Some haematological observations in cardiopulmonary bypass at normothermia using the Melrose oxygenator


From the Cardiopulmonary Bypass Unit, The Royal Infirmary, Edinburgh

Haemostasis is now generally accepted to be a dynamic mechanism. In injury, haemostasis is effected by a series of processes resulting finally in the formation of a blood clot. This process involves capillary retraction whereby the severed vessel end is narrowed; this is followed by the accretion of an occlusive platelet thrombus, and finally the formation of a clot in the now static blood. Progression of this process is probably limited and controlled by the increased production of antithrombin (and probably of other natural inhibitors), and by the fibrinolytic mechanism so that thrombus formation does not undergo retrograde spread to involve the whole vascular tree. The fibrinolytic mechanism is also reparative and is active in the healing process. In normal health a fine balance between the haemostatic and fibrinolytic systems is said to maintain the integrity of the organism (Mole, 1948; Copley, 1954; Astrup, 1956 a and b; Jensen, 1956).

The clotting process is a complex series of reactions in three main stages (Fig. 1). These stages involve the production of intrinsic thromboplastin, which activates prothrombin to thrombin, and this in turn converts fibrinogen to fibrin.

The fibrinolytic system produces plasmin from the soluble globulin precursor plasminogen in the blood. This reaction is controlled by activators which are present in the blood and tissues, and the active enzyme is released in excess at the site of injury. The plasmin so produced normally degrades fibrin (Fig. 2).

An insufficiency of one or more of the clotting factors can occur from (1) consumption, because inadequate anticoagulation may allow clot formation to occur, and (2) destruction, by an excessively activated fibrinolytic mechanism. Either may lead to a haemorrhagic state.

Because more platelets are required to produce an adequate platelet thrombus than to provide enough platelet co-factor 3 in the clotting process, thrombogenesis is impaired earlier than clotting in the presence of thrombocytopenia.

In extracorporeal circuits the maintenance of an incoagulable blood requires the addition of an anticoagulant, heparin being customarily used for this purpose. Post-operatively, the anticoagulant effect of heparin is reversed by an antagonist such as hexadimethrine bromide so that normal haemostasis is restored.

Reports on changes in the coagulation mechanism after the use of an extracorporeal circulation are numerous and variable, a reduction in platelets being the commonest change recorded.
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(Bloom, 1961). Fibrinogenopenia and excessive fibrinolysis, although frequently described, have not been inevitable (Osborn, MacKenzie, Shaw, Perkins, Hurt, and Gerbode, 1956). Depression in blood factor VIII levels (Hoeksema, Mustard, and Mustard, 1959; Smith, Brown, Young, and Sealy, 1959) has not been constant (Bloom, 1961; Matzke, Jensen, and Rygg, 1961), although Perkins, Harkins, Gerbode, Rolfs, and Acra (1961) demonstrated a striking decrease in factor VIII experimentally after the administration of hexadimethrine bromide in large doses to dogs. Many of the haematological variations seemed to be dependent on techniques employed during the procedure. Here we report attempts to achieve adequate anticoagulation in cardiopulmonary bypass and to restore normal haemostasis at the end of operation and in the post-operative period.

MATERIALS

Three groups of subjects were studied.

GROUP I Thirty-two mongrel dogs of both sexes, ranging in weight from 10.5 to 23.8 kg., were subjected to cardiopulmonary bypass.

GROUP II Ten children, aged 4 to 14 years, whose surface area ranged from 0.68 to 1.37 m², underwent cardiopulmonary bypass for the repair of ventricular septal defects, one additionally having congenital pulmonary stenosis. Particulars relevant to these patients are shown in Table I.

GROUP III This group consisted of 36 mongrel dogs of both sexes, ranging in weight from 9.7 to 26.3 kg. Of these, six were subjected to anaesthesia only, 10 to anaesthesia and thoracotomy, and 20 to standard cardiopulmonary bypass.

