the heart-lung machine in vitro and was associated to a greater or lesser extent with destruction of blood cells. In contrast to the findings of Nilsson and Swedberg (1959) and of Gans et al. (1962), working with dogs' blood, but in agreement with those of Perkins, Osborn, and Gerbode (1957), the platelet
loss after recirculation was generally not striking. The differing results may be due to many factors, such as the type of machine used, the flow rate, and the concentration of heparin, but may indicate, as suggested by Perkins and his colleagues, that in some cases much of the platelet loss during clinical perfusion occurs in vivo.

Activation of coagulation by contact is not prevented by heparin (Bloom, 1962) and may contribute to the accelerated clotting times. Didisheim, Hattori, and Lewis (1959) failed to detect an effect of contact with glass on the clotting time of sheep blood. It is wrong to assume, however, that the contact phase is absent in the blood of these animals. The addition of kaolin to intact sheep plasma results in considerable shortening of the silicone calcium clotting time (personal unpublished observation). The fact that the blood was pooled in the oxygenator before sampling and thus exposed to stainless steel may account for the observation that the collection of sheep blood in siliconed or non-siliconed bottles did not appreciably affect the results. In any case the technique used will not distinguish between the effects of contact and those of cellular products, as the addition of brain cephalin to the test system does not entirely exclude the activity of cellular elements. Whatever the mode of enhancement of the coagulation potential, complete coagulation during recirculation was prevented by heparin, and fibrinogen levels remained unaltered. This confirms the observation made by Nilsson and Swedberg (1959) and Gans et al. (1962) that the extracorporeal circuit does not denature this protein, at least during the time limits of the experiments. Fibrinogen loss during and after perfusion thus appears to be a phenomenon in vivo.

Enhanced coagulation potential was also observed in heparinized blood after clinical and experimental perfusion for open-heart surgery. Although the donor blood may have contributed to this effect it was not the only factor responsible. The clotting times of the post-perfusion samples were usually shorter than those of the mixed donor blood.

Although the enhanced coagulation potential was associated with cellular destruction, no relationship could be demonstrated in this small series between the extent of the coagulation changes in individual subjects and that of the platelet counts and plasma haemoglobin levels. This does not necessarily mean that cellular products do not contribute to the coagulation changes. These are no doubt the result of a complicated interplay of factors, including possibly the effects of the donor blood and contact activation of coagulation as well as those of cellular contents.

Recently similar enhancement of coagulation has been described by Ollandorff, Storm, Rygg, and Arnfred (1961) after extracorporeal circulation of human blood both with and without a patient. They were able to detect this by demonstrating an accelerating effect of the test plasma on thromboplastin generation in intact platelet-rich normal plasma. Heparin was neutralized with protamine and toluidine blue (Ollandorff, 1962). The authors considered that activation of factors XII and XI by contact was responsible for the changes detected, but their technique, like that used in the present study, did not exclude the effects of cellular contents, especially as intact platelets were used in their test system. Margolis (1957) has shown that disintegration of platelets increases their activity in coagulation.

The enhanced coagulation potential after perfusion in the present study was usually shortlived and it had been replaced by impaired coagulation in most of the post-polybrene samples. This may merely represent an anticoagulant action of excess polybrene such as that described by Egerton and Robinson (1961) but other explanations are possible. The work of Spaet and his colleagues (Spaet and Kropatkin, 1958; Spaet and Cintron, 1960; Spaet, Horowitz, and Zucker-Franklin, 1960) suggests the existence of a clearing mechanism for plasma thromboplastin and its intermediates which may be associated with the reticulo-endothelial system. Similar clearance of coagulant material may account for the rapid disappearance of activity noted in the present study.

The impaired coagulation may, however, be the result, at least in part, of consumption of coagulation factors following neutralization of heparin while the coagulation mechanism is hyperactive. Appreciable fibrinogen loss occurred during the neutralization of heparin with polybrene and at the same time impaired coagulation was detected in the post-polybrene samples. The available evidence is against precipitation by polybrene as a cause of fibrinogen loss. Unlike protamine, polybrene does not apparently precipitate fibrinogen in plasma (Godal, 1960; Shanberge, Regan, Talarico, and Busiek, 1961). Similar post-perfusion loss of fibrinogen after neutralization of heparin by polybrene was noted by Gans and Krivit (1962). Ollandorff et al. (1961) describe widespread thrombosis occurring at this stage. These findings invite caution in the neutralization of heparin, a point emphasized by Du Bourg et al. (1960). Although it should eventually be complete, slow neutralization in stages may be preferable to the more rapid single dose technique. A recent report of a nephrotoxic effect of polybrene (Hallett, Ransdell, Stowens, and Rubel, 1962), if confirmed, may lead to the abandoning of this drug for use after extracorporeal circulation. Similar considerations of
intravascular coagulation apply equally, however, with the use of the alternative antiheparin agent protamine.

The demonstration of increased coagulation activity after perfusion does not exclude the possibility that fibrinolysis may contribute to the development of haemostatic defects. Increased fibrinolysis is common after using an extracorporeal circulation. This enzyme system can digest not only fibrin, but also fibrinogen and other coagulation factors (Soulier, Alagille, and Larrieu, 1956; Fletcher, Alkjaersig, and Sherry, 1959; Coon and Duff, 1958). A close relationship may exist between the coagulation and fibrinolytic systems. Surface contact and factor XII activation may play a role in the development of endogenous plasminogen activator activity (Niewiarowski and Prou-Wartelle, 1959; Iatridis and Ferguson, 1961) and intravascular activation of coagulation is a potent stimulus to fibrinolysis. Thus the experimental injection of thrombin results not only in intravascular coagulation but also in marked enhancement of fibrinolysis (Brayton and Zucker, 1957; Hardaway, Watson, and Weiss, 1960). Increased fibrinolysis may thus be partly a secondary and perhaps compensatory phenomenon but may well contribute to a bleeding tendency.

Whatever the role of fibrinolysis, however, the sequence of events described in the present study, namely, increased coagulation potential after perfusion, followed, during neutralization of heparin, by impaired coagulation and significant fibrinogen loss, bears a striking resemblance to the more severe changes observed by Osborn et al. (1956) when heparin is not used. It also resembles the ‘positive phase’ of hyperactive coagulation and the ‘negative phase’ of incoagulable blood reported by earlier workers (Martin, 1894; Mellanby, 1909) after the experimental injection of coagulant materials. Although other factors, such as fibrinolysis, the use of stored donor blood, and the presence of residual heparin, may be associated with abnormal bleeding after use of an extracorporeal circulation, the findings reported in this paper suggest that activation of coagulation during perfusion is not completely prevented by heparin and together with clot-accelerating substances liberated from blood cells may contribute to the development of coagulation defects.

I would like to thank Mr. H. R. S. Harley and Mr. D. Thomas for permission to study patients under their care at Sully Hospital, Penarth.

I owe a debt of gratitude to Dr. L. R. West, Sully Hospital, for supplying me with samples of blood from his experimental animals and to the thoracic surgical and cardiology teams for their willing cooperation.

REFERENCES

--- (1909). Ibid., 38, 141.


