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Laboratory Report

Evaluation of Tests Used to Monitor Heparin Therapy during Extracorporeal Circulation

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Two tests, the activated coagulation time test (ACT), and the quantitative protamine titration test (QPT), were examined in detail as representative of a large number of tests potentially useful in determining dose of heparin needed during cardiopulmonary bypass and the dose of protamine needed for reversal of heparin. The variability introduced by the test methods (ACT 6 per cent, QPT 8 per cent) was insignificant compared with the inaccuracy introduced by the variation in patient sensitivities to heparin (> 25 per cent) and the variation in plasma volume (14 per cent). Both of these variables affected not only QPT but also any modification of it that measures the level of heparin by titration with protamine solutions. Tests that measure the effect of heparin on the clotting time, of which the ACT is an example, were unaffected by either population variable when used in conjunction with a simply constructed dose–response curve. (Key words: Blood, coagulation, heparin; Surgery, cardiovascular.)

THE ADMINISTRATION of heparin and protamine according to a set protocol failed in recent studies[1] to anticoagulate safely a significant number of patients. This was due to differences in individual responses to a given dose of heparin—differences detectable only by a monitoring technique. Individual heparin requirements in the group of patients under study varied as much as threefold, while heparin decay rates showed more than fourfold variability.[1] In the light of these data, some form of monitoring seems strongly indicated.

Granted that monitoring is highly desirable, how can the most appropriate test be selected from the more than 20 that are available?[2–4] Before an answer can be given, it is necessary to define precisely the functions such a test must serve. During bypass, the extent of the anticoagulant effect is the crucial information that must be determined; at the termination of extracorporeal circulation the total body load of heparin must be known so that the appropriate neutralizing dose of protamine can be administered. All 20 tests fall into one of two categories: they either 1) measure the *level* of heparin per ml of whole blood or plasma, usually by titration with protamine, or 2) measure the *effect* of heparin by determining the clotting time. As can be seen from table 1, no single test in unmodified form can be used for any test will give at most only half of the information required.

The question can now be rephrased. Assuming only one test is to be performed, is it preferable to modify a test from category I so that it provides all the information needed, or would a candidate from category II serve the purpose better? It is the purpose of this communication to provide a statistical answer to this question.

The Basis for Evaluation of Tests by Categories Rather than as Individual Procedures

As shown in table 2, all of the data items labeled "1" in table 1 have the intrinsic variability of the test that is employed. Any specific test is likely to make a relatively minor contribution to the overall variability of the complete monitoring procedure, as most tests

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TABLE 1. Data Needed in Addition to a Test Result When Monitoring Heparin Therapy

	Data Needed to Determine Prolongation of Clotting Time during Bypass	Data Needed to Calculate Total Body Heparin Load in mg/kg at Termination of Bypass
Test Category I These tests measure heparin level per ml of blood or plasma	Two data items are needed; only the first is provided by an unmodified category I test: 1) The level of heparin in mg/ml 2) The sensitivity of the patient to heparin (mg/kg needed to prolong clotting time a given amount)	Two data items are needed; only the first is provided by an unmodified category I test: 1) The level of heparin in mg/ml 2) The patient's plasma volume at the conclusion of bypass
Test Category II These tests measure heparin by determining changes in clotting time of blood or plasma	One datum is needed and is provided directly by the test result: 1) The prolongation of the clotting time	Two data items are needed; only the first is provided by an unmodified category II test: 1) The prolongation of the clotting time 2) The amount of heparin in mg/kg that would produce this prolongation

methods will show variability (one coefficient of variation) of less than ten per cent.

The data items numbered "2" are all characteristic of the patient population, and this intrinsic variability will remain constant regardless of the specific test employed for monitoring. Furthermore, the variability of these population characteristics is in general larger (coefficient of variation greater than 15 per cent) than the test variability. Thus, the population variability will be dominant in determing the usefulness of any selected approach (table 2). We have therefore measured these population variables under the circumstances that exist in the operating room and in the patient population at risk.

Materials and Methods

Fifty patients admitted over a three-month period to the cardiothoracic surgery service formed the study group. Ten consecutive patients within this larger series formed a special study group.

