The response of the other infections, usually complicated and due to such resistant organisms as *Proteus* and *Ps. pyocyanea*, was disappointing. Temporary improvement was experienced by 6 out of 21 patients, but 12 out of 21 failed to respond.

Strict laboratory control is essential when cycloserine is used for treatment of urinary infections.

We thank our clinical colleagues for their close co-operation throughout this investigation, and Messrs. Eli Lilly and Company for the supply of cycloserine.

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THE NEUTRALIZATION OF HEPARIN AFTER PERFUSION

A COMPARISON OF PROTAMINE AND "POLYBRENE"

BY

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The complete neutralization of heparin is essential after perfusion for open heart surgery because residual heparin can cause or aggravate a bleeding state. The proper correction of heparin by a neutralizer can be ensured by the use of a simple test, the thrombin clotting-time of plasma, which can be made very sensitive to traces of heparin. Its method of performance and use in practice have been previously described (Rothnie and Kinmonth, 1960).

The rapid reversal of the effect of heparin and the restoration of haemostasis after operation requires an efficient heparin neutralizer. The variation and uncertainty of adequate heparin neutralization which we have found with protamine led us to an investigation of the use of the new antiheparin agent "polybrene" (hexadimethrine bromide). We report in this paper the results with this antidote in a series of experimental perfusions in the dog and in patients undergoing perfusion for the correction of varying cardiac abnormalities. These results are compared with those obtained when protamine was used as the antidote. From this investigation we have determined the most satisfactory amount of polybrene needed for complete heparin neutralization after perfusion.

Methods

In all the perfusions the Melrose rotating disk oxygenator was used. The blood samples were fixed with 3.8% (w/v) of trisodium citrate in the ratio of 9 volumes to 1. They were obtained through a small cannula in the inferior vena cava inserted through the saphenous vein in the groin (Rothnie *et al.*, 1960).

Citrated platelet-poor plasma was obtained by centrifuging the blood at 3,000 r.p.m. for 5–10 minutes.

The thrombin clotting-time was obtained by adding a thrombin-calcium solution to citrated plasma and timing the clot formation. If the thrombin clotting-time was prolonged beyond the control time of 10–12 seconds, the test was repeated after adding toluidine blue to the plasma sample. If the result was more normal this was taken to indicate that the original prolongation had been due to heparin (Rothnie and Kinmonth, 1960).

The control pre-perfusion thrombin clotting-time was obtained in each case from a sample of plasma taken before heparinization, and subsequent clotting-times after perfusion were compared with this.

Heparin was given intravenously to each case immediately before caval and femoral cannulation and its antidote by the same route after removal of these cannulae, about 10–15 minutes after stopping perfusion.

Results with Protamine

In a series of 19 perfused dogs, which were given heparin 2.5 mg./kg. (donor blood 25 mg./500 ml.) and protamine as the antidote, 11 had troublesome bleeding afterwards due to residual heparin. Of these 11 bleeders, five died of continuous oozing after operation and had received protamine 2.5 or 3.75 mg./kg. (ratio of protamine to heparinizing dose of 1:1 or 1.5:1). Haemothoraces occurred in the other six bleeders and contributed to the death of two of them. These six cases were given varying doses of protamine from 2.5 to 7.5 mg./kg. (1:1 to 3:1). The remaining eight dogs in the series were non-bleeders, and most of them received protamine 7.5 or 10 mg./kg. (3:1 or 4:1).

Thus as a result of increasing the dose of protamine and ensuring that there was no residual heparin by means of the thrombin clotting-test, we were able to reduce the incidence of oozing after perfusion and eliminate the immediate mortality from this cause. There was, however, a variation in the amount of protamine necessary for the complete neutralization of heparin. In some dogs a ratio of 2:1 was sufficient and in others a ratio of 4:1 was required.

A similar variation in protamine requirements was found in six patients (average weight 30 kg.) who underwent perfusion for the correction of varying cardiac abnormalities. They received heparin 2.5 mg./kg. and doses of protamine varying from 3.75 mg./kg. (1.5:1) to 7.5 mg./kg. (3:1). After perfusion two of them had troublesome bleeding due to residual heparin and had received a total dose of protamine of 5 mg./kg. (2:1) and 7.5 mg./kg. (3:1). Details of the thrombin clotting-times in these two patients were previously reported by us in stressing the importance of the proper correction of unneutralized heparin (Rothnie and Kinmonth, 1960). One other patient, who had a trace of heparin after the administration of protamine 5 mg./kg. (2:1), had an increased post-operative drainage compared with the three non-bleeders. None of these six patients, who were perfused for an average time of 32 minutes, had any evidence of a defect of a major clotting factor. The average post-operative drainage in 24 hours was 40 ml./kg. (lowest 15 ml./kg.; highest 80 ml./kg.).

