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Is Protamine a Clinically Important Anticoagulant?

A Negative Answer

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The effects of moderate overdoses of protamine on the coagulation mechanism were studied in heparinized patients following cardiopulmonary bypass and in healthy volunteers who had received no heparin. In heparinized patients the dose of protamine (in mg) necessary to neutralize heparin (in mg) ranged from 0.25 to 0.72 times the total amount of heparin administered to the patient–pump system. The mean dose was 0.52 times the dose of heparin for eight patients with the Travenol bubble oxygenator and 0.30 times the dose of heparin for six patients with the Mayo-Gibbon vertical-screen oxygenator. Overdoses of protamine of as much as 800 mg/70 kg had minimal effects on the coagulation mechanisms of both patients and volunteers. These effects consisted of a slight increase in the Lee-White coagulation time without increase in the partial thromboplastin time. The effects were transient, and none persisted for as long as 60 minutes. Since relatively small amounts of unneutralized heparin produce anticoagulation, it is recommended that more than the minimal amount of protamine needed be administered when re-establishment of normal coagulation in a previously-heparinized patient is attempted. More than adequate neutralization can be achieved without protamine-induced anticoagulation by the following doses: for pumps with small primes, an amount of protamine equal to the total dose of heparin; for pumps with large primes, an amount equal to half the total dose of heparin. (Key words: Protamine; Heparin neutralization; Extracorporeal circulation.)

PROTAMINE'S anticoagulant effect had been reported [1] 36 years before Chargaff and Olson [2] introduced it as a heparin antagonist in 1937. Subsequent reports that protamine had anticoagulant activity [3-7] presented clinicians with a dilemma when reversal of heparin following cardiopulmonary bypass was necessary. Too small a dose of protamine leaves some residual heparin, and bleeding may continue. However, excess protamine may produce its own anticoagulant effect. The correct protamine–heparin ratio for reversal of anticoagulation is a matter of general disagreement. Protamine doses of zero [8] to three [9] or four [10-11] times the original dose of heparin have been recommended. Therefore, we designed a study to identify the minimal dose of protamine necessary to just neutralize heparin following cardiopulmonary bypass and to investigate the anticoagulant effects of protamine given in excess of this amount.

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Studies in Volunteers

METHODS

To quantify the coagulation defect of excess protamine, we utilized paid, informed human volunteers who underwent no surgery and were given no heparin. Two dosage schedules were used. One group of six conscious volunteers each received four small doses of protamine to simulate a common method of administration to patients following cardiopulmonary bypass.[9] Four intravenous doses of 200 mg of protamine/70 kg body weight were given at half-hourly intervals. Each dose was given over a period of 4 minutes (50 mg/70 kg/min). Partial thromboplastin time,[12] Lee-White coagulation time,[13, 14] and combined heparin–protamine titration were determined before injection, 5 minutes after each of the first three injections, and 5, 30, and 60 minutes after the final injection.

To simulate the single-large-dose method of administering protamine,[15] four of the previ-

ous volunteers and two others were given 600 mg of protamine/70 kg over a period of 12 minutes (50 mg/70 kg/min). Clotting tests were performed before and 5, 30, 60, and 90 minutes after administration.

A polyethylene catheter in the radial artery of each of three volunteers permitted direct blood-pressure measurement and blood sampling. Three milliliters of blood were removed from the catheter and discarded prior to taking the test samples to prevent contamination from the heparin flush. Blood from the other volunteers was obtained by repeated venipunctures using a two-syringe technique. The blood collected in the first syringe was used to determine the partial thromboplastin time; the blood in the second syringe was used for the Lee-White coagulation time and the combined heparin–protamine titration. In those volunteers who did not have intra-arterial catheters, blood pressure was measured by the Riva-Rocci sphygmomanometer. The volunteers' oral temperatures were checked before and 5 minutes after each injection.

Tests of Coagulation

Since there is considerable variation in methods from one laboratory to another, our techniques are described in detail.

Partial Thromboplastin Time.[12] Nine milliliters of blood were mixed with 1.0 ml of 3.8 per cent sodium citrate and centrifuged for 10 minutes at 2,000 rpm. The plasma was removed and 0.1 ml of plasma was mixed with 0.1 ml of activated partial thromboplastin reagent (Hyland). After 3 minutes' incubation at 37 C in a temperature-controlled heating block, 0.1 ml of 0.03-M calcium chloride was added and the mixture allowed to incubate for an additional 30 seconds. The tube was then removed from the controlled-temperature block and gently tilted back and forth until final gel formation occurred. The time from addition of calcium chloride to final gel formation was measured.