The quantitative protamine titration (QPT) test measures the level of heparin in a blood sample (a Category I test). This test was selected because it enjoys wide popularity in variously modified forms. The activated coagulation time (ACT) of whole blood (a Category II test) measures the effect of heparin on the clotting time. It was chosen because the method involved is simple and the test adapts

TABLE 2. The Components of Variability in Heparin Monitoring

	Overall Variability Anticipated during Bypass	Overall Variability Anticipated at Termination of Bypass
Category I tests (level of heparin)	1) Inherent variability of test method—probably small (less than 10 per cent) 2) Inherent variability of population sensitivity to heparin—probably large (more than 15 per cent)	1) Inherent variability of test method—probably small (less than 10 per cent) 2) Inherent variability of plasma volume in patient population—probably large (more than 15 per cent)
Category II tests (effect of heparin)	1) Inherent variability of test method—probably small (less than 10 per cent)	1) Inherent variability of test method—probably small (less than 10 per cent) 2) Inherent variability of population sensitivity to heparin—probably large (more than 15 per cent)

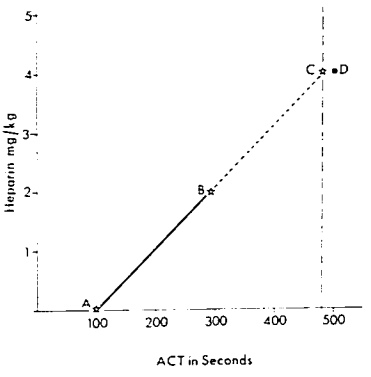

FIG. 1. Preparation of a dose–response curve. The initial ACT result prior to administration of heparin is plotted (A), the ACT after 2 mg/kg is then added (B), and the line joining A and B extrapolated to C to determine total dose for a 480-second ACT. After the additional heparin has been given, the resultant ACT is plotted at D.

well to the environment of the operating room.

The QPT was performed as follows.[10] A 7-ml sample of blood was drawn from a heparinized patient into a tube containing 3.8 per cent sodium citrate, centrifuged, and the plasma used in the test. Dilutions of protamine ranging from 200 μg/ml to 1 μg/ml were prepared and used to determine the lowest concentration of protamine that shortened the clotting time of the plasma to a normal range. A 0.1-ml sample of the solution containing protamine 200 μg/ml was incubated with a 0.1-ml sample of plasma containing heparin for three minutes. A 0.1-ml amount of topical thrombin was then added to the mixture, and the formation of a clot was detected by a Fibrometer (Bio-Quest). More dilute solutions of protamine were then used in sequence until a dilution that inadequately neutralized the heparin, causing delay in clot formation, was found. To calculate the amount of heparin present in the patient's plasma, it was assumed that 10 mg of protamine neutralized 1,000 U.S.P. units of sodium heparin. The accuracy and reproducibility of this test were checked by titrating the series of dilutions of protamine with coded samples of heparinized plasma prepared in duplicate.

The ACT test was performed on whole blood as described by Hattersley.[11] In brief, a 2-ml sample of whole blood was placed in a glass tube containing 12 mg of celite (Becton-Dickinson #3206 × F532), and a stopwatch was started. The tube was inverted once a second for the first 30 seconds to mix the contents thoroughly, then placed in a heat block (Thermoline-Drybath #5900), held over a 40-watt light bulb, and rocked slowly until clotting occurred. The stopwatch was stopped when the first clearly defined clot was visible. The reproducibility of the ACT test was checked by duplicate determinations on patient samples containing widely varying amounts of heparin.

Since previous work had shown that the protocol approach to anticoagulant management was decidedly inadequate, and since the usefulness of the QPT in this regard was unknown, the ACT was used to monitor and control the anticoagulant therapy of all 50 patients.[12] On blood samples from each patient ACT's were performed (A) prior to heparinization and (B) after a dose of 2 mg/kg heparin. The heparin administered in mg/kg was plotted on a graph against the ACT in seconds (fig. 1).

A line drawn between points A and B was then extrapolated to intersect the line drawn at the 480-second ACT point. From this third point (C), a dose of heparin that would produce an ACT of 480 seconds was calculated. For example, in the case illustrated, 4 mg/kg of heparin were needed to produce a 480-second ACT. This was 2 mg/kg in addition to the 2 mg/kg originally given. To each of the 50 patients, the dose of heparin calculated in this way was administered; after allowing five minutes for mixing, another ACT was determined (D). The objective was a clotting time of 480 seconds at inception of bypass.

