Studies on Blood Coagulation and the Effect of Digitalis

By George C. Sutton, M.D.

METHODS for the measurement of the coagulation characteristics of the blood have been extended and refined markedly in recent years. Studies of the effect of container surface upon clotting\textsuperscript{1-4} have led not only to further understanding of the underlying process but to clinical tests based upon prolongation of the coagulation time by certain plastic surfaces. Deceleration of the coagulation of blood in vitro by the addition of heparin has also been developed into a standardized procedure.\textsuperscript{5-8} Similarly, the use of diluted plasma in the prothrombin determination\textsuperscript{9-11} and serial measurements of blood clotting time following an injection of heparin, “the heparin tolerance curve,”\textsuperscript{12-14} have been employed as means of increasing the accuracy of measurements of small changes in the plasma or blood clotting constituents.

With the use of both the older methods for measuring blood coagulation and these refinements, the results of certain studies on alterations in blood clotting have been at considerable variance. This is well illustrated by investigations of the influence of digitalis on the clotting mechanism. Tanaka,\textsuperscript{15} in 1928, reported that parenteral strophanthin shortened the blood coagulation time in dogs. A group of investigators, including Werch,\textsuperscript{16} Macht,\textsuperscript{17} De Takats and Gilbert,\textsuperscript{18} Massie,\textsuperscript{19} and De-court,\textsuperscript{20} have presented results in support of this concept that digitalis hastens blood coagulation and have attached clinical significance to this observation. On the other hand, numerous studies, including those of Sokoloff and Ferrer,\textsuperscript{21} Moses,\textsuperscript{22} Ramsey and Haag,\textsuperscript{23} and Taltavull,\textsuperscript{24} also made in both experimental animals and human beings, have not been able to verify this purported action of digitalis.

These contradictory conclusions concerning one of the effects of digitalis led to the following investigation. In addition to studying the influence of digitalis upon the rapidity of blood coagulation, an examination of the reliability of the Lee-White test for blood clotting was made.

METHODS AND RESULTS

Standardization and Establishment of the Reliability of the 3-Tube Lee-White Method of Determining Blood Coagulation Time.

Technical Procedure. With a clean dry 5 cc. syringe and size 16 needle, 3.5 cc. of venous blood were withdrawn from an antecubital vein after removal of the tourniquet. Only cases in which there was a clean, easy venopuncture were used. After withdrawal from the vein, the needle was removed from the syringe and 1 cc. of blood was introduced, without frothing, into each of three dry glass Wassermann test tubes resting in a water bath maintained at 37 C. The first tube only was tilted every thirty seconds until complete inversion caused no flow; the second and third tubes were tilted successively in similar manner. The blood coagulation time was calculated to be the time blood first appeared in the syringe until clotting of the third tube. Unless specifically noted, this procedure (blood withdrawal and introduction into test tubes, water bath at 37
C., tube tilting and timing) was employed in all subsequent portions of the investigation.

Subjects. One hundred and seventy-three adult individuals, the majority of whom were ambulatory or clinic patients, or hospital personnel, of Cook County Hospital. All were in apparent good health and receiving no medication whatsoever.

Group I: Sixty adult individuals. The determination was done simultaneously on both right and left arms on two separate days, approximately one week apart. The mean of right and left arm determinations was recorded as the clotting time for each individual.

Group II: One hundred adult individuals. The determination was done on one sample of blood obtained from each person on a single occasion.

Table 1.—Blood Coagulation Times of Normal Individuals Determined by the Three Tube Lee-White Method.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Range</th>
<th>Mean Time</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min.</td>
<td>Max.</td>
<td></td>
</tr>
<tr>
<td>I. 60 individuals. Mean time of bilateral determinations</td>
<td></td>
<td>7.2</td>
<td>14.2</td>
<td>7.0</td>
</tr>
<tr>
<td>First Day</td>
<td></td>
<td>7.2</td>
<td>14.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Second Day</td>
<td></td>
<td>7.7</td>
<td>16.6</td>
<td>8.9</td>
</tr>
<tr>
<td>II. 100 individuals. Single determinations</td>
<td></td>
<td>6.8</td>
<td>15.6</td>
<td>8.8</td>
</tr>
<tr>
<td>III. 13 individuals. Repeated (total 99) determinations</td>
<td></td>
<td>6.8</td>
<td>15.6</td>
<td>8.8</td>
</tr>
<tr>
<td>Mean of Groups I, II, III</td>
<td></td>
<td>7.1</td>
<td>15.5</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Group III: Thirteen adult individuals. The coagulation time of single samples of blood was determined on from four to ten random occasions for each individual.

