CLOTTING OF PLASMA AND SILICONE SURFACES

By Thomas B. Patton, M.D., Arnold G. Ware, Ph.D., and Walter H. Seegers, Ph.D.

The use of silicone surfaces for blood clotting studies offers new technical approaches to the problems concerned. In their pioneer work, Jaques, Fidlar, Feldsted and Macdonald indicated the possibility that plasma may clot independently of platelet action. They suggest, as others have, that plasma may contain a soluble factor which will initiate clotting. In their studies, the platelet concentrations were lowered by centrifugation at low speeds only to about 3,000 per mm³.

We believed that with the use of high speed centrifugation and silicone surfaces blood plasma would remain fluid indefinitely. All the platelets would be removed, and even if some should rupture, the thromboplastin released might be expected to be removed by sedimentation in accordance with the work described by Chargaff et al. We followed the venipuncture technic described and clotting was observed at unpredictable intervals. The poor results were apparently due to collapse of the vein with injury to the intima and subsequent liberation of thromboplastic substances. Slowness of withdrawal of blood also increased chances for contamination.

An improved technic was therefore devised which would eliminate tissue trauma insofar as possible. The common carotid artery of a dog was exposed, using meticulous sharp dissection. Saline was flushed over the wound and the artery, after which the wound was draped, leaving only the artery exposed. A special syringe holder (fig. 1) was designed to prevent blood from being forced into the syringe. When puncture was made, 10 cc. of physiologic saline contained in the syringe were first injected into the artery to wash away thromboplastic substances from the needle. After thirty seconds, the blood was permitted to enter the syringe at the pressure of the blood vessel. It was not necessary to pull on the plunger. The needle and special syringe were next removed and, with the syringe in a vertical position, blood was allowed to flow against the side of the silicone centrifuge tubes. A portion was oxalated for hematocrit and control prothrombin determinations. The tubes were then placed in the rotor of a multi-speed attachment (International Equipment Co.) and spun for varying periods of time at 22-23,000 R.P.M. After the runs were completed, the tubes were removed and the clear plasma could be seen above the closely packed red cells which were overlaid by a whitish gray layer. To avoid contamination of the supernatant layer with the middle layer, about two thirds of the centrifuge tube was immersed in a mixture of dry ice and alcohol. The red cells, the superimposed gray layer and the lower portion of plasma were frozen solid so that the clear supernatant plasma could be decanted. Samples for prothrombin determinations were taken immediately after centrifuging and at varying periods thereafter.

Our technic eliminated many of the previously encountered erratic results; how-
ever, it still did not enable us to obtain incoagulable plasma with any degree of certainty. We found that plasma would stay fluid for varying periods—in some runs longer than seventy-two hours at room temperatures. There would usually, however, be some fibrin formed. This occurred in silicone and in glass tubes with about equal frequency, provided centrifugation was carried out for sixty minutes or more.

The rotor of the multi-speed attachment accommodates six tubes and the contents of each one would usually give somewhat different results as to time and quantity of fibrin deposited as judged by visible inspection. This fact discouraged us from making quantitative measurements of the fibrin deposits. At the end of twenty-four hours, any fibrin was removed, thrombin was added to the plasma and a clot invariably formed.

Measurements of prothrombin concentration by the two-stage method indicated that this factor does not change appreciably in concentration if the high speed centrifugation is continued for fifteen minutes or more (table 1). Consequently, if thrombin causes the fibrin deposits mentioned above, only a small quantity is involved. It must be kept in mind that rupture of only a few platelets would liberate thromboplastin, which would perhaps not all be removed by centrifugation. Even if it were completely removed, a small amount of thrombin might first be liberated which would eventually form a fibrin deposit. If a soluble plasma factor, not concerned with platelets, initiates clotting, its action must be considered to be extremely feeble. If that view is not accepted, then it must at least be admitted that the soluble factor was sedimented in the centrifuge, or that it requires an activator removed by centrifugation.

Prothrombin analyses showed no significant change in prothrombin concentration. However, evidence for thrombin formation in the silicone plasma was obtained indirectly from Ac-globulin studies. This factor is transferred from plasma type Ac-globulin to serum type Ac-globulin through the action of thrombin. Even small amounts of thrombin will produce an effect which is easily detected. For
example, a quantity of thrombin which will not produce a clot in one hour will markedly affect plasma Ac-globulin activity. In the experiments discussed above, it was possible to detect serum type Ac-globulin when the two-stage prothrombin analyses showed no significant change. We interpret this as follows: a small amount of thrombin was formed by minute traces of thromboplastin of cellular origin. This amount of thrombin was so small that the plasma prothrombin concentration seemed not to be altered. However, this thrombin was sufficient to transform some plasma Ac-globulin to serum Ac-globulin and also sufficient to form a small fibrin deposit.

Fibrinogen was measured quantitatively in only a few experiments for reasons mentioned above. The changes in fibrinogen concentration during a typical experiment are recorded in table 1. Immediately after centrifugation the fibrinogen concentration, as measured by methods previously described, was lower than in the oxalate control sample. The fibrin was doubtless thrown down as soon as its aggregates formed. While the samples stood at room temperature, only small amounts of fibrin precipitated, and at twenty-two hours about 70 per cent of the original fibrinogen remained. More fibrinogen disappeared during the centrifugation period than at any other equivalent time interval. This suggests the possibility that a few platelets ruptured during the centrifugation.

### Table 1

<table>
<thead>
<tr>
<th>Hours After Centrifugation</th>
<th>Per Cent Prothrombin</th>
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<td></td>
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<td>51</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td></td>
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<tr>
<td>10</td>
<td>68</td>
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</table>

* Minutes in centrifuge. Prothrombin determined on oxalated samples served as control, and this control was considered to have a prothrombin concentration of 100 per cent.
† F = per cent fibrinogen.

Blood was collected with special care and centrifuged in silicone treated glass tubes at 12,000-23,000 R.P.M. for various periods of time. Even after prolonged centrifugation, incoagulable plasma could not always be obtained. When clots formed, only a small portion of the fibrinogen was represented as fibrin. Minute amounts of thrombin cause this fibrin formation. No evidence was found to support the view that plasma may contain a soluble factor which can initiate clotting independent of platelet action.

**Summary**

Blood was collected with special care and centrifuged in silicone treated glass tubes at 12,000-23,000 R.P.M. for various periods of time. Even after prolonged centrifugation, incoagulable plasma could not always be obtained. When clots formed, only a small portion of the fibrinogen was represented as fibrin. Minute amounts of thrombin cause this fibrin formation. No evidence was found to support the view that plasma may contain a soluble factor which can initiate clotting independent of platelet action.
REFERENCES

5 Ware, A. G., Murphy, R. C., and Seegers, W. H.: The function of Ac-globulin in blood clotting. Science, in press.
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