The Standardized Normal Ivy Bleeding Time and Its Prolongation by Aspirin

By C. H. Mielke, Jr., M. M. Kaneshiro, I. A. Maher, J. M. Weiner and S. I. Rapaport

When vessels are cut or injured, platelets adhere to exposed collagen and then to each other to form aggregates called primary hemostatic plugs. These plugs stop bleeding from the small skin vessels that are cut in performing a bleeding time. Therefore, the bleeding time is a test of the ability of platelets to function normally in the first step of hemostasis.

The clinical usefulness of the bleeding time has been limited by difficulties in standardizing the test. As originally described by Duke, vessels in the ear lobe were incised. Ivy and co-workers increased the sensitivity of the test by puncturing the skin of the forearm while applying a back pressure of 40 mm. of mercury to the cut vessels. Borchgrevink and Waller modified the Ivy technic by substituting blade incisions for puncture wounds. Using this technic, we have occasionally obtained different bleeding times from successive incisions in the same patient because of difficulty in reproducing the depth of incisions.

Some years ago, Frick described abnormal bleeding after aspirin in 3 patients whose hemostatic abnormalities were corrected when the aspirin was stopped. Quick recently reported that prolongation of the bleeding time after aspirin could be used to detect patients with von Willebrand's disease who had a normal or only slightly prolonged bleeding time before aspirin. This report prompted us to study further the effect of aspirin upon the bleeding time in various hemorrhagic disorders. The first step in this study, reported herein, was to devise a reproducible Ivy bleeding time technic and to determine the effect of aspirin upon this standardized bleeding time in a normal population.

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Fig. 1.—Template system for the standardized Ivy bleeding time. (A) polystyrene blade handle with blade secured in slot; (B) aluminum measuring gauge; (C) polystyrene template with central slit. Inset 1 shows the use of the handle and gauge to obtain proper blade protrusion. Inset 2 shows the technic used to confirm proper blade protrusion.

MATERIALS AND METHODS

Standardized Ivy Bleeding Time

A template system consisting of 3 components (Fig. 1) was designed to obtain an incision of standard length and depth. The first component, a blade handle (labeled A, Fig. 1), is a rectangular block of polystyrene 4.5 cm. long, 2.5 cm. wide and 0.6 cm thick. A central rectangular slot, 6 mm. wide by 1 mm. deep, is cut in the upper longitudinal face of the block to accommodate a number 11 Bard-Parker® or similar disposable blade. Within this slot, to one side of the center, five holes are placed 2 mm. apart for the insertion of stainless steel screws. Only two screws are needed to secure the blade; the 5 holes are made to accommodate the various types of blades available.

The second component is an aluminum measuring gauge (labeled B, Fig. 1). This gauge is 4.5 cm. long, 3.5 cm. wide, and 0.6 cm. thick. Along one longitudinal edge an L-shaped groove is cut measuring 2.5 mm. wide and 1 mm. deep. A central slot, measuring 1.1 cm. wide and 2.5 mm. deep, is also cut along the entire upper longitudinal face.

The blade handle and gauge permit one to standardize the depth of the incision as follows: The blade is placed in the handle with the sharp end protruding and two screws are loosely set in place. With both the blade handle and measuring gauge on a flat surface, the protruding blade is placed at a right angle to the L-shaped groove of
The use of the blade handle and template to make a standardized incision.

The gauge. Then, the opposing surfaces of the gauge and handle are pushed flush against one another and the blade tip is pushed forward until it touches the inner wall of the L-shaped groove. The screws are then tightened (inset 1, Fig. 1). To confirm that the blade still protrudes the proper distance after the screws are tightened, one stands the handle upon the gauge with the blade protruding into the central slot (inset 2, Fig. 1).

The third component is a polystyrene template (labeled C, Fig. 1) measuring 5.5 cm. long, 2.5 cm. wide, and 1.5 mm. thick, which is used to standardize the length of the incision. This template has a central slit measuring 11 mm. long and 1 mm. wide. When the blade is secured in the handle and placed through this slit, an incision will be made that is 9 mm. long and 1 mm. deep.