Results. The mean blood coagulation times of the three groups of individuals (I, II and III) totalling 173 in all, who were not receiving digitalis, are presented in table 1. The three test tube Lee-White procedure gave very similar mean times for bilateral determinations, for single determinations on different individuals, and for repeated determinations on a small group of individuals. Each of the three groups had a mean time of approximately 10.4 minutes with a range of 8 to 9 minutes, and a standard deviation of ± 2.0 minutes.

A Study of the Effect of Digitalis as Used Clinically upon the Coagulation Time of Individuals with Organic Heart Disease.

Technical Procedure. Identical to preceding section.

Subjects. Group IV: Sixty adult individuals, predominately male, all with established organic heart disease were selected. All the patients had had a definite episode of congestive failure, during which they had received digitalis leaf (USP XII) in therapeutic doses with definite improvement, and were continued on a daily maintenance dose of the drug at the time of the determination. All individuals were compensated and ambulatory at the time and were receiving no other medication. The mean of simultaneous bilateral arm determinations of the coagulation time was used for each patient. In this group of subjects, as well as in all subsequent groups, unless specifically stated, digitalis was the only drug received by the study group. In all cases the control group was receiving no medication.

Group V: Ten adult individuals, both male and female, with organic heart disease were selected. All the patients had received digitalis leaf (USP XII), crystalline digitoxin or lanatoside C in excessive doses and manifested signs of frank digitalis intoxication (scotomata, anorexia and nausea, diarrhea, palpis bigeminus). Sixteen determinations were made while the manifestations of toxicity persisted.

Results. The mean blood coagulation times of the two groups (IV and V) receiving digitalis is presented in table 2. The group of 60 cardiac patients who were receiving a maintenance dose of digitalis at the time of the test had a
mean clotting time of 11.9 minutes (S.D. = ±2.4 minutes) with a range of 11.5 minutes. In the group of 10 persons with frank digitalis intoxication, the mean coagulation time was 11.6 minutes (S.D. = ±2.1 minutes) and a range of 8.0 minutes.

Both of these groups thus had a coagulation time which was longer than in the control groups (I, II and III) by approximately one and one-half minutes, but this time is less than the observed standard deviation for the procedure.

Effect of Digitalis upon the Coagulation Time of Blood Determined under Special Conditions.

Group VI: Coagulation time of blood to which heparin was added in vitro.

Technical Procedure. One cc. of blood was placed in each of three glass Wassermann test tubes in a water bath at 37 C. The first test tube was dry, the second contained 0.5 cc. normal saline, and the third contained 0.5 cc. normal saline with .005 mg. of crystalline heparin dissolved in it. All tubes were inverted three times initially to insure mixing and then every thirty seconds until clotting occurred.

Subjects. Fourteen adult individuals. Control group: 7 normal individuals. Study group: 7 male cardiac patients, all ambulatory and receiving a maintenance dose of digitalis (USP XII), 0.1 Gm. a day.

Group VII: Coagulation time of blood determined in methyl methacrylate (Lucite) test tubes.

Technical Procedure. One cc. of blood was placed into each of three Lucite centrifuge test tubes resting in a water bath at 37 C. and the tubes were inverted successively in the same fashion as the three glass tube Lee-White procedure. The recorded time of coagulation was from the first appearance of the blood in the syringe until the clotting of the third tube.