METHODS

The method of extracorporeal circulation, using a Melrose N.E.P. rotating disc oxygenator, has previously been described (MacKenzie, Davies, Masson, and Wade, 1963).

Healthy mongrel dogs were used as blood donors in the animal experiments. After heparinization (3 mg./kg.) they were exsanguinated under anaesthesia. Blood was collected under sterile conditions through plastic cannulae into siliconed M.R.C. bottles; the anticoagulant used was 30 mg. heparin in 15 ml. sterile normal saline, 400 ml. of blood being drawn into each bottle. Donations were made on the morning of operation. In the early dog experiments, donors and the operation animal were cross-matched and intermatched.

Routine human donor blood was always collected on the morning of operation, 400 ml. being drawn from each donor into similar anticoagulant and containers. The blood was kept at 4°C. until the priming of the machine. Donors had previously been routinely screened for abnormal antibodies, cross-matched with the patient, and intermatched among themselves.

Group III dogs were used to determine the heparin-neutralizing dose of hexadimethrine bromide and to determine the degree of influence, if any, of anees-

TABLE I

<table>
<thead>
<tr>
<th>COAGULATION STUDIES, BLOOD LOSS, AND DURATION OF PERFUSION IN SIX CHILDREN UNDERGOING CARDIOPULMONARY BYPASS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Post-anaesthetic</td>
</tr>
<tr>
<td>At end of perfusion</td>
</tr>
<tr>
<td>Post-hexadimethrine bromide</td>
</tr>
<tr>
<td>Post-anaesthetic</td>
</tr>
<tr>
<td>At end of perfusion</td>
</tr>
<tr>
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</tr>
<tr>
<td>Post-hexadimethrine bromide</td>
</tr>
<tr>
<td>Post-anaesthetic</td>
</tr>
<tr>
<td>At end of perfusion</td>
</tr>
<tr>
<td>Post-hexadimethrine bromide</td>
</tr>
</tbody>
</table>

A flow rate of 2-4 litres/m.²/min. was used in all cases. ¹Pre-anaesthetic.
themia alone, of anaesthesia with surgery, and finally of
anaesthesia with bypass on this ratio.

Subjects, both dog and human, were heparinized
immediately before cannulation of the venae cavae,
using 3 mg./kg. body weight. Hexadimethrine bromide
was given approximately one to one-and-a-half
hours after heparinization in all groups, the drug
being administered diluted in 100 to 200 ml. 5%
dextrose and given over not less than 10 minutes
to avoid circulatory upset (MacKenzie, Wade, Davies,
and Zellos, 1961). Additional doses were given as
indicated in a similar manner. Neutralization of
heparin was thought to be complete when the throm-
bin time had returned to the approximate pre-
heparinization level and was not further shortened
by the prior addition of toluidine blue to the plasma
(Rothnie and Kinmonth, 1960). In all other experi-
ments hexadimethrine bromide was used in the same
manner, and adequacy of neutralization was deter-
mined by the same criteria.

All the subjects, both animal and human, had a full
preliminary blood count and were investigated by
determination of their clotting times both in plain and
in siliconed glass tubes, their prothrombin time,
pro-
thrombin consumption index, thrombin time before
and after toluidine blue, Russell's viper venom time,
thromboplastin generation test, and in some animals
(and in all the humans) by assaying the blood factor
VIII level. Bleeding time and Hess test were done in
humans. Some of these parameters were repetitively
monitored throughout the operation. During perfusion
heparinization was thought to be adequate when a
thrombin time of not less than 60 seconds (the control
being 10 to 12 seconds) and a clotting time (Lee and
White) of not less than 60 minutes were found. At the
end of perfusion a full coagulation screening was
done, and repeated, if necessary, until reversal with
hexadimethrine bromide was considered complete.
Thereafter a modified haematological and coagulation
screening was repeated as required on the day of
operation and then at regular intervals for 14 days
post-operatively.