The results in these experimental and clinical cases showed that for the adequate neutralization of heparin there was a variation in the requirements of protamine from 1.5:1 to 4:1 (see Table).

Results in Experimental and Clinical Cases

Heparin Antidote	Total No. of Cases	Heparin Dose	Ratio of Antidote to Heparin	Non-Bleeders	Bleeders	
					Heparin	Other Causes
Protamine	19 dogs	2.5 mg./kg.	1:1	—	2	—
			1.5:1	—	5	—
			2:1	1	—	—
			2.5:1	1	2	—
Protamine	6 patients	2.5 mg./kg.	3:1	3	1	—
			4:1	3	1	—
			1.5:1	2	1	—
			2:1	1	1	—
Polybrene	25 cases	2 mg./kg.	3:1	—	1	—
			1:1	10	—	3 surgical
			1.5:1	16	—	1 surgical
			1.5:1	7	—	—
Polybrene	60 cases	2.5 mg./kg.	1:1	17	—	1 low fibrinogen
			1.5:1	5	—	—
			1.5:1	5	—	—
			1.5:1	55	—	5

Results with Polybrene

In this investigation polybrene was used in a series of 48 experimental perfusions in the dog and in 12 patients perfused for the open correction of varying cardiac defects.

Experimental Series

In 30 of these perfusions the dogs were given heparin 2 mg./kg. and the donor blood 20 mg./500 ml., and were perfused for a mean time of 37 minutes. The remaining 18 dogs received heparin 2.5 mg./kg. and the donor blood 25 mg./500 ml., and were perfused for an average of 49 minutes. These doses of heparin gave satisfactory anticoagulation and protection of the clotting elements during perfusion.

In all these dogs thrombin clotting-times were assessed on plasma samples taken before heparinization, after perfusion and 5–10 minutes after the administration of polybrene. In 22 dogs, plasma samples were tested at intervals up to two hours after perfusion.

Of the 30 dogs given heparin 2 mg./kg., 19 received an initial dose of polybrene of 2 mg./kg. (ratio 1:1), and 11 a dose of 3 mg./kg. (ratio 1.5:1). Four of the 19 dogs were found to have a trace of heparin as shown by the thrombin-clotting test 5–10 minutes after giving the antidote. They required a further 1 mg. of polybrene per kg., making a total of 2 mg./kg. (1.5:1), for complete neutralization. The remainder (15) of the 19 dogs showed adequate heparin neutralization, but subsequently two of them received a small transfusion (200–400 ml.) of heparinized blood from the heart-lung machine, which necessitated giving more polybrene to a total of 3 mg./kg. (1.5:1). Each of the 11 dogs receiving one dose of polybrene of 3 mg./kg. (1.5:1) showed satisfactory neutralization of heparin.

Thus in 30 experimental perfusions, heparin was completely neutralized by polybrene in a dose of 2 mg./kg. in 13 dogs and of 3 mg./kg. in 17 dogs. The most satisfactory neutralizing dose of polybrene for a heparinizing dose of 2 mg./kg. (see Table) was therefore 3 mg./kg. or a ratio of polybrene to heparin of 1.5:1.

The 18 dogs who received heparin 2.5 mg./kg. were given this ratio of polybrene—that is, a dose of 3.75 mg./kg.—and each was found to have adequate neutralization of heparin.

In 22 of the 30 perfusions (heparin 2 mg./kg.) which showed adequate heparin neutralization 5–10 minutes

after giving the antidote further thrombin clotting-times were assessed on plasma samples taken 30, 60, and 120 minutes afterwards. Ten of these dogs were given polybrene 2 mg./kg. (1:1) and 12 3 mg./kg. (1.5:1). The thrombin clotting-times of each dog up to two hours after administering the antidote were similar to that of the pre-perfusion plasma control. The neutralization of heparin was therefore complete within 10 minutes of giving these doses of polybrene and was satisfactorily maintained. No anticoagulant was found to appear subsequent to adequate neutralization.

In each of the 48 experimental perfusions satisfactory heparin neutralization was achieved with polybrene and there was no instance of bleeding due to residual heparin. In the first 30 dogs there were four cases of abnormal bleeding. They were due to surgical causes and no evidence of a clotting defect was found. In the remaining 18 dogs there was one bleeder. This was due to a much-reduced fibrinogen concentration (ca. 10 mg./100 ml.) after a perfusion lasting one hour in a dog which, before operation, had a low fibrinogen concentration of 100 mg./100 ml. The donor blood was also poor in fibrinogen (180 mg./100 ml.).