Subjects. Twenty-two adult individuals. Control group: 11 ambulatory ward patients without cardiac disease. Study group: 11 male cardiac patients who were receiving only either digitalis leaf USP XII (9 cases) or digitoxin (2 cases) in maintenance doses.

Group VIII: Coagulation time of blood in methyl methacrylate tubes and to which normal saline, water and heparin in saline were added.

Technical Procedure. Five cc. of blood were withdrawn as previously described and 1 cc. was placed in each of four Lucite centrifuge test tubes resting in the water bath. The first tube was dry, the second contained 0.5 cc. normal saline, the third 0.5 cc. distilled water, and the fourth contained 0.5 cc. normal saline and .005 mg. heparin. All the tubes were inverted every thirty seconds until clotting occurred.

Subjects. Twenty adult male individuals. Control group: ten ambulatory ward patients with noncardiac complaints. Study group: ten patients with organic heart disease who were receiving maintenance doses of digitalis leaf USP XII.

Results. Measurements of the coagulation time, as determined in glass and Lucite containers, with and without the addition of heparin, of control and of digitalized patients, (Groups VI, VII and VIII) is presented in table 3. Difficulty in determining the precise time of clotting was encountered in those cases where heparin was added to the blood. The

<p>| Table 3.—Effect of Digitalis upon the Coagulation Time of Blood Determined Under Special Conditions |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Digitalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI. 14 individuals Glass tubes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>8.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Blood + saline</td>
<td>6.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Blood + saline + heparin</td>
<td>35.0</td>
<td>8.1</td>
</tr>
<tr>
<td>VII. 22 individuals Lucite tubes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>25.9</td>
<td>5.3</td>
</tr>
<tr>
<td>VIII. 20 individuals Lucite tubes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>13.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Blood + saline</td>
<td>10.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Blood + water</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Blood + saline + heparin</td>
<td>39.6</td>
<td>12.9</td>
</tr>
</tbody>
</table>

main portion of the blood remained liquid, as judged by the streaming of the cells or the motion of occasional minute bubbles, long after the surface of the blood no longer flowed. The times for heparinized blood presented in the chart are the closest approximation to the clotting time of the bulk of the blood, not the formation of the surface film.

Comparison of the coagulation times of the control groups and of the groups receiving digitalis revealed that, irrespective of the procedure employed, there was no significant time difference between the two categories.
The Effect of Digitalis in Clinical Dosage upon the Heparin Tolerance Curve.

Technical Procedure. Standard heparin (Lederle) in the amount of 0.15 mg. for each kilogram of body weight was injected intravenously. The blood coagulation time was determined by the three glass tube Lee-White method at 37 C. immediately prior to administration of the heparin and at ten-minute intervals thereafter for sixty minutes. The coagulation time of each sample was plotted graphically against the time after heparin injection. The curve was determined for each individual prior to any digitalis administration. Subsequently, digitalis leaf (USP XII) was given in a quantity sufficient to produce cardiac compensation in those ten individuals with cardiac disease. In general this amounted to a minimum of 18 cat units in ten days to a maximum of 45 cat units in eight days (resulting in digitalis intoxication). In the 3 individuals with neurologic disorders, an average total dosage of 26 cat units in ten days was given. The heparin tolerance curve was determined for each individual after such digitalis administration.

Subject. Thirteen adult male individuals, 10 of whom had compensated organic heart disease, and 3 of whom had neurologic disorders.

Results. In each of the 13 cases studied, the injection of heparin produced a definite transient prolongation of the coagulation time. Comparison of the graphic representation of the individual's response to heparin was made before, and after, the administration of digitalis. Criteria for this comparison were, of necessity, rough. A change was considered to have occurred when the several respective coagulation times forming the peak of the curve, or maximum prolongation of clotting, differed by approximately 20 per cent or more.

In the group studied, no difference in the curve before and after digitalis was observed in 5 individuals. Three subjects showed depressed curves of shortened coagulation times, or a lessened response to heparin, after the digitalis. The remaining 5 had elevated curves of lengthened coagulation times, or an increased response to heparin, after digitalis.

Blood Coagulation and Liver Function before and after Cardiac Compensation.

