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Studies on Preserved Human Blood. V. Decrease in Prothrombin Titer During Storage.*

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Recent work has shown that a deficiency in plasma prothrombin is the cause of a bleeding tendency in a number of conditions, most notable of which are disease of the biliary tract, hemorrhagic disease of the new-born, and certain types of liver injury. For patients with such disturbances, blood transfusion is often necessary as an emergency measure. When fresh blood is given to the patient, there can be little doubt that the prothrombin in the transfused plasma can be utilized, and, although greatly diluted by the plasma of the recipient, may be of at least temporary benefit. With the rapidly increasing use of preserved blood for transfusions, the stability of prothrombin under conditions of storage has become a problem of considerable importance.

Rhoads and Panzer[1] reported that the prothrombin content of stored blood, as determined by the method of Quick,[2] diminishes very rapidly, so that after a few days of storage such blood would be useless for treating prothrombin deficiency. In contrast Belk, Henry and Rosenstein[3] found only moderate prolongation of Quick's prothrombin time at 10 days and no prolongation after 5 days of storage. More recently, Lord and Pastore[4] have studied the disintegration rate of the plasma prothrombin in preserved blood by the quantitative prothrombin method of Warner, Brinkhous and Smith.[5, 6] Their results are much more favorable and correspond very closely with the results which we have obtained. In the present paper data are

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1 Rhoads, Jonathan E., and Panzer, Lillian M., *J. A. M. A.*, 1939, **112**, 309.

2 Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

3 Belk, William P., Henry, Norman W., and Rosenstein, Florence, *Am. J. Med. Sci.*, 1939, **198**, 631.

4 Lord, Jere W. Jr., and Pastore, John B., *J. A. M. A.*, 1939, **113**, 2231.

5 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

6 Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

presented on the rate of prothrombin disintegration in blood stored at 3° to 5° in a dextrose-citrate mixture and in sodium citrate alone. These data show that the prothrombin content of the plasma decreases very gradually, reaching 50% of normal in about 3 weeks of storage. The disintegration rate was essentially the same in both preservatives studied.

Procedure. Blood was drawn from 6 healthy adult males into each of 2 solutions. One mixture consisted of 23 volumes of blood and 2 volumes of 3.2% dihydric sodium citrate in distilled water. The other mixture, previously described,[7] was composed of 10 volumes of blood, 13 volumes of 5.4% anhydrous dextrose in water, and 2 volumes of 3.2% dihydric sodium citrate in water. The two blood mixtures, from each of the 6 donors, were apportioned aseptically into a series of cotton-plugged test tubes and stored at 5°. At 5-day intervals a test tube from each series was taken from storage, the blood-mixture centrifugalized at 5° and the plasma titrated for prothrombin by the method of Warner, Brinkhous and Smith.[5, 6]

Preliminary observations indicated need for a slight modification of the usual method of titration. The test was carried out by comparison of unknown plasma with fresh human plasma of known potency. It was found, in the present study on preserved blood, that plasmas containing high concentrations of dextrose, as a diluent, tended to give distorted values when compared with citrated plasmas. The practice was therefore adopted of comparing the preserved plasmas with controls of fresh plasma diluted in the same manner and with the same substances as were the stored bloods. In all cases we adhered to the usual practice of adding a small amount of acacia during titration, to stabilize the colloidal state of the mixture.[6]

Results. A comparison of the values obtained in the 2 types of preservatives revealed no difference in the rate of disintegration, although hemolysis proceeded much more slowly in the dextrose-citrate mixture than when citrate alone was used. In Fig. 1 progressive prothrombin values obtained on 12 samples of stored blood are plotted as individual curves. The curve of the mean goes steadily downward, the prothombin reaching the 50% level between 21 and 25 days of storage. After 10 days of storage the mean prothrombin value was still 91% of normal, as compared with 20% obtained by Rhoads and Panzer, who used the method of Quick.

