MECHANISM OF DEVELOPMENT OF HYPERCOAGULATION IN ACUTE BLOOD LOSS

L. G. Voronyanskaya,
A. V. Zav'yalov, and B. I. Kuznik

UDC 616.151.511-02:[616.151.11-02:
616-005.1-036.11]-092

Acute blood loss accelerates the clotting of the blood and increases its fibrinolytic activity. The mechanism of this reaction however has not yet been discovered. The view has been put forward [1] that hypercoagulation after acute blood loss depends on depression of the antithromboplastin and antithrombin activity of the blood. Meanwhile, D. M. Zubairov [4, 5] found no disturbance of the activity of the anticlotting system after acute blood loss, although he did observe in these conditions an increase in the thromboplastic activity of the blood and the appearance of thrombin in the circulation. The thromboplastic substance could not enter the blood stream via the lymphatic system, because after acute blood loss there was no increase in the clotting of the lymph [6]. It was accordingly suggested that the hypercoagulation in acute blood loss was due to an agent entering the blood stream from the blood vessel wall [5].

A similar conclusion was drawn by the authors from their investigation. They showed that a perfusate of the aorta obtained after acute blood loss significantly shortens the time required for blood clot formation.

These observations were continued in the present study.

EXPERIMENTAL METHOD

Experiments were carried out on 12 dogs weighing from 5 to 15 kg. The effect of acute blood loss (from 10 to 20% of the blood volume) on the thromboplastic agents the natural anticoagulants, and the activators of fibrinolysis entering the blood stream was studied. For this purpose, under thiopental or hexobarbital anesthesia using a modification of I. D. Boenko's technique [2, 3], a part of the carotid artery was isolated humorally. The operation was carried out as follows. Two small incisions (not more than 1 cm long) were made in the skin of the neck, and the part of the carotid artery was mobilized above and below. Its adventitia was incised and stripped off, leaving the nerve fibers running along the course of the vessel intact. Ligatures were passed beneath the adventitia in the upper and lower parts of the artery and the vessel was ligated. Another pair of ligatures was used to fix the cannula through which the vessel was perfused. The humorally isolated portion of the artery was rinsed with physiological saline, heated to 37°, and then filled with perfusate for 5 min. At the end of this time the physiological saline was washed out with 2.5 ml of liquid and used in the experiment. These procedures were carried out three times before and four times after blood loss.

The effect of the perfusate was studied on the recalcification time of the plasma with a low platelet count [14], on the prothrombin consumption by the method of M. A. Kotovshchikova and Z. D. Fedorova [7], as modified by B. I. Kuznik [12], the thrombin time [13], and the fibrinolytic activity of the blood by the method of Kowarzik and Buluk [15], slightly modified. Because of the special features of the experiments, all the methods used were slightly modified. For instance, in the control series (except in the method of determining prothrombin consumption) 0.5 ml of physiological saline was added to the plasma, and in the experimental series the same volume of perfusate was added. This revealed the changes taking place much more clearly. The rate of clotting of the blood was recorded at the same time [16].

EXPERIMENTAL RESULTS

Addition of the perfusate obtained both before and after blood loss caused a significant decrease in the recalcification time of the plasma with a low platelet count and a sharp increase in the prothrombin

Department of Normal Physiology, Chita Medical Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR S. R. Mardashev). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 3, pp. 35-37, March, 1967. Original article submitted April 10, 1965.

construction (see Table).* Since no blood entered the perfusate (as a precaution, the perfusate was centrifuged at 3000 rpm and examined under the microscope), it can be concluded that this action was due to a certain compound secreted by the vessel wall. This compound could only be thromboplastic substance, because the prothrombin consumption was increased only under its influence. The recalcification time was reduced by a particularly marked degree immediately after the blood loss. It was at the same times that the maximal increase in the prothrombin consumption took place under the influence of the perfusate. These facts demonstrate that the secretion of tissue thromboplastic substance is increased in response to blood loss.

Blood loss is accompanied by a sharp increase in fibrinolytic activity. This reaction may be regarded as due to the entry of tissue activators of fibrinolysis from the blood vessel wall into the general blood stream. In fact, the perfusate obtained immediately after blood loss considerably shortened the time required for lysis of the blood clot.

Finally, immediately after and 20 min after blood loss the perfusate slightly increased the thrombin time. Since the experiments were carried out on the same plasma substrate, with the addition of thrombin of approximately the same activity, it may be concluded that antithrombin compounds had entered the physiological saline.

These observations thus showed that the blood vessel wall can secrete at least three compounds into the general blood stream: thromboplastic factor, fibrinolytic agents, and antithrombin.