The connection of points A, B, and D provided a graphic representation of each patient's response to heparin. This dose–response curve was used at hourly intervals throughout operation to determine the heparin needed by the patient. In the case illustrated in figure 1, if the ACT after 60 minutes of by-

TABLE 3. Coefficients of Variation Affecting the Approaches to Anticoagulation

	Sample Size	Sample Range	Sample Mean	Standard Deviation	Coefficient of Variation (C.V.)	Overall C.V. Minus Test C.V.
Quantitative protamine titration (QPT), μ/ml	20	1– 16	6.6	53	8 per cent	—
Activated coagulation time (ACT), sec (by heparin dilution)	100	62–555	248	15	6 per cent	—
Plasma volume, ml/kg	10	28– 58	41.3	6.7	16 per cent	14 per cent
Blood volume (by RISA) ml/kg from Senn and Karlson	22	—	84*	13.1*	15.6 per cent	14 per cent
Heparin response, per cent ACT prolongation at 2 mg/kg dose	100	175–535	350	93	26.5 per cent	25.8 per cent

* These figures are approximately double ours because they refer to whole blood rather than plasma volume in ml/kg.

pass were 350 seconds, the heparin level determined from the graph is 2.8 mg/kg, and 1.2 mg/kg would return the ACT to 480 seconds.

The heparin remaining at the end of operation was determined for each patient by measuring the ACT and noting the corresponding heparin level in mg/kg from that patient's dose–response curve. Although in theory an adequate neutralizing ratio of protamine to heparin is 1:1, a slight excess of protamine appears not to be dangerous, and may prevent heparin rebound.[13] Accordingly, a protamine/heparin ratio of 1.3:1 was selected,[14,15] and on this basis the amount of protamine needed for complete neutralization of the remaining heparin was calculated and administered. If, for example, the ACT at the conclusion of surgery were 325 seconds, the heparin level from the dose–response curve illustrated in figure 1 is 2.4 mg/kg, and 3.1 mg/kg protamine would be required to completely neutralize the remaining heparin.

The precise amounts of heparin and protamine actually received by each of the 50 patients were calculated from the results of the ACT determinations. However, in the cases of the ten patients forming the acute study group, the QPT values were used to monitor and prescribe an alternate anticoagulant program. It was possible in these patient populations to measure the range of variation in plasma volume as well as the range in heparin sensitivity. At the same time, the reproducibility and overall utility of two typical test methods were evaluated.

Results

The variability of the two test methods is listed in table 3. Also listed is the variability in plasma volumes and the responses to heparin of the sample patient population. The coefficient of variation of plasma volumes in the patient population was approximately 14 per cent; the coefficient of variation of heparin effect was in excess of 25 per cent.

The coefficients of variation of the QPT (8 per cent) and ACT (6 per cent) tests were derived from analysis of duplicate samples by standard statistical methods. The procedures for determining the remaining two coefficients of variation were somewhat more complex and deserve further explanation.

A plot of ACT's against administered heparin for all 50 patients (fig. 2) for each of the two initial heparin administrations showed an overall coefficient of variation of 26.5 per cent. In figure 3, 20 representative cases including the high and low extremes are plotted, with the two points that correspond to each patient joined. This plot demonstrates that most of the scatter in figure 2 occurred because some patients were four times more sensitive than others to the same mg/kg dose. This variation in sensitivity to heparin can be quantitated, since the ACT test variability is known to be 6 per cent, and

(overall variability observed)^2

$$- (\text{test variability})^2$$

$$= (\text{intrinsic variability of population})^2$$

Thus the coefficient of variation of heparin effect in the patient population, apart from any variability of the test method, was more than 25 per cent.

The intrinsic variability of plasma volume in hospital patients "with no known circulatory disorders" has been found to be about 14 per cent.[16] Patients undergoing cardiopulmonary bypass usually have a known circulatory disorder at the start of bypass, and it seems reasonable that bypass itself will have an additional highly unpredictable effect on their plasma volumes. This 14 per cent variability must therefore be considered a conservative estimate.

