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STUDIES IN INTRAVASCULAR COAGULATION. I. COAGULATION CHANGES IN ISOLATED VENOUS SEGMENTS¹

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Significant advances have recently been made in our understanding of the biochemistry of blood coagulation. Most of this work has, of necessity, been performed on blood *in vitro*. *In vivo* studies have been hampered by the difficulty of producing clots and thromboses in animals under controlled conditions and by the fact that, unlike the hemorrhagic diseases, there are no known clinical states in which a specific defect responsible for hypercoagulability of the blood has been demonstrated.

We had been impressed by the repeated references in the literature to the effect that a stationary column of blood in a vein included between two carefully applied ligatures will remain liquid for weeks (1, 2). The experiments referred to were carried out more than seventy-five years ago and review of the original data did not indicate clearly whether or not clotting had occurred in these segments (3, 4). It occurred to us that a study of what *did* happen to the clotting mechanism in isolated endothelial-lined segments might provide a bridge between our knowledge of test tube coagulation and of the clotting process as it evolves in a more physiologic environment. Some of the results obtained are herewith presented.

Clot Formation in the Isolated Segment

Healthy dogs of both sexes, 9 to 47 Kg. in weight, were anesthetized with intraperitoneal nembutal, the neck and groin were shaved and cleansed with soap and water and an intravenous infusion of normal saline was begun. Aseptic precautions were not attempted. Both femoral and external jugular veins were widely exposed: the former from Pouparts ligament to within 2 centimeters of the knee; and the latter from the base of the neck to the angle of the jaw. All demonstrable tributaries to these veins were carefully

ligated with No. 3 zero Deknatel silk and then cut so that the vessels were entirely freed from surrounding structures. Continuity of each vein with the remainder of the circulation was interrupted by the careful application of a 27 mm. seraffine² clamp at each end of the freed segment. In larger animals, the isolated femoral veins could be separated into two, and the cervical veins into three segments, each containing approximately 1 cc. of blood. After isolation, the segments were covered with gauze moistened with saline, and the wound closed by apposition of the skin edges with a hemostat. This procedure required one to two hours. Hemostasis was well maintained; rectal temperature, pulse rate, and hematocrit remained constant throughout the operation.

At selected intervals vein segments were removed from the animal and by release of one clamp the contents were emptied into clear shallow glass dishes containing 2 to 3 cc. of 2.5 per cent sodium citrate. These dishes were carefully inspected for the presence or absence of a gross clot. The presence of clot, regardless of size, was taken as the end-point. The empty vein segments were always opened and examined for residual clots.

In fourteen dogs the time interval was observed between the isolation of the vein segment and the recognition of the clot in thirty-four segments. Clot formation was never observed in less than twenty minutes, usually not until after thirty minutes had elapsed and in a few segments not until more than one hour had passed. When these clots were examined promptly by phase microscopy they were found to consist principally of fibrin. These initial clots were very small, but increased only slowly with time. That coagulation was not complete for at least as long as eight hours after isolation could be demonstrated by emptying the contents of such segments into containers devoid

¹ This work was carried out under a contract between the office of the Surgeon General, U. S. Army, and the President and Fellows of Harvard College.

² Manufactured by Arthur H. Thomas, Philadelphia, Pa.

of anticoagulant in which further clot formation could be observed.

Other Alterations in the Blood in the Isolated Segment

The obvious retardation of coagulation afforded by endothelial tissue was further pursued by investigating other clotting functions in these isolated segments; namely—prothrombin consumption, accelerator formation and platelet disappearance. In another group of twelve dogs similarly prepared, the blood was emptied into siliconized centrifuge tubes containing 0.5 cc. of 3.2 per cent sodium citrate. Under these conditions gross clots were not evident unless the blood was obtained from segments which had been isolated for two or more hours.³

³ Small clots, visible in twenty to thirty minutes when blood was diluted with large amounts of anticoagulant in shallow containers as described above, were not recognizable in these centrifuge tubes where blood dilution was minimal.