When Lord and Pastore[4] used Quick's method rather than the 2-stage method they likewise obtained low readings. However, Belk,

7 DeGowin, E. L., Harris, J. E., and Plass, E. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 126.

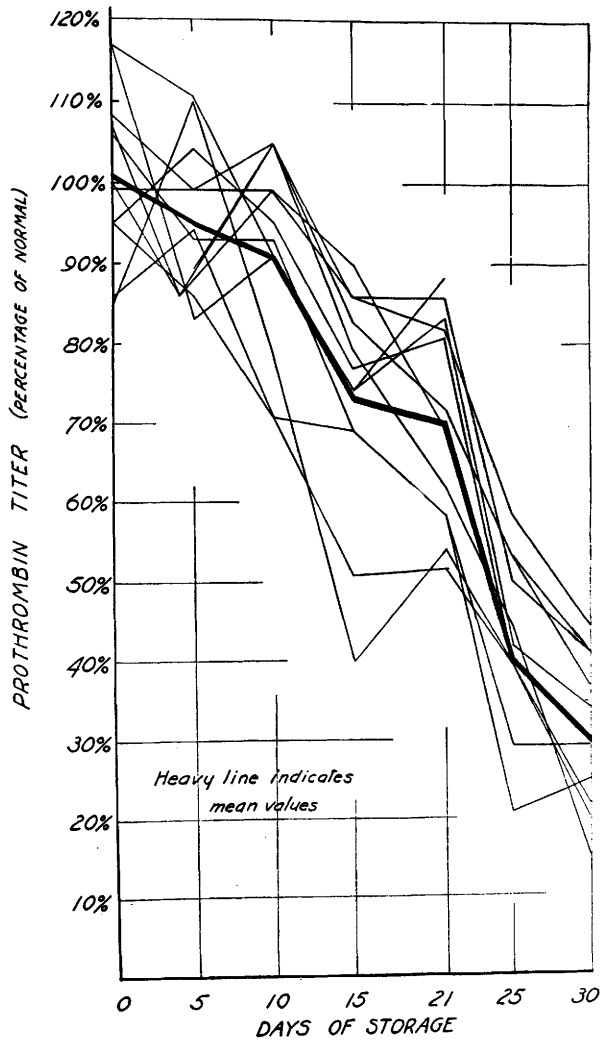

FIG. 1.
Prothrombin Content of Stored Human Blood.

Henry and Rosenstein[3] found only moderate prolongation of Quick's prothrombin time after 10 days of storage. We have used Quick's prothrombin time test on a number of samples of stored blood ranging from 0 to 30 days of age and the results, although rather variable, were only slightly lower than those obtained by the 2-stage method. No instances were found in which the value fell to less than 40% of the control level in 3 weeks of storage. It is possible that slight differences in conditions of storage, or in the anticoagulant used,

may account for the differences in results which have been obtained with Quick's prothrombin test.

Discussion. The quantitative estimation of the prothrombin content of blood is necessarily an assay technic based upon the physiological activity of the thrombin formed from the prothrombin, and no standard method has been generally adopted. Some of the reasons for a disparity in the results obtained by the prothrombin time method of Quick and by the titration procedure of Warner, Brinkhous and Smith have been discussed elsewhere.[8] The latter method eliminates errors due to factors which alter the time required for thrombin formation. Thus, this method at least approximates the ideal of eliminating all variables other than the amount of prothrombin present. In contrast, the prothrombin time of Quick represents a summation of factors which determine the speed with which the blood can clot. It should be pointed out, however, that such a measure of the coagulability of the blood may be of great practical use. It is possible that slight alterations in the reactivity of prothrombin in stored blood may occur under certain conditions and that this may detract greatly from the utility of the transfused prothrombin, even though the total amount present has not appreciably decreased. Clinical evidence will help in answering this question.

Summary. The rate of disintegration of the plasma prothrombin in preserved blood stored aseptically at 5° was studied by the quantitative prothrombin method of Warner, Brinkhous and Smith.

The prothrombin values were found to decrease very gradually, reaching the 50% level at the end of about 3 weeks.

No difference in the rate of disintegration was found between blood stored in sodium citrate and that stored in a citrate-dextrose mixture.

[8] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1939, **125**, 296; PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 197.