THE CHANGES IN CLOTTING POWER OF AN OXALATED PLASMA ON STANDING

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Howell¹ has shown that in testing blood to determine the presence of a hemophilic tendency, it is more satisfactory to obtain the clotting time of the oxalated and centrifugalized plasma after recalcification than to depend upon the time of coagulation of the whole blood, since in the latter case small variations in conditions may make large differences in the figures obtained. In connection with this procedure and also as a matter of general interest, it was thought desirable to ascertain to what extent the coagulating property of an oxalated plasma undergoes alteration upon keeping, and the effect upon this property of temperature and of sterile versus non-sterile conditions. The following experiments were made at the suggestion of Dr. W. H. Howell with the object of testing these points.

It is evident that determinations of the clotting time of blood kept over a period must be made upon oxalate or fluoride plasma. In these experiments, therefore, the following method was adopted:

A cannula was introduced into one of the carotid arteries of an anesthetized cat, and the blood allowed to flow into centrifuge tubes containing one part of 1 per cent sodium oxalate for every eight parts of blood. The blood and oxalate were thoroughly mixed and then centrifugalized for twenty minutes. The cell-free plasma was pipetted off and divided into three parts, one of which was kept at 4°C. during the period of the experiment, another at room temperature and the third at 37°C. The clotting time of these specimens was determined at intervals over a period of twenty-four hours by the method of recalcification. It is well known that if calcium be added to a plasma kept fluid by oxalate precipitation of its ionizable calcium the plasma will readily coagulate, the rapidity of coagulation being determined by the

¹ Arch. Int. Med., 1914, xiii, 76.
amount of calcium added. There is for every oxalated plasma a certain "optimum amount" of calcium, the addition of which will cause coagulation in the shortest time possible for that plasma under given conditions. If calcium be added in amounts under this optimum, the clotting time is appreciably slower, and the same is true for calcium in amounts above the optimum. In order to eliminate the errors arising from recalcifying with uncertain proportions of calcium, a series was carried for each test consisting of five clotting tubes, each of which contained 0.5 cc. of the specimen to be tested. To these tubes were added respectively three, four, five, six and eight drops of a 0.5 per cent calcium chloride solution. The tube clotting first was assumed to contain the optimum amount of calcium chloride. For the cat, using this method, the optimum amount of calcium chloride ranged between four and six drops, for different bloods. One such experiment upon human blood showed an optimum of four drops. The amount of calcium necessary to exert this optimum effect upon coagulation remained fairly constant during the period of each experiment, the slight variations falling well within the limits of experimental error. It is realized, when one considers the very minute quantities of calcium which affect coagulation, that even variations in the size of the drops due to temperature changes during the period of the experiment may influence the clotting time.

The coagulation time of the recalcified plasma was found to lengthen markedly during a period of twenty-four hours. In most cases, the variations in the coagulation time of plasma kept at room temperature were negligible up to about four hours after the blood was drawn, when a marked lengthening occurred and persisted steadily, with the result that after twenty-four hours the clotting time was three to seven times as long as it was at the beginning of the experiment.

The temperature at which the plasma is kept was found to exert a definite effect upon the clotting time. The plasma kept at 4° exhibited a very much less loss of clotting power than did that kept at room temperature. Plasma kept at 37°, on the other hand, showed a very much greater loss than that kept at room temperature (fig. 1).

It was noticed in all cases that evidences of putrefaction were most prominent in the specimens kept at 37°—the ones which exhibited the most marked lengthening of the coagulation time during twenty-

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2 The solutions of calcium chloride used for this purpose should be prepared from the crystal or hydrated preparations rather than from the granulated or fused form.
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four hours. This was suggestive of the possibility that the loss of clotting power might be the result of bacterial action. Accordingly, sterile plasma was collected in the following manner:

The required amount of oxalate was put into small-necked centrifuge tubes which were plugged with cotton and autoclaved. A clean operation laid bare the carotid artery of an anesthetized cat. The cotton plug was removed from one of the centrifuge tubes containing the sterile oxalate and a sterile rubber dam was quickly fitted over the mouth. This dam contained a small slit through which one end