Lee-White Coagulation Time.[13, 14] Immediately after collection, 1.0 ml of blood was placed in each of three clean, disposable, 75 × 12 mm borosilicate test tubes kept at 37 C in the heated plastic block. A digital clock was started when the blood was added to the first tube. Beginning at one minute, the first tube was gently tilted 90 degrees once every 30 seconds until it could be inverted without flowing. This time was recorded, and the same procedure was repeated with the second tube. The third tube was handled similarly after the second tube had clotted.

Combined Heparin–Protamine Titration. Five test tubes were prepared immediately before sampling: Tube A, 1.0 unit heparin; Tube B, 0.5 unit heparin; Tube C, empty (control); Tube D, 10 μg protamine; Tube E, 20 μg protamine. The additives were in solution in microliter volumes, and the tubes were kept at 37 C. One milliliter of blood was added to each tube and the digital clock started. Each tube was gently tilted once every 30 seconds until it could be inverted without causing the blood to flow. With the blood of untreated patients, and again after optimal reversal of heparin, the control tube clots before any of the other tubes. When unneutralized heparin is present, the protamine-containing tubes clot first. Likewise, when there is excess protamine, the clotting times of the heparin-containing tubes are shortened, and those of the control and protamine-containing tubes are prolonged. When the excess of protamine is great, tubes containing heparin may actually clot *before* the control.

Results

The control values for the eight volunteers were 3.28 minutes (SE 0.15) for the one-tube Lee-White procedure, 4.94 minutes (SE 0.14) for the two-tube procedure, 6.63 minutes (SE 0.18) for the three-tube procedure, and 40.7 seconds (SE 1.2) for partial thromboplastin time.

When four doses of protamine were administered, a small increase in the Lee-White coagulation time was observed, reaching significance at 600 mg and 800 mg of protamine (table 1). However, the Lee-White coagulation time was no longer prolonged 30 minutes after the final dose of protamine. The partial thromboplastin time did not increase after any dose of protamine.

The single dose of 600 mg of protamine produced a transient but significant increase in the Lee-White coagulation time (table 2)

TABLE 1. Effect of Protamine on Lee-White Coagulation and Partial Thromboplastin Times in Six Volunteers

	Cumulative Dose of Protamine (mg/70 kg)					30 Minutes after 800 mg
	Zero	200	400	600	800	
Lee-White, three-tube (min)						
Mean	6.70	7.12	7.33	8.21*	9.38*	6.91
SE	0.22	0.24	0.60	0.55	0.71	0.32
PTT (sec)						
Mean	39.7	38.9	40.6†	38.1	37.0	36.5†
SE	1.6	1.3	2.8	1.4	1.7	2.2

* Mean differs significantly from control mean, $P < 0.05$, using two-dimensional analysis of variance.[20]
† Mean of values for five volunteers. One specimen was accidentally destroyed during analysis.

which lasted less than 30 minutes. The prolongation of the Lee-White coagulation time by the single dose of 600 mg protamine/70 kg was similar to that produced by the cumulative 600-mg dose.

The heart rate of one volunteer in the multiple-dose group decreased from 84 to 40 beats/min, with a systolic pressure of 60 torr, when blood was being drawn 5 minutes after the third injection. Intravenous atropine rapidly corrected this hypotension and bradycardia, and the study was abandoned. This volunteer's values are not considered in any averages. It is not clear whether this response was syncopal or secondary to protamine. No bradycardia or hypotension followed the first two doses of protamine. (This subject later participated in another, entirely different, study and had a similar episode immediately after an intramuscular injection of oxymorphone.)

Administration of protamine to the other subjects had no deleterious circulatory effects. The average maximal change in systolic blood pressure was +4.3 torr, with a range of −20 to +20 torr; the average change in diastolic pressure was +1.43 torr, with a range of −10 to +10 torr. With one exception, all vounteers experienced increases in heart rate. The average change was +15.4 beats/min, with a range of −8 to +44.

Flushing and itching were reported as the predominant early symptom by all volunteers. The symptoms increased in intensity after each dose in the multiple-dose experiment and were more distressing in that experiment. The single 600-mg dose did not produce any greater distress than did each of the smaller doses. Most volunteers complained of fatigue and generalized malaise for the remainder of the day. The symptoms are listed in table 3.

