A MODIFICATION OF THE WAUGH-RUDDICK TEST FOR INCREASED COAGULABILITY OF THE BLOOD, AND ITS APPLICATION TO THE STUDY OF POSTOPERATIVE CASES

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In 1944, Waugh and Ruddick1 reported a new test for increased coagulability of the blood, based on controlled deceleration of the clotting mechanism through the use of heparin. While it was appreciated that temperature affected the test, the original work was done at a time when room temperature was constant (i.e., during the winter months), so that special precautions in this direction were unnecessary. Later Whittaker,2 working during the summer months, when room temperature fluctuations were present, experienced some difficulty in obtaining duplicate curves, and worked out in detail the facts concerning the affect of temperature on the test. She found that between 20 and 35 degrees Centigrade, increase in temperature caused a decrease in clotting time. This stressed the fact that comparison of the blood coagulation curves in different individuals, or in the same individual at different times, is possible only if the tests are conducted at the same temperature.

Application of the test to clinical material3 revealed an increased coagulability of the blood under a wide variety of circumstances. These include pneumonia, empyema, peritonitis, and other acute infectious processes: following hemorrhage: after surgical operation, etc. More recently, Ogura and colleagues4 have used the Waugh-Ruddick test to study changes in blood coagulation following coronary thrombosis. They found that in 27 cases, 77.8 per cent showed a decreased coagulation time. This was usually evident by the second or third day following the thrombotic incident, and lasted to about the seventeenth day. Acceleration was prolonged beyond the third week in a few cases, but in every instance, the clotting mechanism was indistinguishable from normal after the fourth week.

The present report deals with (1) a proposed modification of the test, and (2) the application of the modified test to the study of postoperative blood coagulability.

A MODIFICATION OF THE WAUGH-RUDDICK TEST

Using the original method of Waugh and Ruddick,1 blood coagulation studies were carried out on 43 student volunteers. The average curve for this group is shown in figure 1 along with the average curve as determined by the original investigators. It will be seen that although the curves have the same general form, the actual values obtained differ greatly in the two series. There appear to be

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two chief reasons for this, viz., (1) the heparin employed was of different batches and thus possibly of somewhat different potencies, and (2) the endpoint in each tube is not sharply defined, and consequently there arises the question of interpretation of results. These and other reasons for modifying the test can be summarized as follows:
1. To shorten the time needed to complete a test:
2. To perform the test under controlled temperature conditions.

Details of the modified test follow.

Solutions Used. (1) Heparin stock solution containing 2 units of heparin per cc. of saline. The Connaught Laboratory preparation, having a potency of 1000 units per cc., is employed. Six-tenths of a cc. of heparin are added to 300 cc. of physiological saline, and thoroughly mixed. This stock solution is used in making up the various subdilutions of heparin, and can be kept in the icebox when not needed.
Subdilutions of heparin are made up as shown in Table I. These are kept in small glass-stoppered bottles with large mouths (capacity 60 cc.). The potency of the heparin is maintained by keeping these solutions in a refrigerator.

(4) 0.1 M sodium oxalate: 1.34 Gm. of sodium oxalate are dissolved in 100 cc. of distilled water.

Preparation of Test Tubes. Eight Wassermann tubes (100 x 13 mm.) (previously cleaned in potassium-dichromate-sulphuric acid solution, rinsed out in hot water and distilled water, and dried in an oven) are placed in a suitable rack, and are numbered from 1 to 8. To each tube are added 0.2 cc. of 0.01 M calcium chloride and 0.1 cc. of the heparin subdilution from the correspondingly numbered glass-stoppered bottle. The tubes are corked and set aside.

Preparation of Plasma to be Tested. By venipuncture, using a dry 20 to 30 cc. graduated Luer syringe and a large gage (No. 18) needle, 4.5 cc. of blood are drawn and mixed immediately and thoroughly with 0.5 cc. of 0.1 M sodium oxalate by repeatedly inverting the stoppered tube. The blood is centrifuged at the rate of 1000 r.p.m. for five minutes, and during this time the rack containing the prepared tubes is placed in a water bath at 37.5 degrees C. This assures the tubes being at body temperature for the test.

After centrifuging for five minutes, the plasma is removed from the packed cells, and 0.1 cc. is added to each of the eight tubes in a water bath. The actual test now begins.