Complete neutralization was best achieved by giving 1.5 mg. of polybrene for every milligram of the heparinizing dose given to the case—that is, a ratio of 1.5:1.

Clinical Series

This series consisted of 12 patients, seven of whom were given heparin 2 mg./kg. (donor blood 20 mg./500 ml.), and five heparin 2.5 mg./kg. (donor blood 25 mg./500 ml.). Each received 1.5 mg. of polybrene for each milligram of heparin given to the patient (ratio 1.5:1). The duration of perfusion varied from 20 minutes to over two hours.

By means of the thrombin clotting-time complete heparin neutralization was found in each patient 10 minutes after giving this dosage ratio of polybrene, and there was no instance of bleeding due to unneutralized heparin. The average post-operative drainage for 24 hours in six patients (average weight 28.7 kg.), who were comparable to the six patients described above and given protamine, was about 21 ml./kg. in 24 hours (range of 10–40 ml./kg.). This compares with an average of 40 ml./kg. for the patients given protamine which was quoted above.

In five patients in this series thrombin clotting-times were estimated on plasma samples taken 5–10, 30, and 90 minutes after administration of the polybrene. In each patient the clotting-times at these intervals were similar to the pre-perfusion control time, and there was no evidence of the reappearance of heparin (Fig. 1).

In one patient receiving heparin 2 mg./kg., a dose of polybrene 2 mg./kg. (1:1) was given initially. Tests after this showed adequate neutralization (Fig. 2). A small transfusion of 200 ml. of heparinized blood was then given. The thrombin clotting-time after this was increased owing to the presence of heparin, the concentration of which would have been enough to delay coagulation. A further 1 mg. of polybrene per kg. (total dose 3 mg./kg.—that is, 1.5:1) neutralized the residual heparin.

This case further emphasizes the experimental finding that polybrene in a ratio of 1:1 may be only just sufficient for adequate neutralization and provides little reserve to cover the variations in the total circulating heparin that might occur owing to perfusion.

As in the experimental perfusions, polybrene in a dose of 1.5 mg. for each milligram of the heparinizing dose to the patient was found to be consistent and satisfac-

FIG. 1.—Mean thrombin clotting-times charted at intervals from the start of perfusion in five patients. Indicates incoagulable plasma.

FIG. 2.—Thrombin clotting-times before and after perfusion in a patient initially given polybrene 2 mg./kg. and requiring a further 1 mg./kg. after a transfusion of heparinized blood (200 ml.). ∞ Indicates incoagulable plasma.

FIG. 3.—Arterial and venous pressure tracing to show the effects of rapid injection of polybrene (6 seconds) intravenously.

tory. We did not find any evidence that this amount of polybrene produced any adverse effect on the clotting mechanism.

Reaction to Polybrene Injection.—When given rapidly to the perfused dog in about 5 seconds there was a transient drop in arterial pressure, which rapidly returned to normal in about 15 seconds, with no change in venous pressure (Fig. 3). The rapid injection of an equal volume of normal saline under similar conditions produced no such change in arterial pressure, showing that the reaction was most likely due to the polybrene itself. This hypotensive effect of polybrene appears to be less marked and less prolonged than that

found by Hurt *et al.* (1956) after the rapid injection of protamine in perfused dogs.

When polybrene was given undiluted and slowly in two to three minutes, no change in arterial or venous pressure was noted in the perfused dog or patient.

Discussion and Conclusions

The heparin present in the patient after perfusion is derived from two main sources. One is the heparinizing dose given to the patient and the other the perfused heparinized donor blood. In each perfusion the first is a constant amount based on the body weight of the patient; the second is a variable amount depending on how much donor blood is used. When these two sources are mixed during bypass the result is usually a variable increase in the amount of heparin requiring neutralization in the patient at the end of perfusion.

To neutralize this variable amount of heparin it is thus not entirely accurate to base the antidote requirements on the heparinizing dose of the patient. It is, however, convenient to relate the dose of antidote to it, and hence to body weight, provided an allowance is made for the increased heparin. It is usually expressed as a ratio of milligrams of antidote for each milligram of heparin given to the patient.

In practice, we have therefore given at the end of perfusion such a dose of antiheparin agent based on body weight. The presence of any unneutralized heparin after this was detected or excluded by the thrombin clotting-time and further antidote given if necessary. In this way we have determined the most satisfactory dose of antidote necessary for complete heparinization after perfusion.

Although adequate heparin neutralization could be achieved in our series with protamine, this agent was not entirely satisfactory because of its inconsistent and variable neutralizing activity. This could be partly due to the varying amount of heparin present after perfusion, but there was no evidence that such heparin variations predominated in the cases given protamine. The variable activity is more likely to be due to the varying potency of the preparations of this biological product and to competition for the agent, unlike polybrene, between the plasma proteins, especially fibrinogen, and heparin.