Studies in Patients

METHODS

The doses of protamine used in our patient studies did not differ from commonly accepted practices.[9, 10, 15] Eight patients underwent repair of intracardiac defects utilizing the Travenol bubble oxygenator (average prime, 625 ml blood and 1,144 ml Normosol). Six patients had similar operations utilizing the

TABLE 2. Effects of Protamine on Lee-White Coagulation and Partial Thromboplastin Times after Single Doses of 600 mg/70 kg in Six Volunteers

	Control	Time After Injection (min)		
		5	30	60
Lee-White, three-tube (min)				
Mean	6.58	8.89*	6.83	6.58
SE	0.25	0.48	0.53	0.53
PTT (sec)				
Mean	41.5	40.7	41.8	40.7
SE	1.3	1.6	1.8	1.4

* Mean differs significantly from control mean, $P < 0.05$, using two-dimensional analysis of variance.[20]

TABLE 3. Symptoms in Eight Volunteers Following Protamine Administration

Symptoms	Number of Volunteers
Itching/flushing	8
Fatigue/malaise	7
Nausea/vomiting	3
Headache	2
Hyperventilation	1
Temperature elevation (to 100.5 C)	1

Mayo-Gibbon vertical-screen oxygenator (average prime, 4,500 ml blood and 1,433 ml Normosol). The Mayo-Gibbon machine was used when longer bypass times were anticipated. A complete coagulogram (clotting time, prothrombin consumption, clot retraction, platelet count, recalcification time, prothrombin time, factor V assay, and PTT) was obtained preoperatively. Patients were included only when all values were normal before operation. On the day of operation, a polyethylene catheter was placed in a suitable artery, and all blood samples were obtained from this catheter. The methods of blood sampling and coagulation studies were the same as those used in the studies of volunteers.

Just before heparinization, control Lee-White coagulation time, partial thromboplastin time, and combined heparin–protamine titration values were obtained. Patients were then given 3.0 mg/kg of heparin intravenously. The pump prime contained 20 mg (2,000 units) heparin/500 ml blood and 25 mg (2,500 units) heparin/l electrolyte solution. During cardiopulmonary bypass, half the initial heparinizing dose was given each hour unless a protamine titration indicated no need for additional heparin or bypass was expected to be terminated within 15 minutes.

A modification of the protamine titration method of Perkins[16] was used to calculate the dose of protamine necessary to neutralize heparin. Immediately before sampling, 20, 30, 50, 80, and 120 μg of protamine were placed in five test tubes. One ml of the patient's heparinized blood was added to each tube, kept at 37 C in a heating block, and a digital clock was started. Each tube was gently tilted 90 degrees every 30 seconds, starting one minute after the addition of blood. The time needed for each tube to clot was recorded. Using the tube with the shortest clotting time as an index, another five-tube protamine titration was set up with amounts of protamine +20 μg, +10 μg, zero, −10 μg and −20 μg different from that in the index tube. The concentration of protamine which produced the shortest coagulation time provided the basis for calculating the protamine dose for exact reversal. When two or more tubes had identical coagulation times, the tube with the smallest protamine concentration was used to determine the neutralizing dose. This concentration was multiplied by the estimated blood volume (6.5 per cent of body weight), and this dose of protamine, rounded off to the next highest 50-mg dose, was then given.§

We defined complete neutralization as the point at which Lee-White coagulation time and combined heparin–protamine titration both returned to normal. When complete neutralization had been achieved, plasma was collected for partial thromboplastin time determinations, refrigerated, and measured at the conclusion of the study.

Following neutralization, three additional doses of 200 mg/70 kg protamine were given at half-hourly intervals (for a total additional dose of 600 mg protamine). Lee-White coagulation time, combined heparin–protamine titration, and partial thromboplastin time were measured 5 minutes after each increment and, in addition, every 30 minutes after the final injection until all changes had disappeared.

RESULTS

The control values for the 14 patients were 3.43 minutes (SE 0.10) for the one-tube Lee-White procedure, 5.08 minutes (SE 0.15) for the two-tube procedure, 6.64 minutes (SE 0.21) for the three-tube procedure, and 38.1 seconds (SE 0.9) for partial thromboplastin time.

§ In only one of 14 patients did the calculated dose fail to return the Lee-White coagulation time and the combined heparin–protamine titration to normal. An additional dose of 10 μg protamine/ml estimated blood volume (50 mg) effected complete heparin reversal.