Fig. 1. Showing the loss of clotting power in a non-sterile plasma. One-half cubic centimeter specimens of the plasma were recalcified at intervals with the optimum amount of calcium. Ordinates show the clotting time (in minutes). Abscissae show time lapse (in hours) after withdrawal of blood from artery. Continuous line represents plasma kept at room temperature. Broken line represents plasma kept at 4°C. Dotted line represents plasma kept at 37°C.
of a sterile cannula was plunged, and the other end quickly and carefully inserted into the artery. When the required amount of blood had entered the tube, the cannula was withdrawn and a second sterile rubber dam replaced the one with the slit. The blood was centrifuged. This method was found of value because it was quite impossible to prevent a cotton plug from being drawn into the tube during centrifugation. After centrifugation, the rubber dam was pierced by a long, sterile hypodermic needle and the clear plasma was drawn up into the sterile syringe. Two small flasks had been previously capped with rubber dams and autoclaved. The dam of each of these was now pierced, under sterile precautions, by the hypodermic needle and the plasma was delivered into each. When the needle was withdrawn, the elasticity of the rubber closed the hole to the exclusion of bacteria. Whenever a sample of plasma was needed for clotting time determinations, the dam of the little flask was punctured and the required amount drawn into a sterile syringe and transferred to the clotting tubes. One flask was kept at room temperature and the other at 37°. Plates were made daily from each flask. No colonies were found during the period of the experiment, which lasted five days.

The results of this procedure showed that no change whatever occurred in the clotting time of sterile plasma. The clotting time of the plasma at the end of one hundred and twenty hours was practically identical with the clotting time determined at the beginning of the experiment. This was true for the plasma kept at 37° as well as for that kept at room temperature. The slight variations during the period of the experiment were well within the limits of experimental error. After seventy-two hours, a portion of this sterile plasma was exposed to the air at 37°, and its clotting time at once began to lengthen steadily, so that forty-eight hours after exposure the addition of the optimum amount of calcium caused no coagulation in three hours. The unexposed plasma, however, remained constant in its clotting time (fig. 2).

An experiment was made with the object of determining the cause of the loss of coagulating power in exposed plasmas. Fresh oxalated plasma was heated to 54° and the fibrinogen precipitate filtered off. This fibrinogen-free plasma was then recalcified with the optimum amount of calcium, an active fibrinogen solution was added and the clotting time determined. The same test was made on the fibrinogen-free plasma kept at 37° for forty-eight hours. It is clear that any marked
difference in the clotting times so determined will indicate a change in the prothrombin content of the plasma. Another test made also upon fresh and forty-eight hour plasma consisted in the addition of an active thrombin solution to an unheated plasma. The clotting time of such a mixture will give a relative idea of the condition of the plasma-fibrinogen.

Fig. 2. Showing the retention of clotting power in a sterile plasma. One-half cubic centimeter plasma was recalcified at intervals with the optimum amount of calcium. Ordinates show the clotting time in minutes. Abscissae show time lapse (in hours) after withdrawal of blood from artery. The continuous line represents plasma kept at 37° (sterile). At X some of the plasma was exposed to the air at 37°. The change in clotting power of this exposed plasma is followed by the dotted line beginning at X. The broken line represents plasma (sterile) kept at room temperature during sixty hours.

The results were as follows:

0.5 cc. fresh oxalate plasma + 5 drops 0.5 per cent CaCl₂. Firm clot, 7 minutes, 30 seconds.

0.5 cc. oxalate plasma heated 54° + 5 drops 0.5 per cent CaCl₂ + 9 drops fibrinogen. Firm clot, 5 minutes.

0.5 cc. fresh oxalate plasma + thrombin 8 drops. Firm clot, 3 minutes.

Forty-eight hours later

0.5 cc. oxalate plasma + 5 drops 0.5 per cent CaCl₂. No clot, 24 hours.

0.5 cc. oxalate plasma heated 54° + 5 drops 0.5 per cent CaCl₂ + 9 drops fibrinogen. No clot, 24 hours.

0.5 cc. oxalate plasma + thrombin 8 drops. Poor floating clot, 10 minutes.

It is seen that there was a considerable alteration of fibrinogen during the forty-eight hours and a very marked destruction of prothrombin.

CONCLUSIONS

The coagulation time of non-sterile plasma lengthens steadily during the lapse of time after the blood is drawn. This loss of clotting power
is caused by bacterial action upon the prothrombin and fibrinogen and is not manifest in sterile plasma.

Coagulation determinations made on non-sterile plasma after a lapse of several hours from the time of obtaining the blood do not indicate the true coagulating power of the circulating blood. Low temperatures will greatly lessen the change that takes place, but an accurate test of the true clotting power of the circulating blood after a lapse of time, can be made only upon sterile plasma.