Technical Procedure. (a) Coagulation Time: Three glass tube Lee-White method identical to that described for Groups I, II and III. (b) Bromsulfalain Excretion: Five milligrams per kilo of body weight were injected and the per cent of retention at 30 and 60 minutes determined. (c) Prothrombin Time: (1) Standard Quick procedure using undiluted plasma and Difeo thromboplastin. (2) Prothrombin time of saline diluted plasma (12.5 per cent).

Subjects. Fourteen adult individuals, all male, and all of whom had organic heart disease with unequivocal right heart failure with hepatic enlargement were studied. None of these men had any clinical indications of intrinsic liver disease. Studies were carried out at the time of hospital admission for heart failure, before any medication and, if initially abnormal, after compensation by the use of bed rest, salt restriction, digitalis, and diuretics.

Results. Comparison of the coagulation time, bromsulfalain retention test, and undiluted plasma prothrombin time of the group of fourteen individuals before and after compensation is presented in Table 4. The coagulation times before (10.5 minutes) and after (11.2 minutes)

| TABLE 4.—Effect of Cardiac Compensation upon Blood Coagulation Time and Liver Function (Mean results of 14 cases) |
|---|---|---|---|
| | Coagulation Time (minutes) | Bromsulfalain Retention (% retention) | Prothrombin Time (%) (undiluted plasma) |
| | Mean | Standard Deviation | 30 mins. | 60 mins. |
| Before compensation | 10.5 | 2.5 | 30 | 17 | 82 |
| After compensation | 11.2 | 1.4 | — | — | 72 |

compensation cannot be considered significantly different, for both figures fall well within the normal limits set by the larger control studies. The bromsulfalain retention test was not performed after compensation in all the cases because it was originally within normal limits in all but one case. Determination of the undiluted plasma prothrombin times before (82 per cent) and after (72 per cent) compensation also gave results which are considered to be negative. The state of cardiac decompensation did not per se alter the prothrombin time, nor did the state of improvement, with its attendant therapy, produce any change.

Difficulty was encountered in the use of the prothrombin time of the saline diluted plasma (12.5 per cent) by the Link method. Although the end-point of the test was sharp, repeated determinations on a group of five
normal individuals selected as a control group were scattered so much as to make comparison of the control to any other group difficult. The mean diluted plasma prothrombin time of this small group was 56.5 seconds. Accordingly diluted plasma prothrombin time determinations were not employed in the study of the cardiac patients.

In the analysis of all results, the standard deviation, \( \sigma \), was calculated according to the formula \( \sigma = \sqrt{\frac{\sum X^2}{n-1}} \) when “\( X \)” is the deviation of the observations from the mean, and “\( n \)” the number of observations.

**Discussion**

Comparison of the coagulation times of groups of individuals by means of the three tube Lee-White method gives consistent and reliable results. There is no significant difference between the times obtained by simultaneous right and left arm determinations or single determinations. The daily variation observed in individuals was very close to the range found in single determinations on a group of normal adults. Both tests gave a mean time of 10.4 minutes with a standard deviation of ± 2.0 minutes. Recognition of this large standard deviation is essential in the interpretation of the results of the test. The coagulation time of an individual must differ by at least twice the standard deviation from the mean in order to be considered probably abnormal. On such a basis normal blood coagulation times would range at least from a minimum of 6.4 minutes, to a maximum of 14.4 minutes. These figures, which are only minimal limits, emphasize the limitations of the determination, the large experimental error and the great individual variation. They also reveal the impossibility of detecting small changes in the coagulation time of either individuals or groups of individuals by means of this method.

This experimental limitation was encountered in the studies on individuals receiving therapeutic digitalis administration. The mean coagulation time of this group was 11.85 minutes; an increase of 13.5 per cent over the control group. This figure falls well within the expected range, however, and cannot be considered a significant variation from the normal. Similarly, the mean coagulation time of the group with frank digitalis intoxication is not significantly different from the control group. These results can be compared with those of Sokoloff and Ferrer21 in their study of 10 cardiac patients, also using the three tube method. Before digitalization the group had a mean coagulation time of 9.45 minutes and S.D. of ± 1.55 minutes; during therapeutic digitalization the mean was 9.50 minutes with a S.D. of ± 1.41 minutes.