Standard blood counts were done according to the
methods of Dacie (1956). The glass and siliconed-treated
glass clotting times, Ivy bleeding time, Hess test, Quick
prothrombin time, prothrombin consumption index,
thrombin time before and after toluidine blue, and
recalcification time of oxalated plasma were done by
the methods of Biggs and Macfarlane (1962). Russell's
viper venom time was done according to Fullerton
(1940) and factor VIII essay and thromboplastin
generation test as described by Biggs, Eveling, and
Richards (1955) and Biggs and Douglas (1953) respec-
tively. The 'fibrinolytic' and/or 'activator' activity was
measured by a plasma euglobulin lysis time (Macfarlane
and Pilling, 1946) giving in our laboratory a normal
value of more than 80 minutes. The antifibrinolysins
were measured by their ability to inhibit the action of
serial dilutions of plasmin (in our case 'thrombo-
lysin') on a clot indicator system (Biezenski, 1960).
The normal range in our hands was found to be 1/18
to 1/10 titres. The technique of modified methods as
applied in this study has been recently described
(Kamel, 1963). The fibrinogen was assayed by a
Kjeldahl technique (N = 200–500 mg.%).

The results of our experiments are summarized in
the accompanying figures and tables.

**DISCUSSION**

Platelets occupy a central position in relation to
the problem of haemostasis in cardiopulmonary
bypass. In vivo they function in both thrombo-
genic and coagulation systems; in the former, by
undergoing viscous metamorphosis, they produce
a platelet plug, and in the latter they release
platelet factor 3 (co-factor 3), which participates
in the early stages of the clotting mechanism
(Alexander, 1962). As long ago as 1938 Best,
Cowan, and Maclean found that inhibition of
viscous metamorphosis required a much larger
dose of heparin than that required to inhibit the
release of co-factor 3. Heparin, which is still the
most suitable anticoagulant for cardiopulmonary
bypass, is limited in dose by the need to neutralize
it post-operatively by either protamine sulphate or
hexadimethrine bromide, both of which are poten-
tially toxic in excess, and are themselves anti-
coagulants in vitro in high concentrations (Preston,
1952; Perkins et al., 1961). The neutralizing ratio
of hexadimethrine bromide, now most generally
used as the heparin antagonist, has steadily risen
since the first clinical studies by Preston, Hofh, and
Trippel in 1956. These authors recommended a
0·7:1 dose ratio of hexadimethrine bromide to
heparin; Weiss, Gilman, Catenacci, and Oster-
berg (1958) advocated the increase of this dose
to 1:1, and Rothnie and Kinmonth (1960b) to
1·5:1. Table II shows that even this latter dose

<table>
<thead>
<tr>
<th>Hexadimethrine Bromide/Heparin Neutralizing Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadimethrine Bromide/Heparin Neutralizing Ratio</td>
</tr>
<tr>
<td>Less than 1 to 1</td>
</tr>
<tr>
<td>Six intact dogs</td>
</tr>
<tr>
<td>Ten dogs undergoing thoracotomy</td>
</tr>
<tr>
<td>Twenty dogs undergoing perfusion</td>
</tr>
</tbody>
</table>

All dogs were initially given heparin in a dose of 3 mg./kg. I.V.

ratio may not always be adequate. Heparin, 30
mg., in 400 ml. of blood is still inadequate to
prevent platelet loss (Table III), and there is a
further progressive loss on storage (Baldini,
Costea, and Dameshek, 1960). It is still preferable,
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### Table III

<table>
<thead>
<tr>
<th>Time Collected</th>
<th>At Collection</th>
<th>After Pump Priming</th>
<th>Percentage Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within one hour of priming</td>
<td>305</td>
<td>190</td>
<td>38</td>
</tr>
<tr>
<td>On day before priming</td>
<td>320</td>
<td>140</td>
<td>56</td>
</tr>
</tbody>
</table>

Counts are in $10^9$ mm$^3$. The figures given in each group are the average of each of four pooled priming volumes (approximately 15 dogs in each group).