TABLE 4. Summary of Average Heparin and Protamine Requirements with the Travenol and Mayo-Gibbon Oxygenators

	Dose of Heparin (mg)				Bypass Time (min)	Protamine–Heparin Ratio
	Initial		Supplemental	Total		
	Patient	Pump				
Travenol (8 patients)	191	56	81	313	97	0.52
SE	11	3	28	26	12	0.04
Mayo-Gibbon (6 patients)	233	216	112	561	176	0.30
SE	16	6	24	37	15	0.02

Table 4 summarizes the average doses of heparin and the doses of protamine necessary for their neutralization. A large amount of heparin remains in the Mayo-Gibbon machine at the conclusion of bypass, whereas in the Travenol oxygenator the residual volume (and hence residual heparin) at the end of bypass is usually quite small. This is the most likely reason for the lower protamine–heparin ratio in patients whose bypasses were accomplished with the Mayo-Gibbon oxygenator. The ratios ranged from 0.34 to 0.72 (mean 0.52) for patients on the Travenol bubble oxygenator and from 0.25 to 0.37 (mean 0.30) for patients on the Mayo-Gibbon vertical-screen oxygenator.

In table 5, the effects of excess protamine on the Lee-White coagulation and partial thromboplastin times are indicated. With the second overdose of protamine (400 mg/70 kg) there was a trend toward increasing the Lee-White coagulation time, and with the third overdose (600 mg/70 kg) this reached significance. However, even this slight increase persisted for less than 30 minutes.

The most sensitive indicator of excess protamine was the heparin–protamine titration. With excess protamine, clotting occurred in the heparinized tubes in 5 minutes or less (fig. 1). Thus, this test showed evidence of excess protamine from the first protamine overdose, in the form of reduced clotting time in the heparinized tubes. The protaminized tubes, whose chief purpose was to confirm the adequate neutralization of heparin, reflected

TABLE 5. Effect of Excess Protamine on Lee-White Coagulation and Partial Thromboplastin Times

	Control	Excess Protamine (mg/70 kg)				30 Minute after 600 mg
		Zero	200	400	600	
Travenol pump. (8 patients)						
Lee-White three-tube (min)	6.69	5.66	6.13	7.03	8.38*	6.47
SE	0.29	0.14	0.09	0.31	0.79	0.09
PTT (sec)	38.0	44.9	37.6	37.9	42.4	39.4
SE	1.3	3.5	1.8	1.3	2.8	0.8
Mayo-Gibbon Pump (6 patients)						
Lee-White three-tube (min)	6.58	6.00	6.38	7.29	8.90*†	6.92
SE	0.33	0.19	0.19	0.44	0.65	0.23
PTT (sec)	37.4	45.3	39.8	40.5	40.1	40.0
SE	2.0	2.9	2.1	2.5	1.3	1.4

* Mean differs significantly from control mean, $P < 0.05$, using two-dimensional analysis of variance.[20]

† Mean of values for five patients. For the sixth patient, blood in the control tube of the combined heparin–protamine titration did not clot for 15 minutes immediately after the third additional dose of protamine; the corresponding three-tube Lee-White coagulation time was more than 20 minutes. However, this marked effect was no longer demonstrable 30 minutes later.

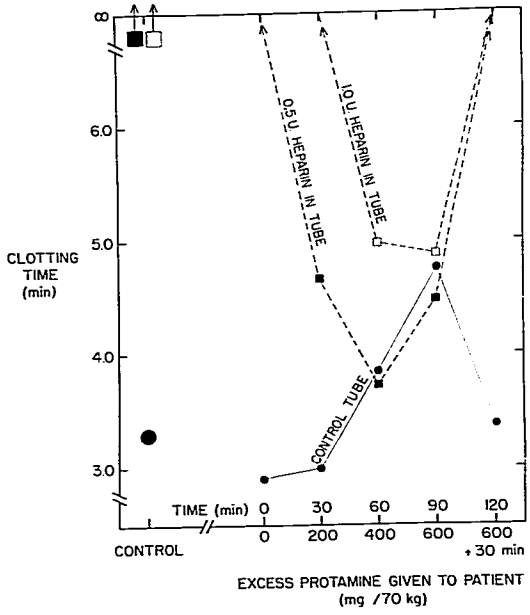

FIG. 1. Effects of excess doses of protamine on heparin titration (Travenol pump, eight patients). The progressive prolongation of the control-tube coagulation times illustrates the mild anticoagulant effects of moderate overdoses of protamine. The progressive shortening of the heparinized tubes' coagulation times indicates the presence of excess protamine in the patient's blood which is neutralized by the heparin. Indeed, evidence of excess protamine appears first in the marked shortening of the 0.5-U heparinized tube's coagulation time with the initial overdose of only 200 mg protamine. ● = control tube; ■ = 0.5 U heparin/tube; □ = 1.0 U heparin/tube.