Further examination of the effect of the therapeutic use of digitalis upon the coagulation time of blood under special conditions also revealed no alteration. The coagulation times of the control and digitalis groups determined in both glass and Lucite test tubes, with and without the addition of heparin, showed no significant difference. The prolongation of the clotting time observed when the blood is heparinized or placed in a plastic tube causes the element of surface drying and evaporation to become an important factor in altering the accuracy of the determination. This is shown by the observation that the apparent coagulation time of heparinized blood in a plastic tube was not greater than the time in a glass tube, although it would be expected that the effects would be additive. The increase in the coagulation time with both procedures (plastic container or heparinization) favors the development of a dried surface film which does not flow, although the blood beneath the surface frequently remains liquid. The formation of the film makes the determination of the true clotting time difficult and inaccurate and also emphasizes the importance of the changes of the blood-air surface in blood coagulation time measurements. As the clotting time was prolonged over twenty or twenty-five minutes, ascertaining the end-point became progressively more difficult. Attempts to lessen this drying on the blood-air surface, by stoppering the tube or layering of mineral oil, were unsuccessful.

The heparin tolerance curves before and after digitalis administration revealed no consistent change. This observation is in accord with the work of Moses22 and others21 that digitalis as
used in clinical dosage does not alter the heparin response.

The studies before and after compensation were undertaken with the view that cardiac failure and attendant liver engorgement might alter production by the liver of constituents essential for the blood clotting process. Thus improvement of liver function with compensation could contribute to an improvement in the coagulation properties of the blood, quite independent of drug administration. In this control investigation no changes were detected in the blood coagulation time or bromsulfalein excretion before and after compensation. The mean standard prothrombin time of the uncompensated individuals was normal.

Satisfactory standardization of the 12.5 per cent saline diluted plasma prothrombin time was not obtained in the control group of five normal individuals. There was considerable variation in the times and the mean for the group was 56.5 seconds. This mean is a rather marked variation from the times of 41.4 and 31.5 seconds obtained by Shapiro and Unger. This discrepancy is, of itself, in accord with some indications that satisfactory comparative standardization of the prothrombin measurement is difficult and that a conservative view must still be taken of the dilution refinement. Fisher obtained a steadily increasing standard deviation with increasing dilution of the plasma used in the test; when 10 per cent plasma was used the average time was 58.4 seconds, with a standard deviation of ±9.1 seconds for his procedure.

Conley and Morse, in a comparative study of the effects of various types of prothrombin-free plasmas and thromboplastins on the prothrombin determination, demonstrated that great variations in the observed prothrombin time are produced by differences in the type of thromboplastin and diluting substance employed. They were able to show that simple saline dilution of the plasma involves not only the relative prothrombin concentration, but the level of other substances involved in the clotting process as well. Using saline as a diluent and Brambel’s acetone treated rabbit brain thromboplastin emulsion (Difco), as in this investigation, they found the prothrombin times of all dilutions of plasma below 30 per cent to exceed 35 seconds, and obtained a time of 123 seconds at 10 per cent concentration. The results obtained with the present experiment are interpreted as being in keeping with these views that dilution of the plasma for prothrombin determinations involves more than one factor, i.e., the dilution of prothrombin, and that the value of using diluted plasma for the prothrombin determination is doubtful.

**Summary**

1. The results of the determination of the blood coagulation times of a group of normal individuals by the Lee-White method and the reliability of this test are presented.

2. Studies on groups of individuals of the effect of digitalis in therapeutic and toxic doses upon the coagulation time of blood determined by several different methods and upon the "heparin tolerance curve" revealed no detectable change from the normal. Change in clinical status from cardiac failure to compensation similarly produced no alteration in blood coagulation times, bromsulfalein retention test and prothrombin times.

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GEORGE C. SUTTON

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