Therefore, to collect blood on the morning of operation rather than the night before, and this is in agreement with recent findings (Botha and Barnard, 1962). The extensive area of foreign surface in the extracorporeal circuit, despite the effect of siliconization to reduce water wettability, produces a pronounced platelet loss through platelet adhesion even with freshly collected blood (Table III), but we found that further platelet loss does not follow proportionately to the duration of bypass (Fig. 3), in contrast to the findings of Gans and Krivit (1962). The prime loss of platelets on the distal end of the 'trombone' of the oxygenator could have serious potentialities for the patient post-operatively by depriving him of their action in the thrombogenic phase of haemostasis. However, this platelet adhesion might also release a quantity of co-factor 3 into the extracorporeal circuit during the bypass and thereby minimally activate the coagulation mechanism with consumption of clotting factors and thus possibly activate the fibrinolytic mechanism. Neither the clotting factor loss nor the increased fibrinolysis might be evident until neutralization of heparin had been effected at the end of bypass.

The minimal platelet count post-operatively occurs at a varying time (Table IV), ranging in these four patients from day 1 to day 4–5, and this could be attributed to mechanical damage to the platelets in the pump with their subsequent shortened survival. We found no evidence, by inference from red cell compatibility tests (Table V), that this might be a platelet antigen/antibody reaction, and our results (Fig. 4) suggest that the red cells suffer trauma in the pump that is directly related to perfusion time, the rise in plasma haemoglobin being proportional to the length of perfusion.

A shortening of the thrombin time is indicative of loss of circulating heparin, presumably due either to its utilization or to its shift into the tissues. Thus a shortened thrombin time, if not corrected by the addition of further heparin, will presage the activation of the clotting mechanism and the consumption of clotting factors including fibrinogen and factor VIII. Using heparin in a dose

### Table IV

<table>
<thead>
<tr>
<th>No. of Dogs</th>
<th>Pre-perfusion</th>
<th>Mixed Donor Blood</th>
<th>Post-b-hexademethrine Bromide</th>
<th>Duration of Perfusion (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 fully intermatched</td>
<td>245 (105-490)</td>
<td>148 (58-245)</td>
<td>64 (45-90)</td>
<td>40 (20-63)</td>
</tr>
<tr>
<td>24 not intermatched</td>
<td>240 (145-400)</td>
<td>139 (39-430)</td>
<td>104 (20-220)</td>
<td>43 (13-62)</td>
</tr>
</tbody>
</table>

Counts are in $10^9$ mm$^3$. ; ranges are given in parentheses.

### Table V

| TABLE V

<table>
<thead>
<tr>
<th>EFFECT ON PLATELETS OF MATCHED AND UNMATCHED PRIMING BLOOD IN DOGS UNDERGOING CARDIOPULMONARY BYPASS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Dogs</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>8 fully intermatched</td>
</tr>
<tr>
<td>24 not intermatched</td>
</tr>
</tbody>
</table>

Counts are in $10^9$ mm$^3$. ; ranges are given in parentheses.

---

**Fig. 3. Percentage fall in platelet count in dogs undergoing cardiopulmonary bypass.**

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**Table IV**

<table>
<thead>
<tr>
<th>Post-anaesthesia</th>
<th>Perfusion</th>
<th>Post-b-hexademethrine Bromide</th>
<th>Post-operative Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beginning</td>
<td>End</td>
<td>20 min.</td>
</tr>
<tr>
<td>265</td>
<td>245</td>
<td>175</td>
<td>160</td>
</tr>
<tr>
<td>195</td>
<td>170</td>
<td>110</td>
<td>104</td>
</tr>
<tr>
<td>365</td>
<td>185</td>
<td>101</td>
<td>103</td>
</tr>
<tr>
<td>175</td>
<td>190</td>
<td>105</td>
<td>105</td>
</tr>
</tbody>
</table>

Counts are in $10^9$ mm$^3$.  