Only 4 measurements are critical in the construction of the parts. The width of the L-shaped groove and the depth of the cut along the longitudinal face of the measuring gauge must be exactly 2.5 mm. The thickness of the polystyrene template used to standardize the length of the incision must be 1.5 mm. Then, the depth of the incision will be 1 mm. Also, the length of the slit in the polystyrene template must be exactly 11 mm. to obtain a cut 9 mm. long.

The subject is seated with his elbow slightly flexed and his forearm resting on a steady support with the volar surface exposed. A syphgmomanometer is placed above the antecubital fossa. The forearm is gently cleansed with an alcohol sponge and permitted to air dry. The cuff is then inflated to 40 mm. Hg. After a wait of 30 sec., the template is placed on the forearm about 5 cm. distal to the antecubital fossa with the longitudinal slit parallel to the antecubital crease. The template is firmly pressed against the skin to flatten the skin surface. This is important to obtain a uniform incision. The blade is introduced at right angles at one end of the slit and the skin is penetrated (Fig. 2). With the flat blade end of the holder flush against the template surface, an incision is made with a smooth, rapid movement along the entire length of the slit. Three separate incisions are made approximately 1.5 cm. from each other. (We have since found that 2
incisions suffice. See Results.) Care is taken to avoid superficial veins. Stop watches are started immediately after each incision is made. The incisions are blotted with Whatman number 1 filter paper strips, without touching the wound edges, every 30 sec. until blood no longer stains the filter paper. The mean of the times for the individual cuts is the bleeding time.

Faint linear scars may persist with this or other bleeding time technics that use an incision instead of a puncture wound. Moreover, the rare possibility of forming a keloid also exists with any technic using an incision. The subject should be advised of these possibilities.

The measuring gauge and template are washed in warm, soapy water after each use, stored in a 70 per cent alcohol solution, and dried with sterile sponge immediately before use again. Only the blade actually contacts exposed tissue, and the blade is discarded after each use. Since the wound does not begin to bleed until between 5 or 10 sec. after the cut is made, blood should not contaminate the template or blade holder. If contamination occurs due to an error of technic, the equipment should be washed as above and then autoclaved with gas.

**Double Blind Aspirin Tolerance Test**

The subjects were 60 normal male volunteers 18 to 45 years old who were physicians, students, or paramedical personnel at the Los Angeles County-University of Southern California Medical Center. The tests were usually performed in the morning since the subjects were required not to eat or smoke for 8 hours prior to the test. A history was taken to rule out recent infections, use of medications, and personal or family history of a bleeding disorder. The bleeding time was determined, following which each subject ingested 3 capsules containing either a total of 1 Gm. of aspirin or lactose as a placebo. Neither the tester nor the subject knew the contents of the capsules. The subject was then allowed to leave the laboratory after being instructed not to eat or smoke. After 2 hours, the test was repeated in exactly the same manner on the same forearm. All bleeding times were carried out by two testers (CHM and MMK). Each tester evaluated 30 subjects and, by chance, each tested 15 subjects receiving aspirin and 15 receiving placebo.

**In Vivo Platelet Adhesiveness**

A secondary goal of this study was to evaluate the effect of aspirin upon the in vivo platelet adhesiveness test. Therefore, in the first 30 subjects, this test was also performed before and 2 hours after the administration of the capsules. Borchgrevink's technic was used with these modifications: the venous blood specimen was collected in a B-D vacutainer® tube containing 6 mg. EDTA anticoagulant, and the blood from the incisions was collected in a B-D Unopette® containing 1 per cent ammonium oxalate anticoagulant. Platelets were counted by a slight modification of the technic of Brecher and co-workers.

**Statistical Methods**

Because the control times plotted on log probability paper, but not on arithmetic probability paper, were normally distributed, bleeding times were converted to log values for statistical calculations. For final expression the values were usually reconverted to minutes and rounded off to the nearest one-half minute.

The significance of the difference of means was determined by a t test. A p value was obtained from a t test table. The value of p < 0.05 was preselected as the level of significance.