One-stage prothrombin values were obtained by the modification of Rosenfield and Tuft (5) in which pooled barium sulfated dog plasma was used as the diluent. Two-stage prothrombin determinations were obtained by the method of Ware and Seegers (6) except that the blood was not defibrinated prior to measurement. Platelets were counted by the direct method with Rees-Ecker diluting fluid (7). Control 1 cc. blood samples carefully obtained from superficial veins with Arquad⁴ coated needles and siliconized syringes, were transferred promptly to siliconized centrifuge tubes containing 0.5 cc. of 3.2 per cent citrate. This volume and concentration of anticoagulant was found to give consistent prothrombin values with 0.5 to 4 cc. of blood. Because of the small amounts of blood available for analysis and the relatively large volumes of anticoagulant in the collecting tubes, the hematocrit was routinely determined to correct for the initial dilution.

⁴ A non-wetting agent generously provided by Armour Laboratories, Chicago, Ill.

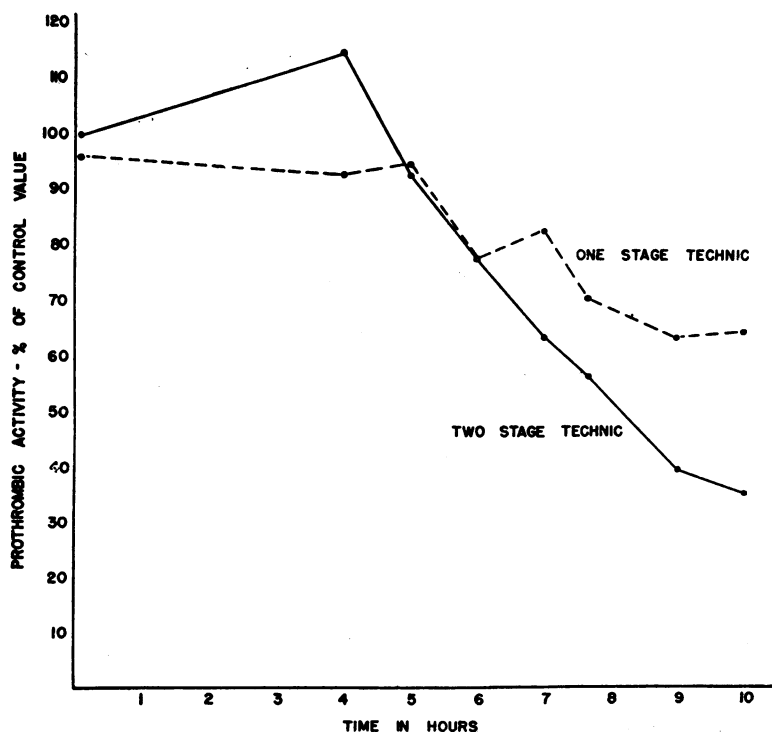


FIG. 1. ONE- AND TWO-STAGE PROTHROMBIN VALUES DETERMINED IN PARALLEL ON THE SAME ANIMAL AT INTERVALS OVER A TEN-HOUR PERIOD

Grossly visible clots appeared sometime before the fourth hour.