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of 3 mg./kg. body weight (Blumberg, Winterscheid, Dillard, Vetto, and Merendino, 1960) in the patient and 30 mg./400 ml. donor blood for priming the machine, platelet loss has been a constant but not serious factor: there has been no appreciable activation of the clotting process as shown by the maintenance post-operatively of relatively normal fibrinogen and factor VIII levels (Table VI). This is in contrast with the fall in factor VIII levels described by other workers (Hoeksema et al., 1959; Smith et al., 1959).

Fibrinolysis has likewise been a minor problem and in no case has the total post-operative blood loss been excessive (range 23–77 ml./kg., mean 43, in post-operative period). A further increase in the initial heparin dosage might therefore be advantageous were it not for the fact that the use of hexadimethrine bromide post-operatively in a 2 to 1 neutralizing ratio itself constitutes a potential hazard in that this drug can cause toxic effects in large doses. However, the thrombin time should be checked at regular short intervals while the patient is on the pump, and further doses of heparin should be given if it falls below 60 seconds.

If a shortened thrombin time heralds a minimal activation of the clotting process, it may likewise herald a compensatory activation of the fibrinolytic mechanism. Our results show that, in two out of three patients in whom the thrombin time had shortened to less than 60 seconds at the end of bypass and who were given no further heparin, the fibrinolytic activity had increased when the heparin was subsequently neutralized, as shown by a marked fall in the euglobulin lysis time; and indeed in these patients fairly rapid lysis of the whole blood clot was the first indication of it. However, the excess fibrinolytic activity subsided spontaneously within 10 minutes to a level such that bleeding was not excessive.

These observations suggest that a progressive shortening of the thrombin time in the heparinized patient on cardiopulmonary bypass may, if allowed to continue, indicate activation of the clotting mechanism and thus the fibrinolytic mechanism so that fibrinolysis could be a real problem as soon as heparinization has been reversed. Significant consumption of clotting factors may not have to occur to produce clinically significant fibrinolysis. The need regularly to check the thrombin time during bypass and to maintain adequate heparinization becomes obvious, and we now give additional heparin to such patients where necessary. Since instituting this régime, we have had no marked post-operative fibrinolysis and no excessive post-operative haemorrhage.

**SUMMARY AND CONCLUSIONS**

Our experimental work suggests that the neutralizing dose of hexadimethrine bromide for heparin should be based on a 2:1 ratio. The role of platelets in the haemostatic mechanism and in particular in relation to extracorporeal cardiopulmonary bypass is discussed. We suggest that adequate heparinization must be maintained to minimize platelet loss and to minimize activation of the clotting mechanism and hence the fibrinolytic mechanism. We think that regular thrombin

**TABLE VI**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-anæsthesia</th>
<th>Primed Blood</th>
<th>Perfusion</th>
<th>Post-operative Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Perfusion</strong></td>
<td><strong>Immediately</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>During</strong></td>
<td><strong>After</strong></td>
</tr>
<tr>
<td>1</td>
<td>87</td>
<td>91</td>
<td>68</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>129</td>
<td>78</td>
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<td>3</td>
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<tr>
<td>9</td>
<td>68</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>97</td>
<td>100</td>
<td>--</td>
<td>103</td>
</tr>
</tbody>
</table>

Levels of factor VIII are given in per cent of normal.
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ADDENDUM

Since this paper was written hexadimethrine bromide has been withdrawn from the market because of possible toxic renal effects.
Some Haematological Observations in Cardiopulmonary Bypass at Normothermia Using the Melrose Oxygenator

R. A. Cumming, S. H. Davies, K. Kamel, G. J. Mackenzie, A. Masson and J. D. Wade

Thorax 1964 19: 170-175
doi: 10.1136/thx.19.2.170

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