The correlation between the before and after bleeding times were evaluated by the calculation of regression lines. The 95 per cent confidence interval for \( \beta \), the slope of the universal population regression line, was calculated as follows:

\[
b \pm t_{0.025, \nu} \frac{1}{S_{yx} \sqrt{\frac{\sum (X - \bar{X})^2}}}
\]

where \( b \) = slope of the sample regression line.
Fig. 3.—The distribution on log probability paper of the control standardized Ivy bleeding times of 60 subjects (Line 1); of the bleeding times after placebo of 30 subjects (Line 2); and of the bleeding times after aspirin of 30 subjects (Line 3).

\[ t_{97.5} = t \text{ distribution for 95 per cent confidence interval for known degrees of freedom} \]

\[ S_{XY} = \text{standard error of the regression line and is defined by:} \]

\[ \sqrt{\frac{\sum (Y - \bar{Y})^2 - b^2 \sum (X - \bar{X})^2}{N - 2}} \]

\[ X = \text{observed before aspirin bleeding time.} \]

\[ Y = \text{observed after aspirin bleeding time.} \]

\[ \bar{X} = \text{mean of before aspirin bleeding time.} \]

\[ \bar{Y} = \text{mean of after aspirin bleeding times.} \]

\[ N = \text{number of subjects.} \]

**RESULTS**

The control bleeding times of the 60 subjects were normally distributed when plotted on log probability paper (Fig. 3, line 1), with a mean of 5 min. and a range (mean ± 2 st. dev.) of 2 min., 30 sec. to 10 min. Since the 30 subjects who received a placebo had the equivalent of two control bleeding times, the reproducibility of the test performed twice on the same subject by the same tester could be evaluated. The distribution of the second bleeding times in the 30 placebo subjects closely approximated the distribution of the initial bleeding times for all 60 subjects (compare lines 1 and 2 of Fig. 3). The mean bleeding time after placebo was 5 min., 30 sec. with a range of 2 min., 30 sec. to 11 min. When log bleeding time before placebo was plotted against log bleeding time after placebo, a regression line with a slope of 0.96 was obtained (Fig. 4).
Fig. 4.—A log plot of bleeding time before placebo against bleeding time after placebo. The solid line is a calculated regression line; the dotted line is the theoretical line of identity.

Two testers did the bleeding times; each performed the tests on 30 subjects. Therefore, it was also possible to evaluate the reproducibility of the test as done by different testers. The mean control bleeding times obtained by testers CHM and MMK were both 5 min. with ranges of 3 min. to 9 min., 30 sec. and 2 min., 30 sec. to 11 min., respectively. The lines obtained by plotting the distribution of the control bleeding times obtained by each tester were almost superimposable.

Inspection of the times for individual incisions suggested that the mean time of 2 incisions would give as accurate a bleeding time as the mean time of 3 incisions. Therefore, in 57 subjects the control bleeding times calculated as the mean of all 3 incisions were plotted against the mean for incisions 1 and 2, the mean for incisions 1 and 3, and the mean for incisions 2 and 3. (Three early subjects in the group of 60 were excluded because of a known error in technic in making one of the incisions). The 3 plots were similar; the plot demonstrating the most variability is illustrated in Figure 5. The clustering of the points around a line of identity is obvious.

The 30 subjects who received aspirin had a mean bleeding time 2 hours later of 9 min., 30 sec. with a range (mean ± 2 st. dev.) of 4 min. to 21 min. However, the highest observed bleeding time after aspirin in a normal subject did not exceed 16 min. (Fig. 3, line 3 and Fig. 6). The mean bleeding time after aspirin differed significantly from the mean bleeding time after placebo, p < 0.001.

These calculations were based upon an assumed normal distribution of
Fig. 5.—A log plot of the mean bleeding time of three incisions against the mean bleeding time of two incisions.

Fig. 6.—A scatter plot of control bleeding times, bleeding times after placebo, and bleeding times after aspirin.
log bleeding times after aspirin. However, a scatter plot of the bleeding times after aspirin (Fig. 6) showed two clusters of values. On a log probability plot the straight line fitting the group of values below 10 min. did not fit the group of values exceeding 10 min.

To examine whether or not the degree of prolongation produced by aspirin was predictable from the control bleeding time, we plotted log before aspirin bleeding time for each subject against log after aspirin bleeding time (Fig. 7). The shift upwards of the regression line from that for the placebo group (Fig. 4) confirms that the overall effect of aspirin is to prolong the bleeding time of a normal population.