The methodology of the QPT test lends itself to an estimate of the plasma volume. When the administered heparin in mg/kg is plotted against the heparin recovered in mg/ml, the plasma volume in which the administered heparin must have been diluted can be determined. This was done for each of the test patients and showed a range of 35–51 ml/kg with a coefficient of variation of 14 per cent.

Discussion

As expected, the contribution of the patient population dominates the overall variability of any monitoring scheme (table 4). Unless the sensitivity to heparin can be measured for each patient in the population, then any monitoring system that requires this information will have a minimal variability of 25 per cent. This is true no matter what test is employed.

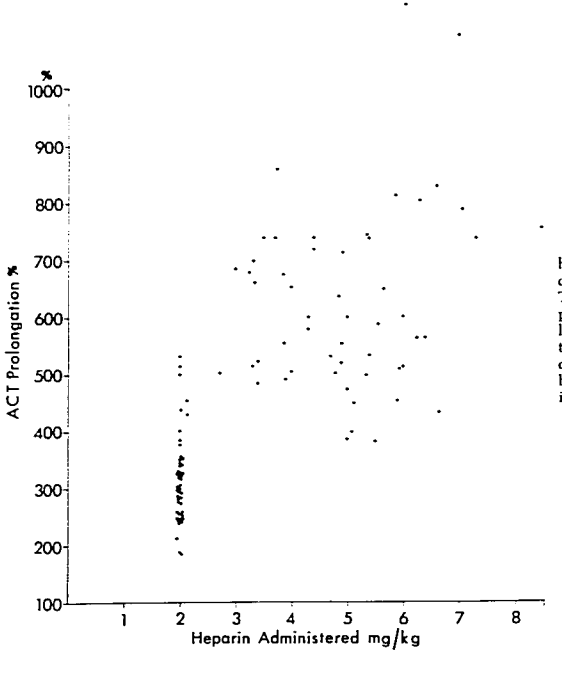

FIG. 2. The effect of heparin on the activated coagulation time (ACT). Two results for each of 30 patients are plotted. The large scatter evidenced by the population is due to a combination of test variability and patient variability.

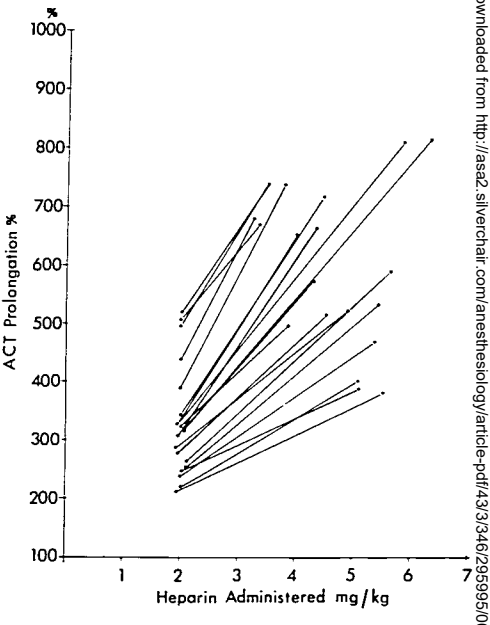

FIG. 3. A replot of 20 representative cases from figure 2. The two points corresponding to each of the 20 cases are joined to demonstrate that virtually all of the scatter is due to differences in the ways individual patients respond to heparin (population variability) rather than to test variability.

The test contribution to the overall variability, since it will usually be of the order of 10 per cent or less, can be virtually ignored. This is because a coefficient of variation of 10 per cent added to one of 25 per cent gives an overall coefficient of variation of 27 per cent.

