IN THE LABORATORY

Stabilization of Prothrombin Activity by Freezing: Application to the Standardization of Prothrombin Determinations

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A review of the literature shows that many reports have appeared describing the loss of prothrombin activity in stored plasma. This loss is thought to be due to alteration of the fibrinogen (*5, 8*), the prothrombin (*7, 10*), or factors activating prothrombin (*2, 6, 9, 12, 13*). Deterioration is delayed, but not prevented, by storage of plasma at low temperatures (*7, 10, 12*). This deterioration is troublesome in that it complicates the clinical use of the one-stage test. Furthermore, it prevents the use of a control plasma of known activity for standardization of thromboplastin solution beyond a single day. Careful standardization requires the testing of four or more normal subjects each day. It was mainly for the purpose of avoiding this that a study was made of the feasibility of preserving prothrombin activity by freezing plasma. In the present report it is shown that storage without loss is possible and practical if certain precautions are taken.

Prothrombin times of various plasmas were determined, using the Quick method (*11, 14*). Nine ml of blood was drawn into a syringe containing 1 ml of 0.1 M sodium oxalate. This was mixed and centrifuged, and the separated plasma treated as described below. Rabbit brain thromboplastin (Bacto Thromboplastin, Difco) was used throughout the study, and this preparation showed the uniform qualities described by other authors (*3, 4*). The thromboplastin solution was prepared as described by Campbell (*1*). A fresh solution was used each day. All determinations were made in a glass-walled water bath at 37° C. This permitted observations of clotting without removing the solutions from the bath, thereby obviating effects due to temperature change.

In several instances plasmas to be tested were examined at different dilutions. These were made using 0.9% sodium chloride solution, and the concentration expressed as per cent plasma in salt solution.

Plasma samples to be preserved were placed in glass tubes of 3-mm internal diameter and sealed in a flame. A tube of convenient length held 0.5 ml of plasma, or enough for several determinations. These tubes were placed in an ordinary "deep freeze" unit at −20° C. Freezing took place within an hour, and the samples were kept frozen until thawed for testing. The plasma was thawed rapidly by placing the tubes in a beaker of water at 37–40° C. Tests were made without delay, the thawed plasma being kept in ice water until examined.

The values given in the tables represent the mean of at least two determinations, further measurements being made in the event that the first pair were not in good agreement.

Three series of experiments were carried out. In the first (Table 1), fresh plasmas were divided into two portions. One of these was tested immediately, and the other was stored. After the time indicated in the table, the stored specimen was thawed and the prothrombin time again measured. At the same time a fresh specimen, drawn from the same subject, was also tested. Values in the table refer only to the 50% dilution, but agree with values obtained at 25% and 10% on the same subjects. It is to be noted that one specimen in this series was kept 50 days without change in activity.

TABLE 1

Date determined (1946)	50% Plasma prothrombin time (sec)			
	Days stored	Fresh sample	Stored sample	Fresh sample
Nov. 25	..	26	...	...
Dec. 2	7	...	26	25.5
Nov. 26	..	25.5	...	...
Dec. 5	9	...	25	25
Nov. 26	..	25.5	...	...
Dec. 3	7	...	26.5	...
Oct. 15	..	32	...	...
Dec. 3	50	...	31.5	...

TABLE 2

Date drawn (1947)	Jan. 23	Jan. 24	Jan. 25	Jan. 26	Jan. 27	Feb. 5
Days stored	13	12	11	10	9	Fresh
Prothrombin time (sec), 50% plasma on Feb. 5, 1947	22.5	25	22	22.5	23	24

In a second experiment (Table 2) blood specimens from the same subject were collected and stored on each of 5 successive days. Eight days after collecting the last specimen, prothrombin times of all stored specimens were measured. A fresh specimen was tested at the same time. Although results are given only for the 50% dilution, determinations at 25% and 15% gave similar agreement. It will be noted that deviation from the mean was no greater than 5%.

Finally, a series of 8 specimens from different subjects were obtained. The plasma from each was divided into three portions. One portion was tested immediately, and the others were frozen and stored. Eight days later the second portion of each was thawed and the prothrombin activity measured. This process was repeated after a further period of 13 days, when the third portion was examined. The results for the undiluted plasma, given in Table 3, show agreement with those obtained at 25% dilution. It will be noted that there are only minor differences in the average values. Fresh control plasmas studied at the time of each determination showed no significant differences in the thromboplastin solutions used.

TABLE 3

Subject	Prothrombin time (sec)—undiluted plasma		
	Fresh	Stored 8 days	Stored 22 days
A	16.5	17	16.2
B	16.1	15.2	16.3
C	19.3	19.8	19.1
D	18.9	19	18.8
E	19.1	18.3	18.5
F	19.7	19.1	18.9
G	20.6	20.2	...
H	19.7	19.5	...
Average	18.7	18.5	18.0

The first attempts to store plasma by freezing were only partially successful. Evaluation of a number of techniques led to the method finally devised. Four important points in this technique require emphasis:

(1) The samples were kept frozen. Samples accidentally thawed during storage showed a considerable prolongation of the prothrombin time. Freezing in an ordinary "deep freeze" unit was quite satisfactory, and no improvement was noted when samples were rapidly frozen by means of solid carbon dioxide.

(2) Samples to be used in determinations were always thawed rapidly. If allowed to thaw in ice water or in air at room temperature, a faint cloud of precipitated material was often observed in the plasma, and this was usually accompanied by an increase in the prothrombin time. This may be associated with the fibrinogen change reported by others (*5*, *8*). Such phenomena were not observed with rapidly thawed plasma.

(3) Once thawed, determinations were made without delay. Thawed plasma kept at room temperature or at 37° C deteriorated rapidly, the prothrombin time being doubled in 1½ hrs at the latter temperature. Such changes were noted to be similar to those described by others (*7*, *10*) for fresh plasma, but were found to be more pronounced in thawed plasma. Fortunately, when thawed plasma samples were kept in ice water during the period between thawing and determination, no appreciable deterioration occurred for at least 4 hrs.

(4) Storage was most satisfactory when tubes of small lumen were used. The more rapid heat transfer of such tubes presumably aided the necessary rapid thawing of the plasma. The tubes used were easily sealed in a flame, thereby avoiding spillage and loss or contamination. No precautions were taken to avoid bacterial contamination, aside from ordinary cleanliness.

It might also be noted that much of the work was done during hot weather and that this did not demonstrably influence the results.

It is believed that the method described makes possible (1) the use of stored standard plasma samples as controls in the prothrombin test, and (2) storage of plasmas, if desired, until a convenient time for determination is at hand.

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An Improved Method of Precipitating and Drying Vinyl Copolymers[1]

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In the course of synthesizing several 1-kg batches of vinyl copolymers there arose the problem of precipitating and drying the large batches in the laboratory.

For most of the copolymers prepared, precipitation in a large vat with methanol gave rise to materials with a molasses-like consistency which were very difficult to dry. This method failed with a number of copolymers including methyl acrylate-maleic anhydride, ethyl acrylate-maleic anhydride, styrene-butyl acrylate (these copolymers were prepared in toluene solution), methyl vinyl ketone-methyl methacrylate, methyl vinyl ketone-butyl methacrylate (prepared in 70% methanol solution), vinyl acetate-vinyl

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