the modest overdose of protamine with prolongation of the coagulation time (fig. 2). By comparison, protamine-induced changes in the Lee-White coagulation time did not occur until after the second overdose, reaching statistical significance with the third tube, and protamine did not change the partial thromboplastin time (table 5).

Only two of the 14 patients showed evidence of persistent excess protamine effect by the titration method 30 minutes after a total of 600 mg "excess" protamine/70 kg. These values had returned to normal by 60 minutes.

No changes in heart rate or blood pressure were clearly related to protamine. However, protamine was always administered during that critical period shortly after the termination of cardiopulmonary bypass when blood loss is brisk and changes in myocardial contractility and vascular tone are frequent. Should protamine produce a decrease in blood pressure by vasodilation,[17, 18] it would be most difficult to separate this cause from others acting simultaneously.

Unlike the response in awake volunteers, there was only occasional slight flushing in anesthetized patients when protamine was injected.

Discussion

PROTAMINE–HEPARIN RATIO

Our results indicate that neutralization of heparin requires protamine–total heparin ratios averaging 0.52 for patients using the Travenol oxygenator and 0.30 for patients with the Mayo-Gibbon oxygenator. These figure become 0.85 (range 0.68 to 1.25) and 0.77 (range 0.67 to 1.02), respectively, when only the initial heparinizing dose is considered.

The variability in protamine requirement following cardiopulmonary bypass reflects the many factors which may influence the prot-

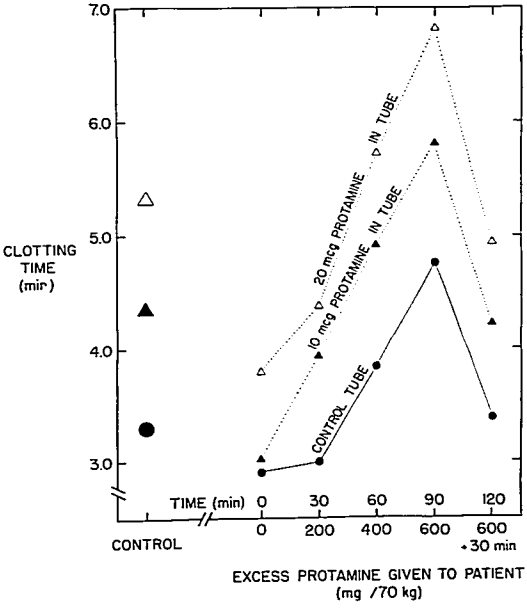

FIG. 2. Effects of excess doses of protamine on protamine titration (Travenol pump, eight patients). The progressive prolongation of the coagulation times in all three test tubes illustrates the mild coagulation effects of moderate overdoses of protamine. The consistently longer coagulation times of the 20-μg protamine tube *vs.* the 10-μg protamine tube (and for both when compared with the control tube) reflect the anticoagulant activity of protamine *in vitro*. ● = control tube; ▲ = 10 μg protamine/tube; △ = 20 μg protamine/tube.

amine–heparin ratio necessary for exact reversal: decay of blood heparin levels [19] as related to duration of bypass and body temperature [20]; supplemental doses to insure adequate heparin levels; additional heparin given in the form of heparinized blood [21]; or excessive heparin flush for arterial and central venous lines. Thus, it is extremely difficult to give an exact figure or ratio for the amount of protamine necessary to neutralize heparin following cardiopulmonary bypass. Ideally, the amount of heparin remaining in the patient after cardiopulmonary bypass might be calculated by subtracting that left in the pump, on the drapes, and in the suction bottles. Since this figure is not readily obtained, Perkins [16] suggested using the protamine titration to calculate the minimum dose of protamine needed. While we have found this method to be accurate, it is not necessary, as the danger of moderate over-protaminization is not great.