The regression line of Figure 7 has a slope of 0.66. A hypothesis was proposed that no relation existed between the before and after bleeding time. If true, the 95 per cent confidence interval for \( \beta \), the slope of the universal population regression line, should include 0. The calculated 95 per cent confidence interval for \( \beta \) was 0.66 \( \pm \) 0.33; therefore, this hypothesis was untenable. A second hypothesis was proposed that the bleeding time after aspirin was prolonged by a constant proportion of the control bleeding time. If this were true, the 95 per cent confidence limit of \( \beta \) should include 1. Since 1 was just outside 0.99, the upper limit of the confidence interval, the data did not support this hypothesis either but suggested the need for a series with a larger number of subjects. Examination of the points of

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Fig. 7.—A log plot of bleeding time before aspirin against bleeding time after aspirin. See legend to Figure 4.
Figure 7 suggested to us that normal subjects have a ceiling (about 16 min.) to the bleeding time after aspirin that is independent of the control bleeding time.

The initial in vivo platelet adhesiveness test result is plotted against the second in vivo platelet adhesiveness test result in Figure 8. As is illustrated, no relation between the before and after values could be demonstrated for either the subjects receiving placebo or the subjects receiving aspirin.

**DISCUSSION**

A standardized Ivy bleeding time technic has been described in which incisions measuring 9 mm. in length and 1 mm. in depth are reproducibly obtained with a simple template system. The components are easily made from inexpensive materials and can be used by either a right or left-handed tester. Because disposable blades are used, sterility has not been a problem.

The mean bleeding time for 60 normal male volunteers was 5 min. with a range of 2 min., 30 sec. to 10 min. For each test 3 incisions were made and their mean was taken as the bleeding time. Subsequent analysis (see Fig. 5) showed that the mean of any 2 incisions gave the same bleeding time as the mean of 3 incisions. Consequently, only 2 incisions are needed after one becomes familiar with the technic. The test was reproducible when performed twice 2 hours apart in the same individual by the same tester (see Figs. 3 and 4). Moreover, no difference was found between testers. Therefore, this
standardized technic should permit one to obtain accurate bleeding time data in man.

Normal values for an aspirin tolerance test in which a standardized bleeding time was done 2 hours after the administration of 1 Gm. of aspirin to male subjects were: mean, 9 min., 30 sec.; mean ± 2 standard deviations, 4 min. to 21 min. This mean was significantly longer (p < 0.001) than the mean bleeding time after placebo (5 min., 30 sec.) which confirms, with a reproducible bleeding time method, that aspirin prolongs the mean bleeding time of a normal population.\textsuperscript{5,6,12,13,16} The longest bleeding time observed after aspirin in 30 subjects was 16 min. Probably, this difference between the observed and calculated upper limit of normal was obtained because the bleeding times after aspirin were not normally distributed (line 3, Fig. 3). Our mean and range are very similar to those reported by Blatrix,\textsuperscript{12} who found a mean Ivy bleeding time of 9 min. and a range of 4 to 20 min. in 100 subjects given 40 mg. per Kg. of aspirin daily for 3 days. It would appear, therefore, that a single 1 Gm. dose of aspirin can prolong the bleeding time of normal individuals as much as larger doses given over several days. (Note that capsules of aspirin powder rather than tablets were used in the tolerance test to minimize variability of absorption with tablets).

Aspirin interferes in vitro with platelet aggregation induced by a weak concentration of collagen and with a second wave of aggregation induced by a critical concentration of ADP or by epinephrine.\textsuperscript{13-16} Each of these reactions is mediated by ADP released from the platelets. Moreover, Weiss and Aledort\textsuperscript{13} have demonstrated a decreased release of ADP from platelets on incubating with connective tissue the platelet-rich plasma of subjects given aspirin. Consequently, it is tempting to attribute the lengthening of the bleeding time by aspirin to interference with release of ADP from platelets in vivo.

The distribution of the bleeding time values after aspirin (Figs. 3, 6, and 7) suggests that normal subjects do not respond to aspirin as a single population. A larger series of normals must be studied to confirm this. However, the hypothesis receives support from the striking difference in prolongation of bleeding times after aspirin observed in hemophiliacs by Quick\textsuperscript{17} and confirmed by us in a larger series to be published elsewhere. It will be important to attempt to relate the presence or absence of a marked effect of aspirin upon the bleeding time in hemophiliacs to the in vitro effects of aspirin upon platelet aggregation in these patients.