It is certain that the acceleration of blood clotting after acute blood loss depends on the secretion of thromboplastic factor by the vessel wall. It should be noted, however, that maximal secretion of thromboplastic substance took place immediately after acute blood loss, whereas the blood clot was formed most rapidly 15 min after blood loss. The authors consider that these results are perfectly consistent. In the intact organism the secretion of tissue thromboplastic factor leads to the appearance of active thromboplastin in the blood stream (this reaction involves the participation of plasma factors V, VII, and X), changing prothrombin into thrombin. Part of the thrombin thus formed in inactivated in the blood stream, but the appearance of fresh amounts of new thrombin leads, on the one hand, to activation of the plasma compound (factors V, VIII, and IX), and on the other hand, to the formation of fibrin clots. The evolution of the cycle of chain reactions takes place gradually. That is why the maximal shortening of the clotting time of the blood took place not immediately, but after a definite interval.

The authors consider that the secreted thromboplastic factor as a stimulus to blood clotting. The amount of prothrombokinase liberated by the vessel wall may subsequently diminish, but the chain reaction of blood clotting, once started, increases in intensity, so that the time of clot formation continues to diminish.

The above remarks evidently apply equally to fibrinolysis. Immediately after blood loss the vessel wall begins to secrete tissue lysokinases, but the increase in fibrinolytic activity only develops 10-30 min after the beginning of bleeding. This depends on the cycle of intermediate reactions leading to activation of the system of fibrinolysis in the intact organism.

It must be emphasized once again that in these experiments a slight increase in the concentration of antithrombin was found in the perfusate after blood loss. Evidently initially the reaction of the vessel wall to the blood loss was undifferentiated. The increase in the concentration of antithrombin 20 min after the blood loss probably depended on the appearance of thrombin in the blood stream [4, 5], activating the release of the components of the anticlotting system into the blood stream [8-11].

The problem of the intimate mechanism of the reaction revealed by these experiments has not been discussed. It must be noted, however, that Shimanoto and Ishioka [17], who perfused a humorally isolated segment of aorta with physiological saline, found that thromboplastic factor appeared in the perfusate. If the results obtained by these authors are taken into account, no further discussion of our findings is necessary. Blood loss is accompanied by an increase in the tone of the sympathetic division of the autonomic nervous system, leading to secretion of the thromboplastic and fibrinolytic substances into the blood stream.

The increase in the clotting power of the blood and in its fibrinolytic activity after acute blood loss is thus associated with the entry of tissue compounds from the vessel wall into the general blood stream.

* Table omitted in original Russian.

LITERATURE CITED

1. V. P. Baluda and N. A. Gorbunova, Pat. Fiziol., No. 2, 46 (1961).
2. I. D. Boenko, New Facts on the Physiology of Interoception, Doctorate dissertation, Ryazan' (1955).
3. I. D. Boenko, In the book: Problems in the Nervous Regulation of the Functions of the Animal and Human Body in Normal and Pathological Conditions [in Russian], No. 1, p. 34, Chita (1956).
4. D. M. Zubairov, Byull. éksp. Biol., No. 11, 33 (1962).
5. D. M. Zubairov, Physiological and Pathological Mechanisms of Regulation of the Clotting Power of the Blood, Author's Abstract of Doctorate Dissertation, Kazan' (1964).
6. D. M. Zubairov and V. I. Kurochkin, Byull. éksp. Biol., No. 3, 28 (1964).
7. M. A. Kotovshchikova and Z. D. Fedorova, Lab. Delo, No. 1, 18 (1961).
8. B. A. Kudryashov, Klin. Med., No. 10, 3 (1958).
9. B. A. Kudryashov and P. D. Ulitina, Doklady Akad. Nauk SSSR, 120, 677 (1958).
10. B. A. Kudryashov, Problems in Blood Clotting and Thrombus Formation [in Russian], Moscow (1960).
11. B. A. Kudryashov, In the book: Abstracts of Proceedings of the 10th Congress of the I. P. Pavlov All-Union Physiological Society [in Russian], 1, p. 144, Erevan (1964).
12. B. I. Kuznik, Probl. Gematol., No. 12, 15 (1962).
13. E. Sirmai, Prob. Gematol., No. 6, 38 (1957).
14. H. D. Bergerhof and L. Roka, Z. Vitamin-Hormon-u. Fermentforsch., Bd 6, S. 25 (1954).
15. H. Kowarzik and K. Buluk, Acta physiol. Pol., T. 5, N1, 35 (1954).
16. R. J. Lee and P. D. White, Am. J. med. Sci., 145, 495 (1913).
17. T. Shimamoto and T. Ishioka, Circulat. Res., 12, 138 (1963).