We have not encountered hypotensive reactions (Hurt *et al.*, 1956), provided the protamine is given slowly, nor have we observed any anticoagulant effect (Andersen *et al.*, 1959) with the doses we have used in the cases reported above. Neither of these side-effects of protamine appears to us to contraindicate its use after perfusion. However, dissatisfaction with its efficiency as a heparin neutralizer led us to investigate the suitability of other antiheparin agents.

Toluidine blue has been used clinically but is slow and uncertain in its action, with side-effects which preclude its use after perfusion (Allen *et al.*, 1949; Bickers, 1953; Weiss *et al.*, 1958). The more recently introduced anti-heparin agent is polybrene (Preston and Parker, 1953). It is a basic polymer of a quaternary ammonium salt with the generic name hexadimethrine bromide.

A dose of 1 mg. of polybrene for each milligram of the heparinizing dose given to the patient (ratio of 1:1) is the amount recommended by Weiss *et al.* (1958) and by Keats *et al.* (1959) after perfusion. These workers used the whole-blood clotting-time to detect adequate

heparin neutralization. We have previously pointed out that this clotting-test is a poor indicator of heparin activity (Rothnie and Kinmonth, 1960). With the use of the more sensitive thrombin clotting-time some of our experimental cases showed incomplete neutralization of heparin with this 1:1 ratio of the antidote. Evidence was found in other cases that such a dose was only just enough to complete neutralization. This slight variation in polybrene requirements is most likely due to the variable amount of heparin present after perfusion. Since it is important to neutralize the anticoagulant completely, we have used a polybrene-to-heparin ratio of 1.5:1, and this has consistently been satisfactory.

With this dose of polybrene we have noted no inhibitory effects of the drug on coagulation. The problem of the anticoagulant effect of polybrene, and of protamine (Andersen *et al.*, 1960), has been investigated and will be the subject of another report. There appears to be a wide margin of safety with both agents and no danger of inhibiting coagulation with the doses used clinically.

Neutralization by polybrene, as with protamine, is prompt, and once the heparin has been completely neutralized we have found no evidence of the reappearance of the anticoagulant in the circulation. This theoretical reappearance must not be confused with the term "heparin rebound" which has been given to a period of hypercoagulability of the blood after prolonged heparin administration (Cate *et al.*, 1954).

If a transfusion of heparinized blood is given to the patient after perfusion, we have found it advisable to cover this extra heparin with polybrene. A dose of 1 mg. of polybrene for each milligram of heparin in the transfused donor blood is a convenient and adequate amount.

The slow administration of polybrene produced no hypotensive effects, but careful observation of the arterial pressure should be made during the injection, which should be temporarily stopped if the pressure falls.

Although our comparison of the amount of post-operative drainage after each antidote involved only a small number of patients, our findings that polybrene was superior to protamine are in agreement with those of a larger comparative series studied by Lillehei *et al.* (1960).

This investigation has shown that polybrene, when compared with protamine, is more consistent as a heparin neutralizer after perfusion and more effective in eliminating residual heparin as a cause of abnormal bleeding.

Summary

An investigation of the neutralization of heparin by protamine and "polybrene" (hexadimethrine bromide) after perfusion of experimental and clinical cases has been made, using the thrombin clotting-time as the indicator of heparin activity.

Protamine was found to be variable and uncertain in its neutralizing activity and the reasons are briefly discussed. Polybrene was the more consistent and effective antiheparin agent, and a dose of 1.5 mg. for each milligram of the heparinizing dose given to the patient was found to be satisfactory.

The hypotensive and anticoagulant side-effects of these agents were not noted with the doses and method of administration recommended.

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TRANSVENTRICULAR MITRAL VALVOTOMY DURING PREGNANCY

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The surgical relief of rheumatic mitral stenosis now meets with universal acceptance. In pregnancy the mechanical impediment of the narrowed valve is further aggravated by the physiological alterations in the circulation, particularly during the second trimester. The place of mitral valvotomy during pregnancy is still under discussion, and references to the operation in such circumstances in the world literature are still sparse. The earliest reports were by Brock (1952), Cooley and Chapman (1952), and Logan and Turner (1952). These three papers described a total of seven cases of valvotomy during pregnancy. Since then the total has risen to 133. All these operations were performed by the finger method or by a suitable knife via the atrium.

Present Series

The purpose of this communication is to add a description and an analysis of a further 10 patients operated on during pregnancy by the transventricular route assisted by a mechanical splitter, first used and described by Logan and Turner (1959).

The Table summarizes the salient features of the 10 patients in this series.

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