We were unable to evaluate the effect of aspirin upon in vivo platelet adhesiveness. Fourteen of our subjects had abnormally low initial in vivo platelet adhesiveness of less than 24 per cent.\textsuperscript{7} Moreover, we failed to obtain consistent data when the test was performed twice on those subjects receiving placebo. The reason for these difficulties is not clear. It seems unlikely to us to result from the minor modifications of the test that we employed (see Methods).

The drops of blood that formed during the second bleeding time were much larger in some subjects than in others. When the code was broken, the
individuals with the large drops were found to have received aspirin. Thus, aspirin can not only lengthen the bleeding time of a normal subject but can strikingly increase the amount of blood lost. The impairment of hemostasis observed in some subjects seemed to us sufficient to account for clinical reports of increased bleeding after tonsillectomy in patients given aspirin. Aspirin has also been shown to increase occult blood loss from the gastrointestinal tract of normal subjects, even when given intravenously to avoid local irritation. Possibly, the use of oral aspirin converts a mildly bleeding gastrointestinal tract lesion into a heavily bleeding lesion by the combination of local irritation plus impairment of the formation of the primary hemostatic plug.

In 16 of the 30 subjects the bleeding time after aspirin exceeded 10 min., which is our upper limit of normal for the standardized Ivy bleeding time. Apparently, therefore, the ingestion of small amounts of aspirin will result in an erroneous “abnormal” bleeding time in a large number of normal males. The duration of the effect of aspirin upon the bleeding time is unknown, but the effect upon platelet aggregation has been shown to persist for up to 7 days. Clearly, one must know about the use of even small amounts of aspirin in assessing the status of a patient with a suspected bleeding abnormality. In planning for elective tests, one should instruct a patient to avoid the use of aspirin in any form for at least one week.

SUMMARY

A standardized, reproducible Ivy bleeding time technic has been described which permits one to obtain accurate bleeding time data in man. The technic was used to standardize an aspirin tolerance test in which 60 normal males had a control bleeding time; were given, on a double blind basis, either placebo or 1 Gm. of aspirin, and had a second bleeding time 2 hours later. The control values were: mean, 5 min.; mean ± 2 st. dev., 2 min., 30 sec. to 10 min. The values after placebo were: mean, 5 min., 30 sec.; mean ± 2 st. dev., 2 min., 30 sec. to 11 min. The values after aspirin were: mean, 9 min., 30 sec.; mean ± 2 st. dev., 4 min. to 21 min. The difference between the mean bleeding time after placebo and after aspirin was highly significant (p < 0.001). The distribution of the bleeding times after aspirin suggested that normal subjects do not respond to aspirin as a single population. The degree of prolongation of the bleeding time and the large size of the drops of blood observed in some subjects suggested to us that small amounts of aspirin may exert a significant effect upon hemostasis in normal individuals.

SUMMARIO IN INTERLINGUA

Es describite un technica standardisate e reproducibile pro le determination del tempore de sanguination de Ivy. Iste technica permitte le obtention de accurate tempores de sanguination in humanos. Le technica eseva usate pro standardisar un test de tolerantia pro aspirina in le qual 60 masculos normal con valores normal in determinaciones de controlo del tempore de sanguination recipieva, a base de un technica experimental a dupe anonymato, 1 g aspirina o un placebo. Duo horas plus tarde le tempore de sanguination eseva redetermine. In le determinaciones deescontrolo, le valor medie eseva 5 min durante
que le valor medie ± 2 DS esseva 2 min 30 sec a 10 min. Post placebo, le valor medie esseva 5 min 30 sec e le valor medie ± 2 DS esseva 2 min 30 sec a 11 min. Post aspirina, le valor medie esseva 9 min 30 sec e le valor medie ± 2 DS esseva 4 min a 21 min. Le differentia inter le tempores medie de sanguination post le placebo e post le aspirina esseva altemente significative (P < 0,001). Le distribution del tempores de sanguination post aspirina suggestionava que subjectos normal non responde a aspirina como un sol population homogenee. Le grado del prolongation del tempore de sanguination e le grande dimensiones del guttas de sanguine observate in certe subjectos suggestionava a nos que micre quantitates de aspirina pote exercer un effecto significative super le hemostase in individuos normal.

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