The Test Proper. As soon as the plasma has been added to the heparinized tubes, they are agitated slightly to ensure complete mixing of the fluid and the plasma. A stop watch is started when plasma has been added to the last tube.

Starting at about two and a half minutes, the first tube is gently partially withdrawn from the rack, and the plasma is noted for clotting. If this has not occurred, the tube is replaced and examined at half-minute intervals until a complete firm clot has formed. The time is noted. The same procedure is then carried on for tube
2, then tube 3, etc. until coagulation has occurred in all tubes. The results are plotted on graph paper, the coagulation time against the amount of heparin in each tube.

Some Observations Using the Modified Test. Tests were done on 36 samples of normal plasma. The mean, standard deviation, maximum and minimum values for each tube are given in table 2. The average curve is shown in figure 2. As is to be expected, some individual variation is seen, but this is of the same order as that described in the original Waugh-Ruddick method. That the test is reliable in any given case was shown by doing checks on the same plasma samples (figs. 3, 4, 5, 6).

Discussion Regarding the Modified Test. The modification proposed is in reality a combination of Quick's method of determining the clotting time of recalcified plasma and the Waugh-Ruddick test. As stated by Chargaff, Bancroft and Stanley-Brown, working on methods for the measurement of inhibition of clotting by various substances including heparin, such a method "can only have the accuracy of the biological test, and not that of a quantitative chemical procedure. The chief reason is the fact that the endpoint, namely the formation of a clot, is comparatively ill-defined." The average curve (fig. 2) is simply that—an average; and while some tests vary considerably from this, the results obtained in any one case are fairly constant. The use of plasma instead of whole blood has rendered the endpoint easier to determine, but other factors equally as important must be taken into consideration. As Chargaff points out, to be successful in studying the action of inhibitors on plasma coagulation the following must be considered.

1. The reaction volume must be kept constant, as the numerous substances contained in plasma respond to dilution in different ways. The addition, to a series of plasma samples, of increasing amounts of inhibitor solution leads to discordant results.

2. Extreme care must be taken to disturb the plasma as little as possible. Attempts to increase the precision of the endpoint may adversely affect its accuracy. Plasma which coagulates in the presence of an inhibitor in general gives rise to clots that are very soft, and may easily be broken up beyond recognition if shaken before their formation is complete.

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\text{Table 2. Modified Waugh-Ruddick Test} \\
\begin{array}{|c|c|c|c|}
\hline
\text{Tube} & \text{Minimum} & \text{Mean} & \text{Maximum} \\
\hline
\text{mins.} & \text{mins.} & \text{mins.} & \text{Standard Deviation} \\
\hline
1 & 2.5 & 3.46 & 5.0 & 0.5 \\
2 & 4.0 & 6.46 & 9.5 & 1.3 \\
3 & 5.8 & 10.8 & 22.0 & 3.45 \\
4 & 8.2 & 16.8 & 39.0 & 7.18 \\
5 & 12.0 & 23.8 & 50.0 & 9.64 \\
6 & 15.0 & 33.1 & 55.0 & 10.9 \\
7 & 24.5 & 38.2 & 62.5 & 10.2 \\
8 & 22.7 & 49.5 & 69.0 & 12.3 \\
\hline
\end{array}
\]
3. The period of time over which the determination of inhibitor activity takes place should not be too extended, as the clotting properties of plasma may change quite considerably with time.

It is felt that the modification outlined fulfills these conditions, and retains the advantages of the Waugh-Ruddick test while avoiding some of the disadvantages.

**Fig. 7.** Average curves preoperatively (P), and 1, 2, 3, 4, and 10 days postoperatively.

**Fig. 8.** Case 1. Right inguinal herniorrhaphy. Curves obtained preoperatively and 1, 2, 3, 4, and 11 days postoperatively. Note the increased coagulability postoperatively, present on the first day. Coagulability back to normal by the eleventh day.

**Fig. 9.** Case 2. Right inguinal herniorrhaphy. Curves preoperatively and 1, 3, 6, and 14 days postoperatively. Increased coagulability is seen within 24 hours after operation.

**Fig. 10.** Case 3. Appendectomy. The increased coagulability seen postoperatively is indicated by a clockwise shift of the curves.

**Fig. 11.** Case 4. Appendectomy. Clockwise shift of coagulability curves postoperatively, i.e., increased coagulability.