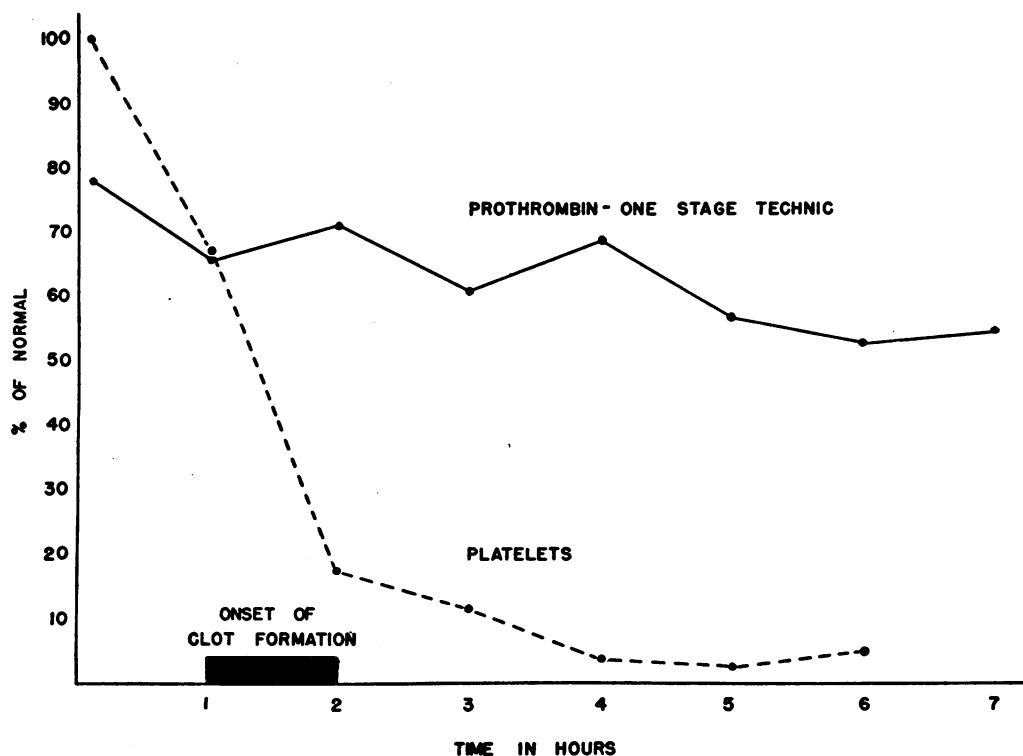


FIG. 2. PLATELET COUNTS AND ONE-STAGE PROTHROMBIN VALUES DETERMINED IN PARALLEL ON THE SAME ANIMAL AT INTERVALS OVER A SEVEN-HOUR PERIOD

Grossly visible clots appeared sometime between the first and second hour.

In a series of 70 segments from 12 dogs, no significant changes in one- or two-stage prothrombic activity were noted until after a segment had been isolated for four hours. Thereafter, the activity decreased although in some instances the change was not apparent until after seven hours. Even after ten hours of isolation a considerable amount of prothrombic activity was still demonstrable. This is consistent with the finding that clots after hours in blood-filled isolated segments are small and increase in size when the blood is subsequently emptied into uncoated glass vessels. The decrement in prothrombic activity as measured by the two-stage method was always greater than that found with the one-stage technic.

In Figure 1 are shown the one- and two-stage values determined in parallel on the same animal at intervals over a ten-hour period; grossly visible clots appeared sometime before the fourth hour—several hours before any demonstrable change occurred in prothrombic activity. Figure 2 represents determinations, over a seven-hour period, of

the platelet counts and one-stage prothrombin values in an animal in which clot formation began before the second hour. Although platelet disappearance is delayed markedly over that found when blood is allowed to clot in glass tubes, a correlation can be seen between the onset of platelet disappearance and the development of a grossly visible clot. Again, the one-stage prothrombin values were unchanged throughout the period during which samples were collected.

DISCUSSION

Although prolongation of both the clotting (8) and prothrombin (9) times of blood in silicone tubes have been demonstrated, it is evident that coagulation occurs far more slowly in blood in contact with the endothelial-lined surfaces of isolated venous segments than in coated vessels currently employed in various *in vitro* technics. This is true even though the blood is completely stagnant and the vascular segment cut off entirely from the circulation.

The interpretation of the observed discrepancy between the one and two-stage prothrombin values inevitably depends upon one's viewpoint regarding the specificity of these two measures of the prothrombin moiety. The observed difference is entirely consistent with the view that during the coagulation process changes occur, whereby there is an increase in the velocity with which prothrombin is converted to thrombin. Since this velocity effect is manifest only in the one-stage method (10), the difference between the one- and two-stage values reflects the presence of accelerators.

Of particular interest in this study was the insight afforded into the early phases of the coagulation sequence. From these experiments it is apparent, for instance, that a fibrin clot can develop prior to the disappearance of demonstrable amounts of prothrombin or the elaboration of measurable quantities of clot accelerators. This observation is of potential clinical significance since induced hypoprothrombinemia is currently employed to inhibit or retard intravascular coagulation in man. It becomes important, therefore, to ascertain whether induced hypoprothrombinemia actually causes retarded fibrin deposition *in vivo* under the conditions of these experiments. Work in this direction is in progress.

SUMMARY

The influence of the isolated venous segment of the dog on intravascular coagulation has been characterized with respect to clot formation, prothrombin consumption, accelerator formation and platelet disappearance. Under the conditions of

these experiments it has been shown that a fibrin clot may be deposited intravascularly prior to the demonstrable utilization of prothrombin. The isolated venous segment provides an additional and more physiologic means for investigating the early phases of intravascular coagulation, because endothelial tissue replaces artificial membranes.

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