The exact amount of heparin given to the patient and to the patient–pump system is a figure which can be readily obtained and used as the basis for calculating a safe protamine dose. Using this figure for patients with oxygenators of small prime volumes, such as the Travenol, such a safe dose may be calculated using a protamine–heparin ratio of 1.0, *i.e.*, an amount of protamine equal in milligrams to the total dose of heparin. For patients with oxygenators of large prime, or whenever the total amount of heparin administered to the pump and in the form of supplemental doses to that patient is greater than the initial heparinizing dose, such a safe dose may be calculated using a protamine–heparin ratio of 0.5.

When the adequacy of reversal is in doubt, a "two-tube protamine titration" can be performed. In this test, 10 μg protamine are added to one test tube and the second serves

as a control.¶ One ml of blood is added to either tube and a conventional Lee-White coagulation time procedure is performed. If the control tube clots first, the reversal is adequate and the anticoagulant effect of protamine *in vitro* is being seen in the protaminized tube. On the other hand, if the protaminized tube clots first, heparin is still present and additional protamine is needed. A dose of protamine of 100 mg/70 kg can safely be given in such cases.

Anticoagulation by Protamine

Protamine has been shown to produce transient thrombocytopenia *in vivo* and *in vitro*.[5, 17, 22] Since thrombocytopenia is common after cardiopulmonary bypass, protamine might be expected to aggravate this situation. Gans and Castaneda showed that patients whose platelet counts decreased 45 per cent by the end of bypass experienced a further 10 per cent decrease after protamine.[23] However, there was considerable scatter in their data, and the biological effect of the additional 10 per cent decrease in platelet count probably was not important compared with the 45 per cent decrease already present. In the presence of platelet deficiencies, the effects of even small amounts of heparin are greatly enhanced.[24] If the goal is normal coagulation time, the importance of neutralizing all residual heparin greatly exceeds any hazard from small excesses of protamine.

When anticoagulation due to excess protamine doses of 600 mg and 800 mg was demonstrated in our studies, duration of action was in keeping with the results of other studies. Jaques[17] reported that 95 per cent of protamine had disappeared from the circulation within 5 minutes of injection in unheparinized dogs. In part, this rapid disappearance may be a result of the protaminolytic activity of plasma described by Fantl and Everard.[25] More recently, Frick and Brogli[26] demonstrated a progressive inactivation of protamine sulfate, but not protamine chloride, by a naturally occurring plasma factor. Protamine sulfate was used in all our studies.

¶ Use of a microliter syringe (Model #710) with repeating dispenser (Model PB 600-1), available from Hamilton Company, P. O. Box 307, Whittier, California 90608, permits accurate delivery of protamine in 10-μg increments.

Tests of Coagulation

Although the Lee-White coagulation time is one of the oldest tests of coagulation, it has frequently been criticized as being relatively insensitive, and many authorities have recommended replacing it with the activated partial thromboplastin time as a guide for following heparin therapy.[27-29] Among the advantages of the latter is that the specimen can be taken to the laboratory for testing at a more convenient time and under more carefully controlled conditions. This very advantage, however, limits its usefulness in the operating room. Even the 10 minutes of centrifugation necessary to obtain plasma represents a delay which the anesthesiologist might like to avoid. Similarly, the equipment and reagents necessary for determining partial thromboplastin time are not readily available in the operating room.

On the other hand, the simplicity of the Lee-White coagulation time procedure has contributed to its present unpopularity by promoting improper performance. With rigid control of temperature, size of tube and type of glass, volume of blood, frequency, degree and vigor of tilting, and definition of endpoint, the reproducibility of the Lee-White coagulation time (one-tube, two-tube, and three-tube) compared quite favorably with that of the partial thromboplastin time in our patients.** Equally important, the reproducibility of the one-tube procedure is such that it can be used in lieu of the three-tube procedure, thereby saving additional time and material.††

** Use of a constant-temperature block (Model 212) and an elapsed-time indicator (Model 12), available from Technilab Instruments, Pequannock, New Jersey 07440, greatly facilitates control of these many variables.

†† The control values for the 14 patients and the eight volunteers were quite similar and, when combined, the coefficients of variation were 11.53 per cent for the one-tube Lee-White procedure, 10.33 per cent for the two-tube procedure, 10.24 per cent for the three-tube procedure, and 9.23 per cent for partial thromboplastin time. In a single patient the coefficients of variations were 9.10 per cent for the one-tube procedure, 4.92 per cent for the two-tube procedure 6.92 per cent for the three-tube procedure, and 9.23 per cent for partial thromboplastin time. Thus, the greater contribution to variability appears to originate in the measurement, with a lesser contribution from variability within the normal population.

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