**Application of the Modified Test to the Study of Postoperative Blood Coagulability**

Using the above test, it was decided to study the affect of operation on the coagulability of the blood. Waugh and Ruddick using their test in a wide variety of conditions, applied it to several patients undergoing operation. They found a marked increased coagulability after operation, but did not investigate the time...
at which it first appeared nor its duration. They state: "It would be interesting if finer analysis of this change were studied in order to demonstrate the exact time at which increased coagulability occurs." That is exactly what the present study attempts to accomplish.

In this investigation, tests were performed on 9 patients admitted to the Royal Victoria Hospital for operation. A special effort was made to use patients with no acute illness or any other condition likely to affect the coagulability of the blood. Eight of these were for appendectomy or herniorrhaphy. The ninth was a gastrectomy. All operations were performed under spinal anesthesia. Coagulation tests were done preoperatively and as often postoperatively as possible, commencing the day after operation. The average curves obtained preoperatively and on the first, second, third, fourth, and tenth days postoperatively are shown in figure 7. Some individual records are shown in figures 8-11. In figures 7-9, not all the observed days are recorded, to avoid complicating the graphs. Figures 10 and 11 are complete. Some illustrative cases follow.

Case 1. Mr. P., age 56, admitted for a right inguinal herniorrhaphy. It will be noted that the postoperative coagulability curves are below the preoperative level immediately after operation (i.e., within 2.4 hours), and for several succeeding days. The coagulation time slowly returns to normal, and is within normal limits by the eleventh day (fig. 8).

Case 2. Mr. C., age 49, admitted for a right inguinal herniorrhaphy. The curves in this case are much like those in case 1. There is a marked increase in blood coagulability postoperatively, with a slow return to normal, in this case by the fourteenth day (fig. 9).

Case 3. Mrs. C., age 37, admitted for appendectomy. The relatively short stay in hospital allowed of only three curves being done. Here again, the increased coagulability postoperatively, indicated by a clockwise shift of the curves, is seen (fig. 10).

Case 4. Mrs. D., age 29, admitted for appendectomy. The story is much the same as in case 3. See figure 11 for coagulability curves.

Discussion

The average curves (fig. 7) indicate that there is a definite increased coagulability of the blood postoperatively. This begins within 2.4 hours after operation, and may last, in varying degrees, for a week or more. There does not appear to be a definite order in which the coagulability changes from day to day, but this is hardly to be expected, as the test must of necessity be a qualitative rather than a precisely quantitative affair. In all cases where hospitalization was long enough to permit observations, the coagulation time was normal at the end of two weeks.

Quick7 points out that "thromboplastin is the trigger-substance in the coagulation process. It initiates and determines the speed of the reaction." If this is so, then the decreased coagulation time seen postoperatively can be explained by an increase in the amount of available thromboplastin. There are two reservoirs of this material, namely the platelets and the tissue juices. Let us consider each of these in turn. Hueck8 in 1926 first demonstrated the presence of a postoperative thrombocytosis. This has since been confirmed by many other workers.9-15 All agree that this increase in platelets occurs about the sixth or seventh postoperative day. It therefore cannot explain the increased coagulability of the blood seen within 2.4 hours after operation.
On the other hand, an increase in the circulating thromboplastin, presumably derived from damaged tissue in the operative area, is a much more likely explanation. This view has been favored by Pickering and Mathur, Dougal and others, and is supported by observations that the blood urea and polypeptides are increased postoperatively. Snell and Bancroft point out that after operations on obese patients there may be an increased liberation of thromboplastic lipid substances such as cephalin, due to extensive areas of fat invaded. Waugh and Ruddick showed that the addition of thromboplastin to plasma caused a clockwise shift of the coagulability curves, such as has been shown to occur postoperatively. It would appear that the mechanism is the same in both instances.

**SUMMARY**

1. A modification of the Waugh-Ruddick test for increased coagulability of the blood is described, which employs the use of recalcified plasma in the place of whole blood.
2. Using this modification, studies were carried out on a series of patients undergoing operation. It was found that there was an increased coagulability of the blood present within 24 hours following operation, and that this condition lasted for a week or so. In all cases, the coagulability had returned to normal by the end of two weeks.
3. It is felt that the change is due to an increase in the circulating thromboplastin, presumably derived from damaged tissue in the operative area.

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