In a similar manner, unless the plasma volume of each patient is known, the overall variability of plasma volume in the patient population is the minimal variability of any monitoring scheme that utilizes this information. Category I tests, such as the QPT, because they are affected by both blood volume and heparin sensitivity, are poorer choices as a basis for a monitoring scheme than Category II tests. Furthermore, the determination of heparin levels in the plasma by the standard QPT test requires a skilled laboratory technician; the solutions are unstable and the equipment needed cannot be used in the operating room. The QPT test requires 30–120 minutes for performance even when all the solutions are in readiness. By the time the results are available, the heparin level has already fallen, and a decision concerning the management of the patient has usually been made. The QPT test in standard form therefore serves only as confirmation of decisions already made. The modified semiquantitative QPT methods all have coefficients of variation several times larger than that of the parent method because of the necessarily large step intervals between the protamine solutions used for titration. Further modifications to decrease the time required for the QPT and permit its use on whole blood usually limit its usefulness until it can merely distinguish whether heparin is present or not. An exception is the recently described Hemotensiometer.[17] This instrument can measure the neutralizing effects of four different protamine concentrations simultaneously, thus making

TABLE 4. The Cumulative Variability in Heparin Monitoring

	During Bypass	At Termination of Bypass
Quantitative protamine titration test (a Category I test)	1) Inherent variability of test method C.V. = 8 per cent 2) Inherent variability of population sensitivity to heparin C.V. = 25 per cent Overall variability is *C.V. = 26 per cent*	1) Inherent variability of test method C.V. = 8 per cent 2) Inherent variability of plasma volume in patient population C.V. = 14 per cent Overall variability is *C.V. = 15 per cent*
Activated Coagulation Time (a Category II test)	1) Inherent variability of test method C.V. = 6 per cent Overall variability is *C.V. = 6 per cent*	1) Inherent variability of test method C.V. = 6 per cent 2) Inherent variability of population sensitivity to heparin; this should be a C.V. of 25 per cent but with availability of dose–response curve for each patient it becomes C.V. = 6 per cent Overall variability is *C.V. = 8 per cent*

it possible to obtain a four-step protamine titration curve in less than ten minutes. The technique can thus be used for monitoring. The instrumentation required is complex, however, and the protamine solutions needed are unstable and must be freshly prepared.

The ACT test requires no expensive equipment because it involves only the addition of blood to a tube containing celite and the timing of clot formation. The test can be done in the operating room by a relatively unskilled person or by a technician who is also involved in blood-gas determinations. These characteristics make the monitoring and precise regulation of anticoagulant therapy in the operating room a thoroughly practical procedure. Although the ACT was used in this study, virtually any test from Category II would yield similar statistical results. That is, any test that measures heparin effect by measuring changes in the clotting time (provided a dose–response curve is prepared as an integral part of the monitoring scheme) should prove similarly useful. As indicated in table 4, the use of a dose–response curve with any test from Category II eliminates the only population variable that influences this approach.

The final choice from tests in this category will no doubt depend upon the preferences of the people who will use the results and the circumstances under which the test is to be performed. If the test is to be done in the operating room, then the anesthesiologist is likely to be involved. The rapidity, simplicity, and minimal expense involved in the ACT render it desirable. If, however, the test is to be performed in a nearby laboratory, then some variant of the recalcification time of plasma or whole blood would be likely to prove more useful.[6,7]

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Malignant Hyperthermia

CURARE AND HYPERTHERMIA A number of investigators have claimed that it is probably safe to administer *d*-tubocurarine to a patient susceptible to malignant hyperthermia (MH). This report details the anesthetic management of two patients with a strong family history of MH. A 13-year-old Caucasian boy required surgery to correct bowel obstruction. Although he had no history of muscle abnormalities, 20 of his relatives had had non-rigid MH; eight had died in the perioperative period. The patient was premedicated with atropine and chlorpromazine; during a period of preoperative prophylactic cooling, he received meperidine, chlorpromazine, and promethazine. Rectal temperature was 36.1 C immediately prior to induction of anesthesia with nitrous oxide and oxygen, which was followed by intravenous injection of *d*-tubocurarine. Within five minutes after the administration of *d*-tubocurarine, rectal temperature was 39.7 C. The patient was treated by external cooling and no further *d*-tubocurarine was given. An uneventful recovery ensued. The second patient (with a strong family history of rigid MH) developed a rectal temperature of 40.6 C within 15 minutes after administration of *d*-tubocurarine. Neither patient manifested rigidity at any time. Serum CPK determinations performed several months after the two incidents were normal or minimally elevated. (*Britt BA, and others: Malignant hyperthermia induced by curare. Can Anaesth Soc J 21:37–375, 1974.*) ABSTRACTER'S COMMENT: What about the possibility that N_2O was the triggering agent?