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

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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 anti-thrombin (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

FIG. 1. *The clotting process.*

FIG. 2. *The fibrinolytic system.*

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

¹On a study grant from Ain-Shams University, Cairo

(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 m.², 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 anaes-

TABLE I

COAGULATION STUDIES, BLOOD LOSS, AND DURATION OF PERFUSION IN SIX CHILDREN UNDERGOING CARDIOPULMONARY BYPASS

	Thrombin Time (seconds)	Fibrinogen (mg.%)	Factor VIII (Oxford units)	Euglobulin Lysis Time (min.)	Anti-fibrinolysins (titre)	Duration of Perfusion (min.)	Blood Loss (ml./kg.)	
							Day of Operation	First Day Post-op.
<i>F. 4 yr., 17 kg., ventricular septal defect</i>								
Post-anaesthetic	11	220	62 ¹	135	1/14	40	40	0
At end of perfusion	48	—	100	60				
Post-hexadimethrine bromide	12	195	75	140	1/10			
<i>F. 9 yr., 26.25 kg., ventricular septal defect</i>								
Post-anaesthetic	10	300	114 ¹	> 580	1/16	31	20	3
At end of perfusion	60	261	69	295	1/16			
Post-hexadimethrine bromide	10	262	88	280	1/18			
<i>M. 8 yr., 22.5 kg., ventricular septal defect</i>								
Post-anaesthetic	10	235	92 ¹	> 488	1/20	47	40	5
At end of perfusion	60	288	69	305	1/18			
Post-hexadimethrine bromide	10	180	80	430	1/20			
<i>M. 13 yr., 33.5 kg., ventricular septal defect</i>								
Post-anaesthetic	10	231	100 ¹	> 360	1/6	24	43	34
At end of perfusion	25	199	100	138	1/8			
Post-hexadimethrine bromide	10	141	88	33	1/6			
<i>M. 12 yr., 30.5 kg., ventricular septal defect and pulmonary stenosis</i>								
Post-anaesthetic	11	195	100 ¹	270	1/10	85	33	8
At end of perfusion	60	236	103	145	1/12			
Post-hexadimethrine bromide	10	137	103	95	1/16			
<i>M. 11 yr., 28.5 kg., ventricular septal defect</i>								
Post-anaesthetic	10	289	91 ¹	> 360	1/8	50	30	3
At end of perfusion	48	149	68	81	1/24			
Post-hexadimethrine bromide	11	213	87	228	1/24			

A flow rate of 2.4 litres/m.²/min. was used in all cases.

¹Pre-anaesthetic.

thetia 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 thrombin 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, 1960b). In all other experiments hexadimethrine bromide was used in the same manner, and adequacy of neutralization was determined 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, prothrombin 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 silicone-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 assay and thromboplastin generation test as described by Biggs, Eveling, and Richards (1955) and Biggs and Douglas (1953) respectively. 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 'thrombolylin') 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 thrombogenic 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 potentially toxic in excess, and are themselves anticoagulants *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, Hohf, and Trippel in 1956. These authors recommended a 0.7:1 dose ratio of hexadimethrine bromide to heparin; Weiss, Gilman, Catenacci, and Osterberg (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 II
HEXADIMETHRINE BROMIDE/HEPARIN NEUTRALIZING RATIO IN DOGS

	Hexadimethrine Bromide/Heparin Neutralizing Ratio			
	Less than 1 to 1	1.0-1.5 to 1	1.5-2.0 to 1	2 to 1 or more
Six intact dogs	-	4	2	-
Ten dogs undergoing thoracotomy	-	4	3	3
Twenty dogs undergoing perfusion	3	9	3	5

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,

TABLE III

COMPARISON OF PLATELET LOSS IN FRESHLY DRAWN AND STORED POOLED DOGS' BLOOD USED TO PRIME 'MELROSE' HEART-LUNG MACHINE

Time Collected	At Collection	After Pump Priming	Percentage Fall
Within one hour of priming	305	190	38
On day before priming	320	140	56

Counts are in $10^3/\text{mm}^3$.

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

FIG. 3. Percentage fall in platelet count in dogs undergoing cardiopulmonary bypass.

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

TABLE V

EFFECT ON PLATELETS OF MATCHED AND UNMATCHED PRIMING BLOOD IN DOGS UNDERGOING CARDIOPULMONARY BYPASS

No. of Dogs	Pre-perfusion	Mixed Donor Blood	Post-hexadimethrine Bromide	Duration of Perfusion (min.)
8 fully intermatched	245 (105-490)	148 (58-245)	64 (45-90)	40 (20-63)
24 not intermatched	240 (145-400)	139 (39-430)	104 (20-220)	43 (13-62)

Counts are in $10^3/\text{mm}^3$; ranges are given in parentheses.

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

EFFECT OF CARDIOPULMONARY BYPASS ON PLATELET COUNTS IN FOUR PATIENTS

Post-anaesthesia	Perfusion		Post-hexadimethrine Bromide		Post-operative Days							
	Beginning	End	20 min.	120 min.	1	2	3	4	5	6	7	8
265	245	175	160	70	150	115	87	115	140	138	195	290
195	170	110	104	124	87	87	184	240	320	340	420	435
365	185	101	103	149	235	300	190	170	168	192	210	240
175	190	105	105	95	120	110	88	66	92	160	360	400

Counts are in $10^3/\text{mm}^3$.

FIG. 4. Rise in plasma haemoglobin related to duration of perfusion in dogs undergoing cardiopulmonary bypass.

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 fibrino-

lytic 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
FACTOR VIII (A.H.F.) LEVELS IN PATIENTS UNDERGOING CARDIOPULMONARY BYPASS

Patient	Pre- anaesthesia	Primed Blood	Perfusion			Post-operative Days					
			During	Immediately After	After 2–3 Hours	1	3	4	6	8	10
1	87	91	68	87							
2	62	129	78	78							
3	92	62	76	79							
4	62	79	101	75							
5	68	75	87	62	90	87		100			56
6	69	100	87	75	81	75				83	
7	90	114	69	88		81		88			100
8	—	92	69	80		75	69				75
9	68	100	100	88		66	79		92		94
10	97	100	—	103	100	111			100		100

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

time estimations whilst the patient is on bypass are a good indicator of the control of this process.

The authors wish to thank Miss A. Cameron, of the Blood Transfusion Service, for technical assistance. Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania, kindly supplied the 'Thrombolylin' used in the antifibrinolytic activity assays.

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ADDENDUM

Since this paper was written hexadimethrine bromide has been withdrawn from the market because of possible toxic renal effects.